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Carpeloidy in flower evolution and diversification: a comparative study in *Carica papaya* and *Arabidopsis thaliana*

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- **Background and Aims** Bisexual flowers of *Carica papaya* range from highly regular flowers to morphs with various fusions of stamens to the ovary. *Arabidopsis thaliana sup1* mutants have carpels replaced by chimeric carpel–stamen structures. Comparative analysis of stamen to carpel conversions in the two different plant systems was used to understand the stage and origin of carpeloidy when derived from stamen tissues, and consequently to understand how carpeloidy contributes to innovations in flower evolution.
- **Methods** Floral development of bisexual flowers of *Carica* was studied by scanning electron microscopy and was compared with teratological *sup* mutants of *A. thaliana*.
- **Key Results** In *Carica* development of bisexual flowers was similar to wild (unisexual) forms up to locule initiation. Feminization ranges from fusion of stamen tissue to the gynoeceum to complete carpeloidy of antepetalous stamens. In *A. thaliana*, partial stamen feminization occurs exclusively at the flower apex, with normal stamens forming at the periphery. Such transformations take place relatively late in development, indicating strong developmental plasticity of most stamen tissues. These results are compared with evo-devo theories on flower bisexuality, as derived from unisexual ancestors. The *Arabidopsis* data highlight possible early evolutionary events in the acquisition of bisexuality by a patchy transformation of stamen parts into female parts linked to a flower axis-position effect. The *Carica* results highlight tissue-fusion mechanisms in angiosperms leading to carpeloidy once bisexual flowers have evolved.
- **Conclusions** We show two different developmental routes leading to stamen to carpel conversions by late re-specification. The process may be a fundamental aspect of flower development that is hidden in most instances by developmental homeostasis.

Key words: *Arabidopsis*, *Carica papaya*, bisexual flowers, carpeloidy, ectopic ovules, evo-devo, feminization, floral development, sex conversion, *sup1* mutants.

INTRODUCTION

Flowers are the result of evolutionary innovations, which include bisexuality and determinate flower meristems. The origin of bisexuality from ancestors with indeterminate and unisexual axes of male or female structures relies on the location on the same flower axis of stamen and carpel structures. Determinate flowers result through internode compaction and production of a reduced and fixed number of reproductive organs (Bateman *et al.*, 2006; Baum and Hileman, 2006).

One of the possible scenarios for the separation of stamen from carpel zones in compacted hermaphrodite flowers required the early specification of stamen and carpel identities through the action of class B and C MADS-box homeotic genes associated with specific cadastral genes (Krzek and Fletcher, 2005; Sablowski, 2007). The conserved primary activity of these MADS-box factors in the evolution of angiosperms has largely been documented (Kramer *et al.*, 2004; Zahn *et al.*, 2005). The expression patterns, loss-of-function mutations and morphological effects in ectopic variants of *SUPERMAN* (*SUP*), a transcription factor with cadastral male–female boundary function, appear to be equally

conserved in a few, but phylogenetically distantly related genera of angiosperms, such as *Arabidopsis* (Bowman *et al.*, 1992), *Antirrhinum* (Davies *et al.*, 1999), *Oryza* (Nandi *et al.*, 2000), *Nicotiana* (Bereterbide *et al.*, 2001; Nibau *et al.*, 2011), *Petunia* (Nakagawa *et al.*, 2004) and *Silene* (Kazama *et al.*, 2009). More specifically, loss-of-function mutants, on the one hand, are known only in *Arabidopsis*, *Petunia* and *Antirrhinum* with phenotypes exhibiting alterations in stamen and carpel number and/or placenta/ovule structure (see references above). Experiments with ectopic expression of *SUP* (mainly from *Arabidopsis*), on the other hand, have resulted in juvenile death or dwarf plants (strong expression) and plants with reduced leaf size and/or flower organs (milder expression) in tobacco, rice and petunia (see references above). This indicates that *SUP* may operate at the cellular level by controlling both proliferation and elongation of cells.

Alterations and reversions of bisexuality have frequently occurred during angiosperm evolution and resulted in functional unisexuality (male or female sterility, such as in *Asparagus*) or morphological unisexuality (mono- or dioecious reproductive systems, to name just a few, such as in maize, hops, papaya and white campion) (Negrutiu, 1991; Ainsworth, 1999;

Mitchell and Diggle, 2005). Among such alterations, carpeloidy (also called pistillody), restricted here to the stamen feminization process, is widespread in nature (Storey, 1969; Shealy and Herr, 1973; Islam and Shepherd, 1992; Hufford, 1996; Hama *et al.*, 2004; Drea *et al.*, 2007). However, little is yet known regarding the processes at work in the development of carpeloidy. We describe here a comparative investigation in two species belonging to the Brassicales, *Carica papaya* and *Arabidopsis thaliana*.

The *C. papaya* system consists of male, female and bisexual flower types, with stamen-to-carpel conversions in the last named (see below). Most wild forms and some cultivars are dioecious and produce unisexual flowers with very little evidence of the other sex. In male flowers the ovary has become transformed into an elongated pistillode that is apparently nectariferous, and no trace exists of stamens in pistillate flowers (Ronse De Craene and Smets, 1999). Hermaphrodite cultivars of *C. papaya* have been known for a long time and represent important crops (e.g. Sprecher, 1943; Chateau, 1955; Storey, 1958, 1969; Purseglove, 1968; Lassoudière, 1969).

Hermaphroditic flowers of *Carica* tend to be highly variable in the extent of development of stamens and carpels (Moncur, 1988; Sippel *et al.*, 1989), ranging from flowers with ten stamens and five carpels (*elongata* type), to flowers with five stamens and five or ten carpels (*pentandria* type) (Lassoudière, 1969). Abnormal flowers with 1–5 stamens fused to different degrees with the ovary and with some developing stigmatic tissue on the anthers have been frequently described in the literature (e.g. Sprecher, 1943; Lassoudière, 1969).

The *Arabidopsis* system consists of wild-type determinate hermaphrodite flowers (as typical for the Brassicaceae) with four sepals, four petals, six stamens and two fused carpels, organized as distinct successive whorls. The cadastral mutant class *sup1* (*superman*) flowers are slightly indeterminate male flowers, exhibiting chimeric carpel–stamen structures at the centre of the flower (Bowman *et al.*, 1992; Jacobsen and Meyerowitz, 1997; Sakai *et al.*, 2000; Breuil-Broyer *et al.*, 2004). Such structures are considered to result from congenital fusion of carpel primordia and innermost presumptive stamen primordia (Bowman *et al.*, 1992: p. 603, fig. 1O, P). Although the genetic and molecular nature of the mutation is relatively well understood, little is known about the origin and nature of the mosaic organs and the suggested fusion process. Interestingly, *SUP* is known to perform early cadastral but also additional functions during later stamen and carpel differentiation (Gaiser *et al.*, 1995; Breuil-Broyer *et al.*, 2004).

