

## CHANGES IN FLORET DEVELOPMENT PATTERNS THAT MAY CORRELATE WITH SEX DETERMINATION IN THE PCK CLADE (POACEAE)

Renata Reinheimer,<sup>1,\*</sup> Fernando O. Zuloaga,<sup>†</sup> Abelardo C. Vegetti,<sup>\*</sup> and Raúl Pozner<sup>†</sup>

<sup>\*</sup>Morfología Vegetal, Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, Kreder 2805, S3080HOF Esperanza, Santa Fe, Argentina; and <sup>†</sup>Instituto de Botánica Darwinion, C.C. 22, B1642HYD San Isidro, Buenos Aires, Argentina

We investigated changes in floral developmental patterns and sex determination in the PCK (phosphoenolpyruvate carboxykinase) clade using a comparative approach and SEM. We identified variation in patterns of floral development that may be correlated with sex determination. Nine different patterns of floret development were identified, based on sex of the lower floret, sequence of stamen development, and rate of glume and lemma differentiation. Although staminate florets are always formed by the abortion of the gynoecium, the timing of abortion differs among species. Similarly, the formation of sterile lower florets showed different pathways that may operate at the level of floral meristem or organ identity. Finally, we discuss several reasons why patterns described for maize and closely related species may not be easily extrapolated to other grasses.

**Keywords:** spikelet and floret development, SEM, diversity, sex determination, Poaceae, PCK clade.

**Online enhancement:** appendix table.

### Introduction

Spikelet characteristics are highly diverse in grasses (Clayton and Renvoize 1986; Watson and Dallwitz 1992 and onward). In addition to the number of florets per spikelet, grass species differ in the direction of maturation of florets within the spikelet and the sexuality of the florets. The transition between perfect and staminate or pistillate florets has occurred many times during grass diversification (Columbus 1999; Kinney et al. 2003; Malcomber and Kellogg 2006). It has also been suggested that such a transition may result from the existence of different pathways of sex determination. Studies in *Zea mays* L. (maize) and other members of the Andropogoneae as well as in *Zizania aquatica* L. (Ehrhartoideae) showed that the staminate floret may be formed by different patterns of cell death in the aborting gynoecium (Cheng et al. 1983; Sundberg and Orr 1996; Le Roux and Kellogg 1999; Zaitchik et al. 2000; Orr et al. 2001). However, the sex determination process in grasses is not well understood. Studies that link changes in development patterns with sex determination are not abundant in the literature, but the few that exist are compelling (Irish and Nelson 1993; Irish et al. 1994; Le Roux and Kellogg 1999). Previous studies suggested that the rate of glume elongation may correlate with sex determination, meaning that perhaps the genetic control that regulates sex determination also is involved in glume development (Irish and Nelson 1993; Irish et al. 1994; Le Roux and Kellogg 1999). These studies indicate that further insights can be gained using comparative developmental studies. Unfortunately, previous

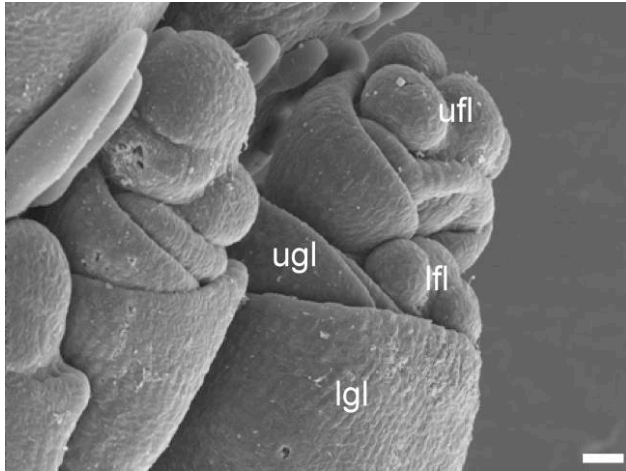
studies were focused on maize and other members of the Andropogoneae tribe, and the applicability of these patterns for other grasses is unknown.