The aim of this study was to perform a comparative analysis of stamen to carpel conversions in hermaphrodite *C. papaya* and in the *Arabidopsis sup1* mutants: (1) to understand the origin of carpeloidy when derived from stamen tissues, including the timing (i.e. critical stage) of its development; and (2) to assess, in the light of these results, the contribution of carpeloidy to innovations in flower and fruit evolution.

MATERIALS AND METHODS

Floral buds of *C. papaya* (collection sample 900062) were collected at the Jardin Botanique de Lyon and kept in 70% ethanol.

Inflorescence buds were dissected under a Wild MZ8 stereomicroscope (Leica, Wetzlar, Germany), dehydrated in an absolute ethanol–acetone series, and critical point dried with a K850 Critical Point Dryer (Emitech Ltd, Ashford, UK). Material was coated with platinum using a K575X sputter coater (Emitech Ltd) and observed with a Supra 55VP scanning electron microscope (LEO Electron Microscopy Ltd, Cambridge, UK).

For *Arabidopsis*, flower samples were mounted on slides covered with double-sided adhesive tape and photographed with a Leica MZ TLIII binocular system.

RESULTS

Floral development in staminate and pistillate Carica flowers

Ronse De Craene and Smets (1999) described the floral development of staminate and pistillate *Carica*. Early stages of flowers including the development of petals was highly similar between pistillate and staminate flowers. Subsequently, differences were found in the absence of the androecium in pistillate flowers, while pistillate flowers had massive ovaries with subequal carpellary lobes and strongly developed intruding parietal placentae. In staminate flowers two stamen whorls surround a central residual area which develops into a spear-like organ that Ronse De Craene and Smets (1999) interpreted as a pistillode. These elements constitute the reference system in the analysis of bisexual flowers below.

Flower development in Carica perfect bisexual flowers

Flowers are enclosed by a bract and two bracteoles. Five sepals are rapidly initiated in a 2/5 sequence starting on the abaxial side next to the bract (Fig. 1A, D). The sepals grow into massive subequal lobes enclosing the bud in a quincuncial aestivation. The five petal lobes arise almost simultaneously and undergo strongly contorted growth by unequal development (Fig. 1B, C). Petals develop into highly asymmetric organs with an inner section intruding between the developing stamens and an outer section enwrapping a neighbouring inner invagination of an adjacent petal (Fig. 1C). Stamens initiate immediately following common basal growth below the petals. They develop on the periphery of a flattened apex. Initiation of antesealous stamens precedes the antepetalous stamens, but often stamens on the abaxial side of the flower arise before those on the adaxial side (Fig. 1E, F). The androecium is diplostemonous with the antesealous stamens overlapping the antepetalous stamens (Fig. 1F, H). This is caused by the continuing growth of a common primordium lifting stamens and petals into a tube (Figs 1H, 2D and 3A, B). This two-tiered arrangement of the stamens is maintained and developing stamens form a long tube around the developing ovary. In later stages hairy filaments are produced leading to erect tetrasporangiate stamens (Fig. 3A–C). The central apical area differentiates into a bulging pentagon and five carpellary lobes develop simultaneously on the periphery (Figs 1G and 2A). They grow upwards as five carpels around a central residue (Fig. 2B, C). Carpels often have an unequal shape or develop irregularly (Fig. 2B–D). While a

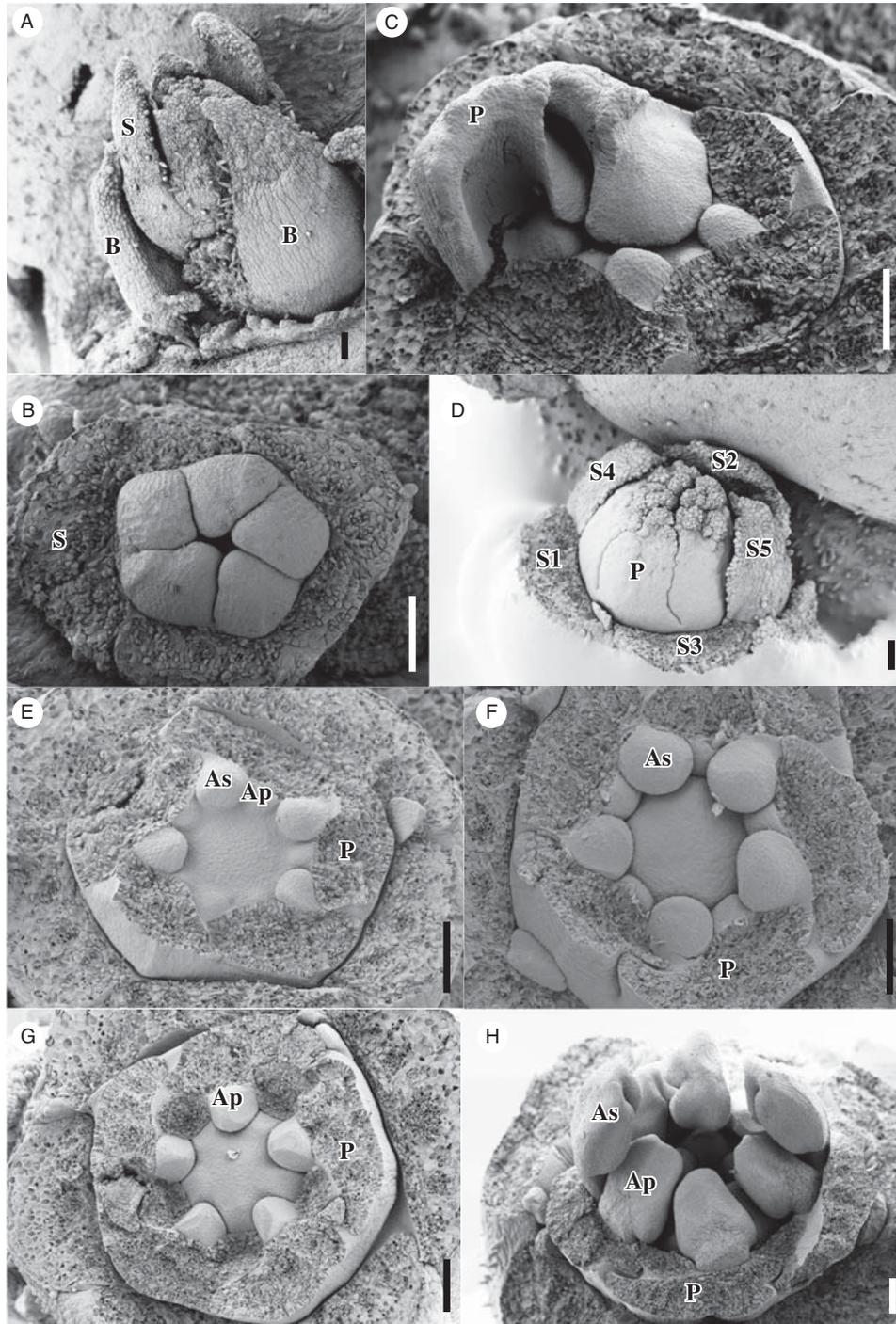


FIG. 1. Early floral development of bisexual *Carica papaya*. (A) Lateral view of young bud at sepal initiation. Lateral flowers are initiated in the axil of a broad bract and are enclosed by two bracteoles. (B) Floral bud from above, showing young petals; sepals removed. (C) Lateral view of two imbricate petals covering young stamens; three petals and sepals removed. (D) View of lateral floral bud; two sepals removed. Sepal lobes are rapidly covered with papillae, especially on the margins. Note the differentiation of petals and basal connection. They become rapidly lifted by common growth at the base and enclose the bud in an imbricate contorted aestivation. By continuous growth the petals push open the loosely enclosing sepal lobes. At that time the upper part of the petal lobes becomes strongly papillate. (E) Apical view at stamen initiation; petals removed. Note the delay in stamen formation on the adaxial side. (F) Development of two stamen whorls and gynoeceal dome. (G) Apical view of development of gynoeceal dome; antesepalous stamens removed. (H) Lateral view at anther differentiation; two antesepalous stamens removed. Abbreviations: Ap, antepetalous stamen; As, antesepalous stamen; B, bract; P, petal; S, sepal. Scale bars = 100 μ m.

common lower tube leads to a saccate ovary, the carpillary lobes remain apically separate and develop into stylar lobes (Figs 2D and 3A). A characteristic shape resembling antlers

is finally achieved at maturity. Compared with unisexual flowers of *Carica*, the ovary of a bisexual flower resembles an elongated pear.

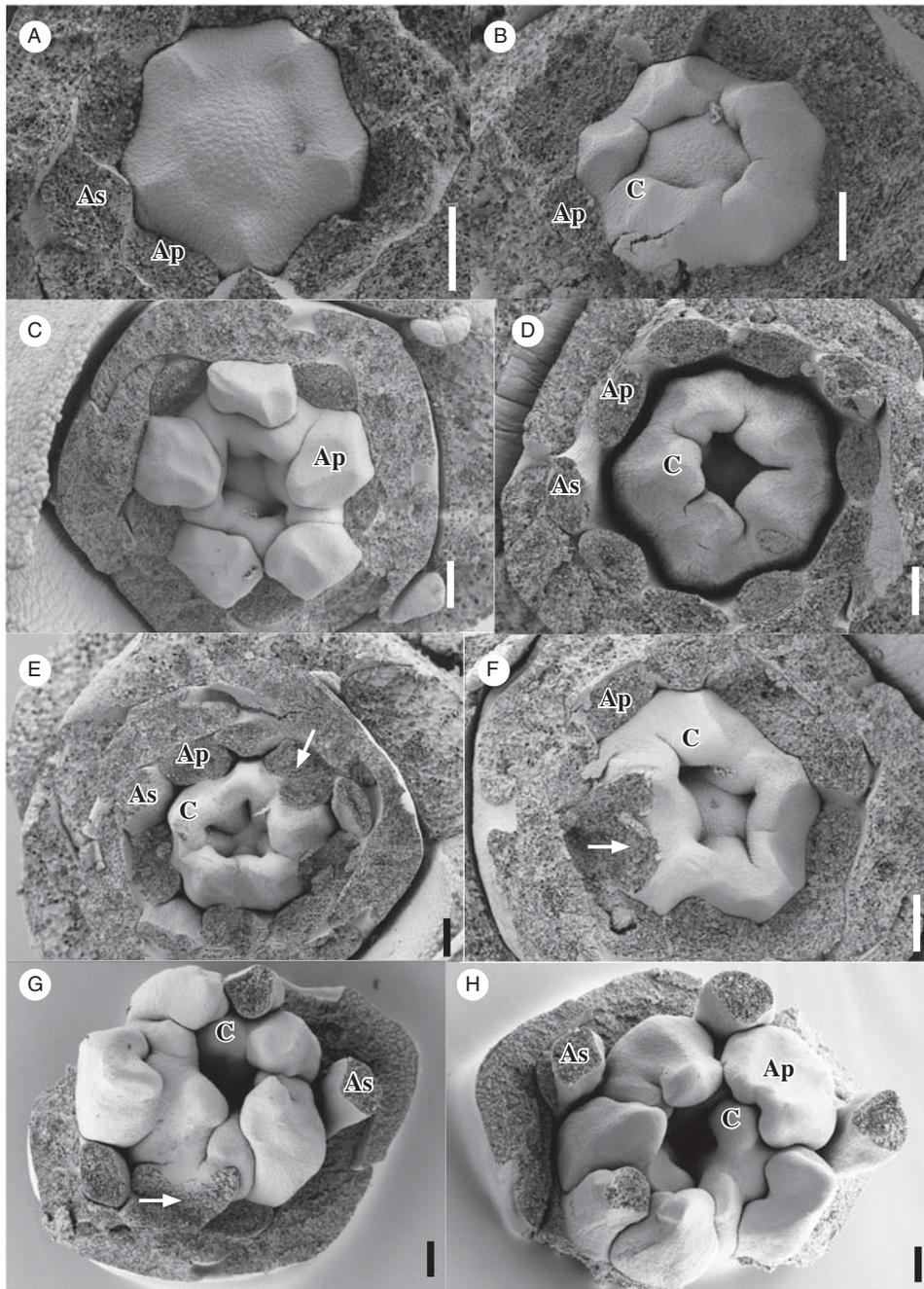


FIG. 2. Differentiation of organs in bisexual *Carica papaya* without (A–D) and with (E–H) carpel–stamen fusion. (A) Initiation of five carpels on apical meristem; all stamens removed. (B) Lateral view of differentiation of peripheral carpels. (C) Apical view of development of carpillary locules; antesepalous stamens removed. (D) Development of stigmatic lobes on top of ovary; note the tube with fused petals and stamens surrounding the ovary. (E,F) Apical and lateral view of two buds at differentiation of carpillary locules. Note scars of antepetalous stamen invading carpel zone (arrows). (G,H) Two views of abnormal buds showing invading antepetalous stamens fused with ovary (arrow). Note hybrid fused stamen–carpels and the development of normal filaments of antesepalous stamens. Abbreviations: Ap, antepetalous stamen; As, antesepalous stamen; C, carpel or stigmatic lobe. Scale bars = 100 μm .

Stamen feminization in Carica flowers occurs through invasive stamen–carpel fusion

We now compare the development of perfect bisexual flowers with floral material from the same cultivar showing highly variable flowers, ranging from fully hermaphrodite morphs to strongly altered teratological flowers.

In a large number of cases (45% of 30 nearly mature flowers investigated), flowers depart from the wild-type situation by being much more distorted, showing a range of transitional morphologies between ovary and stamens. Distortions appear at the time of carpillary locule differentiation by the invagination of antepetalous stamens into the carpillary

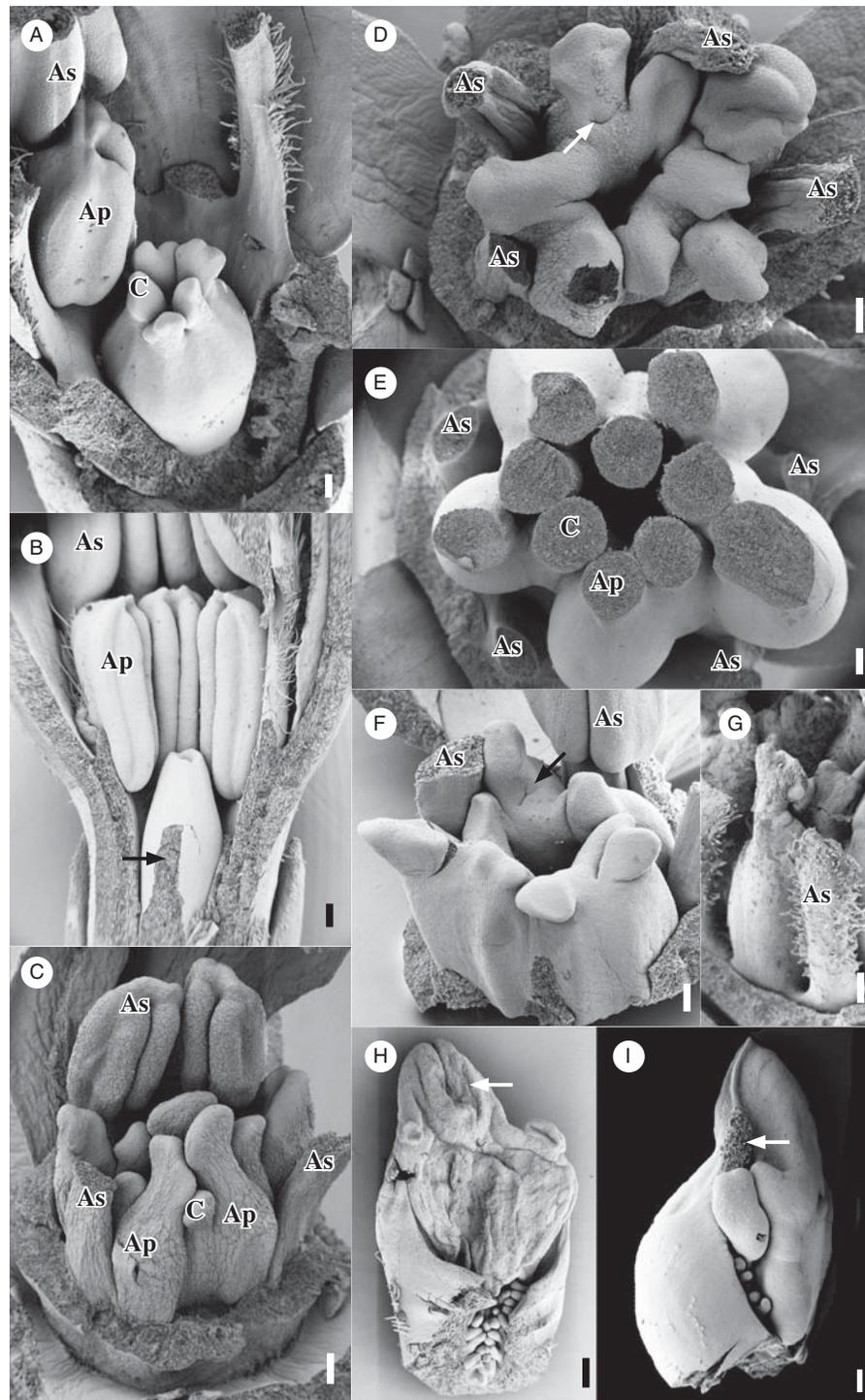


FIG. 3. Carpeloidy in preanthetic buds of *Carica papaya*. (A) Lateral view of partly dissected perfect bisexual flower showing elongated ovary and two levels of stamens. (B) Lateral view of similar stage but with incompletely developed ovary showing fusion scar on one side (arrow). Similar pistillodes are typical for wild-type male flowers. (C) Lateral view of abnormal bud with extensive fusion between aberrant antepetalous stamens and ovary. Note stigmatic-shaped appendages on antepetalous stamens. (D) Apical view of flower with partly feminized antepetalous stamens fused to carpellary tissue. (E) Transverse section through composite ovary–stamen structure. Note the extensive fusion of carpellary and stamen tissue. (F) Lateral view of compound ovary–stamen structure with no evidence of staminal tissue. Arrow points to carpel slit. (G) Lateral view of two stamens; the left with extralocular ovules; the right with normal anther removed. (H,I) Lateral views of feminized stamens. In H note basal ovules and apical remnants of anthers (arrow); in I note lower ovules and higher stigmatic tissue (arrow). Abbreviations: Ap, antepetalous stamen; As, antesepalous stamen; C, carpel or stigmatic lobe. Scale bars: (A, C, D, F) = 100 μm ; (B, E, G, H, I) = 200 μm .

tissue. This invagination occurs by sideways concrescence of ovary and antepetalous stamens (Fig. 2E–G) and corresponds to an invasion of stamen-origin tissue into the confines of the ovary (Fig. 4A–C, G). This was visible during development as a postgenital fusion of young stamens with the developing carpels (Fig. 2E, F). Fusion of stamens with the ovary appears to different degrees; sometimes one side of the flower produces anthers, while the other side becomes completely transformed by fusions with no clear anthers visible (Figs 2H and 3D). The ovary can also appear underdeveloped without clear stigmatic lobes, although ovule primordia are visible (Fig. 3B). In other cases, concrescence is almost complete without evidence of anther tissue (Figs 3C–F and 4D, E, G–I). While antepetalous stamens are fused with the ovary, the antesepalous stamens remain free and produce filaments (Figs 2H, 3C–E and 4D). The fusion of antepetalous stamens is accompanied by a transformation of anthers into plate-like stigmatic lobes (Figs 2G, H, 3C–D, F and 4G–J). In some cases monstrosities arise with slit-like holes (Fig. 3D, F, arrows), hybrid organs with external stylar tissue and anthers (Figs 3D, I and 4B, C, G) or ovule-producing appendages with remnants of sterile anthers (Fig. 3G–I). In most cases fusion was unequal as one or more stamens could be left out (Fig. 4D, G, J). In only a few cases did we find flowers corresponding to the *pentandria* type with an equal fusion of antepetalous stamens to the ovary apparently producing outwardly bulging carpels (Figs 3E and 4E).

Hand-sectioned flowers with such bulging carpels showed that stamen–carpel fusions must have occurred in most cases between the margins of developing primary carpels and the adaxial epidermis of the filaments (Figs 2C, E–H, 3B and 4A–C). As a result the feminized stamens alternate with true carpels at the exterior of the central carpellary locule (Fig. 3C, E). In addition, connective and/or anther tissues can take part in the fusion process (Figs 2G, H and 4A–C), with, for example, tissue proliferation occurring from the anther interocular space (Fig. 4C). In fully feminized fused stamens no trace of anther tissue is visible and circular cavities filled with ovules were produced centrally, representing miniature locular cavities around the central ovary and a dorsal vascular bundle in a similar position as normal carpels (Fig. 4D–F).

In conclusion, the results show that early stages of development were found to be highly conservative and similar to dioecious *C. papaya*, indicating that the developmental alterations commence after organ initiation, with stamens switching their developmental fate during morphogenesis. Despite the relatively variable occurrence of carpeloidy, we define the critical stage as locule differentiation in antepetalous stamens.

Carpeloidy in Arabidopsis occurs exclusively at the flower meristem centre

Contrary to the determinate wild-type flower of *Arabidopsis* (Fig. 5A), *sup1* mutants exhibit imperfect male flowers, with increased numbers of stamens [on average 10–13 (even 15)], a doubling as compared with wild-type flowers, formed as 2–3 whorls initiated successively in time.

Ninety per cent of *sup1* flowers produce only stamens or aberrant stamens, i.e. male flowers. The rest produce carpels

or carpeloid structures at the centres of the flowers, i.e. bisexual flowers (Fig. 5B). Exceptionally, such flowers produce seeds.

An average of 10 % of the stamens in all *sup1* flowers are aberrant, producing ectopic ovules and/or stigmatic tissues, with various degrees of filament, connective and/or apical stamen region expansions, i.e. undergo feminization (Fig. 5B–L). Such feminized stamens are positioned at the flower meristem centre only (Fig. 5C–K) and occasionally show fusion at the base of the filaments. Different stamen tissues are transformed to different degrees, with connective and filament being mostly subject to carpeloidy, while pollen sacs tend to persist on such structures (Fig. 5C–G, I–L). However, one and sometimes both pollen sac(s) per theca are altered (some dedifferentiate, others abort), generating reduced asymmetric anther-derived structures (Fig. 5C, E, L).

Such feminized structures are able to produce mega- and microspores on the same organ, therefore exhibiting combined male and female traits. Overall, the results highlight the important capacity of filament and connective tissues to respond to feminization. Such filaments expand or show hypertrophy (i.e. exhibit fasciation), with ectopic ovules and valve-type structures originating most frequently along the vascular region (Fig. 5C, E–G, L). The connective is equally responsive and, in addition, can produce at its distal region stigma, style and occasionally valve-type tissues (Fig. 5C–L).

Based on the observed alterations, carpeloidy in *sup1* can be classified into three categories. The weak type has more or less normal stamens on which isolated ovules and/or stigmatic tissue appear (Fig. 5B–E, I). The medium type corresponds to stamens with shorter and broader filaments (fasciation), expanded connective tissue with ovules and/or stigmatic papillae and clear-cut pollen sac structures in the right position along the axis (Fig. 5D, E, J–L). The strong type resembles the medium type, except that pollen sacs dedifferentiate to a great extent or are entirely absent and reduced valve structures are formed (Fig. 5F–H).

DISCUSSION

This comparative study of carpeloidy in two plant species shows distinct developmental mechanisms which result in stamen–carpel conversions: (1) stamen feminization through antepetalous stamen–carpel fusion in *Carica* and (2) stamen feminization exclusively at the flower meristem centre in *Arabidopsis*, with stamens at the periphery of the meristem being wild-type. In the two species, such transformations mediated through apparently distinct cellular processes occurred at relatively late stages of stamen development, indicating strong developmental plasticity of various stamen tissues towards acquisition of female traits, including ovule formation.

In *Carica*, our observations on hermaphrodite flower development showed no clear-cut differences from wild morphs (see Ronse De Craene and Smets, 1999) until after stamen initiation, indicating that feminization is not linked to homeosis. In subsequent stages, hermaphrodite flower meristems and buds apparently lack space, as the basal common zone surrounding the pistillode in staminate flowers is less constricted compared with hermaphrodite flowers where the ovary bulges

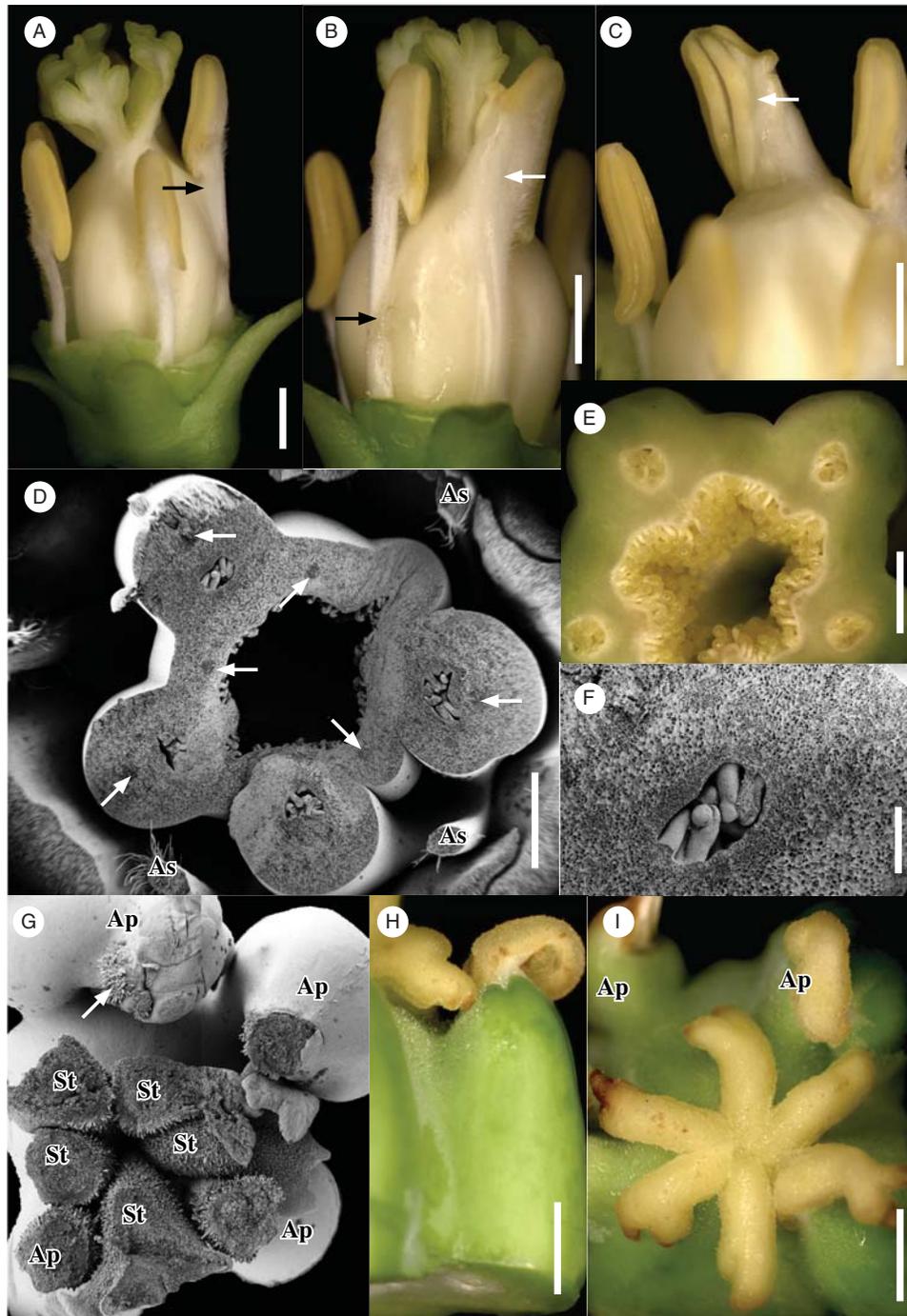


FIG. 4. Carpeloidy in preanthetic buds of *Carica papaya* (cont.). (A–C) Lateral views of bisexual flowers with variable stamen–carpel fusions showing the role played by the adaxial side of stamen filaments in the process (A, B; black arrows). Connective cells are also involved, the result being aberrantly shaped anthers with associated invading tissue growth (B, C; white arrows). (D–F) Transverse sections of entirely feminized stamens fused to the gynoecium. The corresponding carpeloid structures appear as regular locules alternating with the genuine carpels and bear apparently normal ovules. The lobes and cavity of the genuine ovary can be seen in the centre (D, E). In D four feminized stamens are fused to the ovary. Arrows point to dorsal vascular bundles. (E) Slightly older bud showing the secondary locules and central ovary with ovules. (F) Detail of secondary locule of D. (G) Top view of partly sectioned ovary with feminized stamens. Partly fused aberrant stamen bearing stigmatic tissue is visible at top (arrow); the remaining stamens are indistinguishable from carpellary tissue and bear stigmatic papillae. (H, I) Lateral and top views of entirely feminized stamens fused to the gynoecium. In I incomplete fusion and transformation of two antepetalous stamens. Abbreviations: Ap, antepetalous stamen; As, antesealous stamen; St, stigmatic lobe. Scale bars: (A–E, H, I) = 1 mm; (F, G) = 200 μ m.

up and occupies the limited space. Compared with wild pistillate *Carica* flowers, the initiation of carpels in hermaphrodite flowers is irregular with different sizes of carpel primordia.

This and the intruding, invasive growth of the stamens during differentiation suggest that tissue pressure in a closed space favours fusion of inner antepetalous stamens and

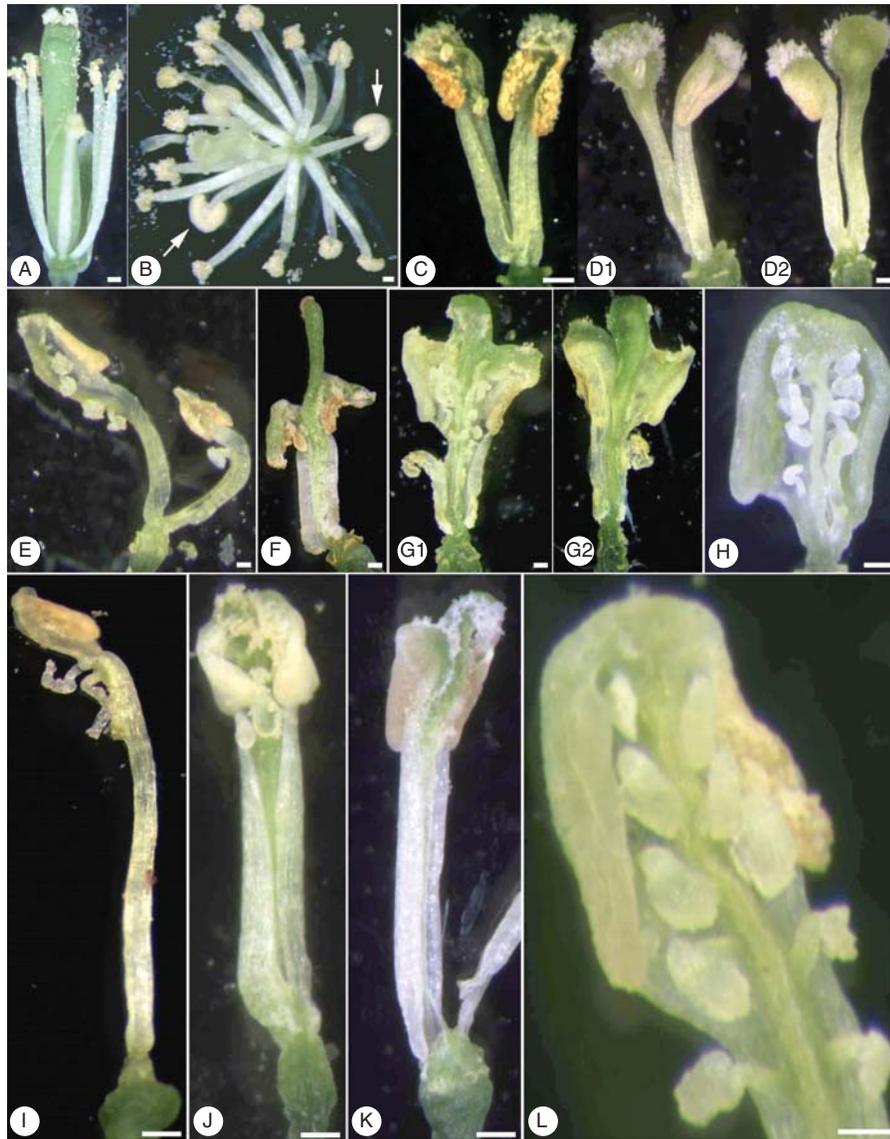


FIG. 5. Carpeloidy in the *Arabidopsis sup1* mutant. (A) Lateral view of a wild-type flower with sepals and petals removed. Five of six stamens and one valve, style and stigma are visible. (B) *sup1* mutant flower with sepals and petals removed. Most stamens are wild-type and have liberated their pollen. Two stamens with enlarged connective (arrows) tissue, indicating weak feminization. A carpelloid structure has formed with narrow base and style and stigma. (C–L) All stamens shown were located at the flower centre, the remainder have been removed, with scars and nectaries visible on the flower bud dome. The pollen sacs, when present, are yellow or brownish. The common trends in feminized stamens are filament fasciation, connective tissue expansion, anther reduction, and production of ectopic ovules and/or stigmatic tissues. (C) Two stamens with weak feminization phenotypes. To the right, the stamen with a compact filament is topped by stigmatic tissue. The connective tissue has expanded. To the left, only one pollen sac is recognizable, two ovules and stigmatic papillae being produced. (D) Two stamens viewed from adaxial (D1) and abaxial (D2) sides. (D1) The stamen at the left has a medium-to-strongly feminized phenotype, lacks pollen sacs and is terminated by a compact green anther-like region topped with stigmatic tissue and one ovule-like structure. The stamen at the right has a weak feminized phenotype, producing stigmatic papillae at its top. The pollen sacs are reduced in size. (D2) The distal abaxial region of the two stamens is greenish and corresponds to a hypertrophied carpelloid connective tissue. (E) Two stamens, a weakly feminized to the right (producing one ovule on the filament) and a medium feminized to the left, producing a row of ovules along the expanded connective tissue and the filament. Only one pollen sac is recognizable (asymmetric feminization); one theca has dedifferentiated. (F) A strongly feminized stamen with hypertrophied filament. The distal connective tissue has formed, and as an extension, a long and narrow carpelloid structure topped with stigmatic papillae. Two reduced brownish split thecae (aborted pollen sacs) with green carpelloid and stigmatoid expansions are visible at the transition region between the filament and the central stigmatoid structure. (G) A strongly feminized stamen viewed from its adaxial (G1) and abaxial (G2) sides. (G1) The hypertrophied filament and connective tissue are producing rows of ovules and green carpelloid tissues topped with stigmatic papillae at distinct positions. The pollen sacs are reduced in number. The structure is fused at its base with a neighbouring curling stamen. (G2) The abaxial side shows the hypertrophied filament extending into a club-shaped valve structure. (H) A strongly feminized stamen with very compact and expanded filament and hypertrophied connective with two rows of ovules along the vasculature and the two narrow-sized pollen sacs undergoing dedifferentiation (as indicated by tissue colour at lateral positions, the latter being 'resorbed'). (I–L) Details of stamen feminization. (I) A stamen with weak feminization phenotype producing two ovules with typical *sup* morphology located at the base of the anther and at the distal part of the filament. (J) A medium feminized stamen with hypertrophied filament and extended connective tissue forming a laminar structure topped with stigmatic papillae. Two reduced thecae with ectopic ovules at their base. Pollen sacs are reduced in number and size. (K) A medium feminized stamen with expanded greenish connective tissue topped with stigmatic papillae. Reduced abortive pollen sacs are present on both sides. (L) A medium feminized stamen with expanded filament, vasculature and connective. Two rows of ovules are formed along the vasculature. The theca on the left is reduced and may undergo dedifferentiation. Scale bars = 125 μ m.

carpels, with a transformation of the former into carpels as a result (also see Sieber *et al.*, 2000 and below).

This fusion is variable and gradual, with identities of antepetalous stamens occasionally maintained on one side of the flower or with a complete transformation and immersion of stamens into carpellary tissue. The various degrees of carpeloidy we observed were variably associated with remaining male attributes, such as small sterile anthers. In this respect, Lassoudière (1969) distinguished, as we describe in detail here, (1) cases with hypertrophied filaments (i.e. fasciation) and more or less developed anthers, (2) cases with the development of a carpellary cavity by the curvature of the filament bearing a terminal anther and (3) cases with flowers with complete absorption of stamens into the ovary tissues. The last appeared as small-scale carpels with a dorsal vascular bundle in a similar position to that of normal carpels (Fig. 4D). Various authors have concentrated on these different types, as they determine the shape of fruits produced, which is of commercial significance (e.g. Storey, 1958, 1969).

The precise nature of carpeloidy at the cellular level in *Carica* remains to be analysed. The conversion process occurs rapidly upon stamen–carpel contact and is complete most of the time, i.e. all stamen tissues undergo feminization. The genetic nature of carpeloidy in *Carica* remains to be understood, possibly within the framework of the genetic system for sex determination, which rests on five pairs of genes occurring in three sex-determining complexes in the sixth chromosome (Purseglove, 1968). Interestingly, Ackerman *et al.* (2008) have investigated the differential expression profile of three B-class genes in the trioecious papaya (bearing male, female and hermaphrodite flowers), suggesting that B-class genes are the main targets of the sex-determination system. Their and our study combined could stimulate further work on the role of B-class genes – extended to C-class and *SUP* genes – in the development of carpeloidy in *Carica* at specific stages and in tissues undergoing fusion.

Postgenital organ fusions have been investigated in a number of species and show that (1) fused organs, organ deformations and protrusions of epidermal cells developed at positions with high mechanical compression, (2) an intact cutin layer prevented fusions between different plant organs and was therefore necessary for normal epidermal differentiation and organ formation, and (3) the junctions of the fusions contained pectic polysaccharides (Sieber *et al.*, 2000) and that diffusible factors were involved (Siegel and Verbeke, 1989). Furthermore, analysis of the genetic bases of the process has made much progress in identifying several of the genes involved in *Arabidopsis* (Lolle *et al.*, 1998; Pruitt *et al.*, 2000; Krolikowski *et al.*, 2003).

In *Arabidopsis*, we show that carpeloidy in *sup1* stamens also arises during stamen development. The transformed structures have an overall stamen morphology, indicating that they had been initiated as stamens (and have genuine stamen identity) and maintain a recognizable stamen organization plan. Female attributes were generated on or from recognizable stamen tissues, namely connective, filament and anther apical region, and, more rarely, from micro-sporangial tissues, which all can exhibit a hypertrophied growth. None of these mosaic morphologies seems to result from stamen–carpel postgenital fusions, because early carpel parts, such as

valves, have been only occasionally observed in our analysis in the strongly feminized phenotypes. Instead, stigmatic and ovule tissues, which differentiate latest during gynoecium morphogenesis, were formed directly and independently on stamen parts. Furthermore, the feminized structures undergoing such alterations were frequently symmetrical, which should not be the case in random fusions. In addition, the fact that *SUP* has been reported as being expressed in developing stamen (primordium, connective) and carpel (septum, ovules) tissues (Ito *et al.*, 2003; Breuil-Broyer *et al.*, 2004) may have significant relevance to these results for the understanding of carpeloidy in this species. We show that the mutation of this gene confers female attributes to stamen parts through filament fasciation, connective hypertrophy and anther reduction. Of note here, Warner *et al.* (2009) have shown by experimental manipulations of waterlily flowers that sepal and petal fates can switch very late in development and argue that sepalness and petalness in angiosperm history were not fixed to particular organs, but were primarily environmentally controlled.

The precise nature of carpeloidy at the cellular level in *Arabidopsis* remains to be understood, the conversion process occurring fast and being partial in *sup1* mutants. The most spectacular and challenging issue is to analyse the conversion of the tissues close to the vascular strand along the filament and in the connective into regular ovule-producing tissues (placental?) and/or valve-like structures. Of note here are the results with petunia *phsup1* mutant flowers in which feminized stamens exhibited excess proliferation of connective tissues around the vascular bundle (Nakagawa *et al.*, 2004).

Carpeloidy occurs in other *Arabidopsis* genetic backgrounds, such as the B-class gene mutations, which transform stamens into carpels, but – at the same time – transform petals into sepals in the majority of analysed alleles (Bowman *et al.*, 1991; Krizek and Meyerowitz, 1996). We have concentrated our study on *sup1*, because the organ transformations associated with this mutation only affect the stamen–carpel balance. However, in both cases, B-class and *SUP* mutations participate in the same developmental process: acquisition of stamen and carpel identities in contingent territories via the expression of both appropriate combinations and/or concentrations of MADS-box transcription factors and of the male–female boundary factor, *SUP* (Bowman *et al.*, 1992; Sakai *et al.*, 2000).

Such considerations might be meaningful in the context of *sup1* mutants undergoing carpeloidy at the floral meristem centre only. Altering *SUP* function appears to modify B-class gene activity at the floral meristem centre, which might interfere with female factors known to operate in that region, such as CRC, STK or SHP (Girin *et al.*, 2009; Prunet *et al.*, 2009), to name just a few. Work with the petunia *phsup1* mutant showing that all stamens undergo weak feminization (Nakagawa *et al.*, 2004) suggests that an altered class-B/C factor balance might suffice to provoke carpeloidy. This appears to be the case with alloplasmic lines of bread wheat exhibiting both carpeloidy and alteration of B-class gene expression (Hama *et al.*, 2004).

In summary, the two species produce stamen-to-carpel transformations within defined genetic backgrounds. While in the bisexual *Carica* organ fusions represent the mechanism

underlying the sexual conversion, in the *Arabidopsis sup1* context this process resulted from the formation of various female attributes directly on stamen parts. In both species, stamen filaments and connective tissues were the most responsive to feminization. In *Carica*, the ovules were formed, most of the time in locules along the vasculature, with ectopic ovules on the outside of transformed stamens also being observed. In *Arabidopsis*, the ovules were formed on the connective or close to where the filament meets the connective tissue. More globally, the stamen developmental plasticity in the two plant systems appeared remarkably strong during organ differentiation.

Evo-devo implications

The results can now be placed within evo-devo scenarios of the derivation of flower bisexuality. The mostly male theory (MMT) predicts that the angiosperm flower is derived from the male reproductive structures of an ancestor, on which ectopic ovules developed. The male unit would thus become bisexual and later some of these modified microsporophylls would enclose the ovules, giving rise to the angiosperm carpel (Frohlich and Parker, 2000). Alternatively, the out of male (OOM) and the out of female (OOF) theories proposed that homeotic changes in gene expression would have given rise to a flower axis with male reproductive units at the basal region and female reproductive units at the apical region (Theissen et al., 2002; Theissen and Becker, 2004).

Stamen–carpel fusions in the *Carica* system do not seem to directly contribute to the issue of the origin of bisexuality because they imply the pre-existence of bisexual flower meristems with compact internodes and whorls, enabling contact between stamen and carpel organs initiated at proximity and in a quite synchronous manner. However, the comparison of the *Carica* and *Arabidopsis* systems enabled us to identify common trends in stamen developmental plasticity, and in particular how ovules could form on stamen parts.

In contrast, the formation of ectopic ovules and stigma–style structures on developing stamens in *Arabidopsis* is highly relevant to the MMT because it implies that stamens have, in the absence of *SUP*, an intrinsic potential to sexual conversion and bisexuality in the absence of physical contacts with pre-existing female tissues. This indicates that somatic tissues of the *A. thaliana* stamen can switch developmental programmes locally and relatively late in development. The fact that such conversions occur at the flower meristem summit only, i.e. in the presumptive female domain, allows us to speculate that female factors mainly controlling ovule and style–stigma formation could have been specifically expressed in organs produced in that region of an ancestral male flower axis, with as a consequence the ectopic formation of female parts on bona fide male tissues.

In conclusion, our results show that carpeloidy has been expressed in different ways for morphological diversification during flower and fruit evolution. The *Carica* results highlight mechanisms that allow direct resource reallocation (for general trends see Wright and Meagher, 2003; Knight et al., 2006, and references therein) between male and female organs through (partial) sex conversion once bisexual flowers have evolved. The *Arabidopsis* results highlight possible early evolutionary

events in the acquisition of bisexuality by transformation of stamen parts into female parts during stamen morphogenesis as a flower axis–position effect, i.e. in the distal region of the flower axis only. This process appears to support the basic claims of the MMT by a change in the expression of female differentiation factors within a male flower axis and on male organ structures. This differs from a reproductive organ identity switch during the initiation stage, i.e. a homeotic transformation, as claimed by the OOM theory.

Obviously, such bisexual organs were inefficient in nature in the short run and the formation of a fully functional bisexual flower would have required further optimizations. A better delimitation of the male–female boundary linked to more compact and determinate flower structures (Baum and Hileman, 2006; Soltis et al., 2007, and references therein) appears as a likely sequence of evolutionary events. We have shown that developmental plasticity during organ formation can circumvent such a sexual boundary constraint.

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