We investigated floret development and sex determination in the PCK (phosphoenolpyruvate carboxykinase) clade using a comparative approach. We selected the PCK clade because it is a well-defined clade within the Paniceae tribe (subfamily Panicoideae), which is sister to the Andropogoneae tribe. More importantly, its members offer interesting variation in the sex of the florets even within the same spikelet. The PCK clade is characterized by having two-flowered spikelets, a character shared with other Panicoideae (fig. 1), in which the upper floret is perfect and the lower floret may be staminate or sterile. The PCK clade includes most C<sub>4</sub> grasses that have phosphoenolpyruvate carboxykinase physiology (Frank 1998; Gómez-Martínez and Culham 2000; Zuloaga et al. 2000; Duvall et al. 2001; Giussani et al. 2001; Aliscioni et al. 2003; Torres González and Morton 2005). Six genera have been confirmed by *ndhF* and ITS markers to be members of the PCK clade (*Moorochloa* Veldkamp (= *Brachiaria* (Trin.) Griseb.), *Chaetium* Nees, *Eriochloa* Kunth, *Megathyrsus* (Jacq.) B. K. Simon and S.W.L. Jacobs, *Melinis* P. Beauv., and *Urochloa* P. Beauv.; Gómez-Martínez and Culham 2000; Zuloaga et al. 2000; Duvall et al. 2001; Giussani et al. 2001; Torres González and Morton 2005). We aim to identify additional variation in the patterns of floret development, different from that observed in maize, associated with the sex determination in grasses.

### Material and Methods

We investigated a total of 20 species of the PCK clade among which we included 14 *Urochloa* species, *Moorochloa eruciformis* (Sm.) Veldkamp, two species of *Eriochloa*, *Megathyrsus*

<sup>1</sup> Author for correspondence; current address: Department of Biology, University of Missouri, One University Boulevard, St. Louis, Missouri 63121, U.S.A.; e-mail: reinheimerr@umsl.edu.



**Fig. 1** Scanning electron micrographs of the two-flowered spikelet basic type that characterizes the subfamily Panicoideae (*Urochloa lorentziana* is an example). *lfl* = lower floret; *lgl* = lower glume; *ufl* = upper floret; *ugl* = upper glume. Scale bar = 20  $\mu$ m.

*maximus*, and two species of *Melinis* (table A1 in the online edition of the *International Journal of Plant Sciences*). Some developmental aspects of the spikelet and floret of *Urochloa plantaginea* (Link) R.D. Webster and *Megathyrsus maximus* have already been described (Reinheimer et al. 2005); nevertheless, we reanalyzed floret development of these species here in the context of other variation shown in the clade. The genus *Chaetium* was not included because of lack of material.

Plant material was grown from seeds obtained from field collections and from a seed bank (Centro Internacional de Agricultura Tropical [CIAT]). Plants were grown during the summer in the greenhouse of the Agronomy School of the University of Litoral (Argentina), under ambient light conditions and daytime temperatures of 30°C. Material was collected in mid-January. When possible, several accessions and individuals were studied per species (table A1). In general, more than five inflorescences per plant were fixed in FAA (formalin : acetic acid : 70% ethanol, 10 : 5 : 85, v/v) and dehydrated in an alcohol series and two final changes of 100% acetone. Dehydrated inflorescences were critical-point-dried with CO<sub>2</sub> as transitional fluid and coated with gold-palladium using a Thermo VG Scientific Polaron SC7620 sputter coater (Balzers, Switzerland). All samples were observed and photographed using a Philips XL30 series scanning electron microscope (Eindhoven, The Netherlands) of the Bernardino Rivadavia Natural Science Museum (Buenos Aires, Argentina). In order to determine plasticity in floret development along the inflorescence, photographs were taken on spikelet at different levels of branching. Although, patterns of floret development vary among species, they showed to be uniform among individuals of the same species as well as along each inflorescence photographed.

## Results

### General Pattern of Spikelet and Floret Development

The development of spikelets within the PCK clade begins with the formation of two glume primordia. The lower

glume initiates first and is followed by the upper glume, then the lemma of the lower floret initiates (fig. 2A) before the lemma of the upper floret (fig. 2B). That is, glume and lemma initiation is acropetal within spikelets.

Palea and stamen formation follows lemma initiation. In all cases, palea and stamen initiation is basipetal within spikelets. That is, the palea of the upper floret develops first (fig. 2C). Following the initiation of the stamens of the upper floret, the palea and stamens of the lower floret initiates (fig. 2D). In *Eriochloa* and *Melinis minutiflora*, the palea of the lower floret aborts early in development, making this pattern difficult to recognize (fig. 2E). We observed a remnant of the meristem above the distal floret that corresponds to the sterile end of the spikelet rachilla. This may be evident before or after stamen initiation depending on the species (fig. 2D).

Lodicules initiate after the stamens do in a basipetal direction along the spikelet (fig. 2F). Lodicules always initiate in between stamens and palea.

Carpels develop last, again in a basipetal direction along the spikelet (fig. 2G). Carpels initiate surrounding the single ovule primordium; at the same time, stamen primordia form anthers before the filament elongation (fig. 2G). At this stage, the lower floret may be protected by the glumes or by the lemma, depending on the species (fig. 2G, 2H).

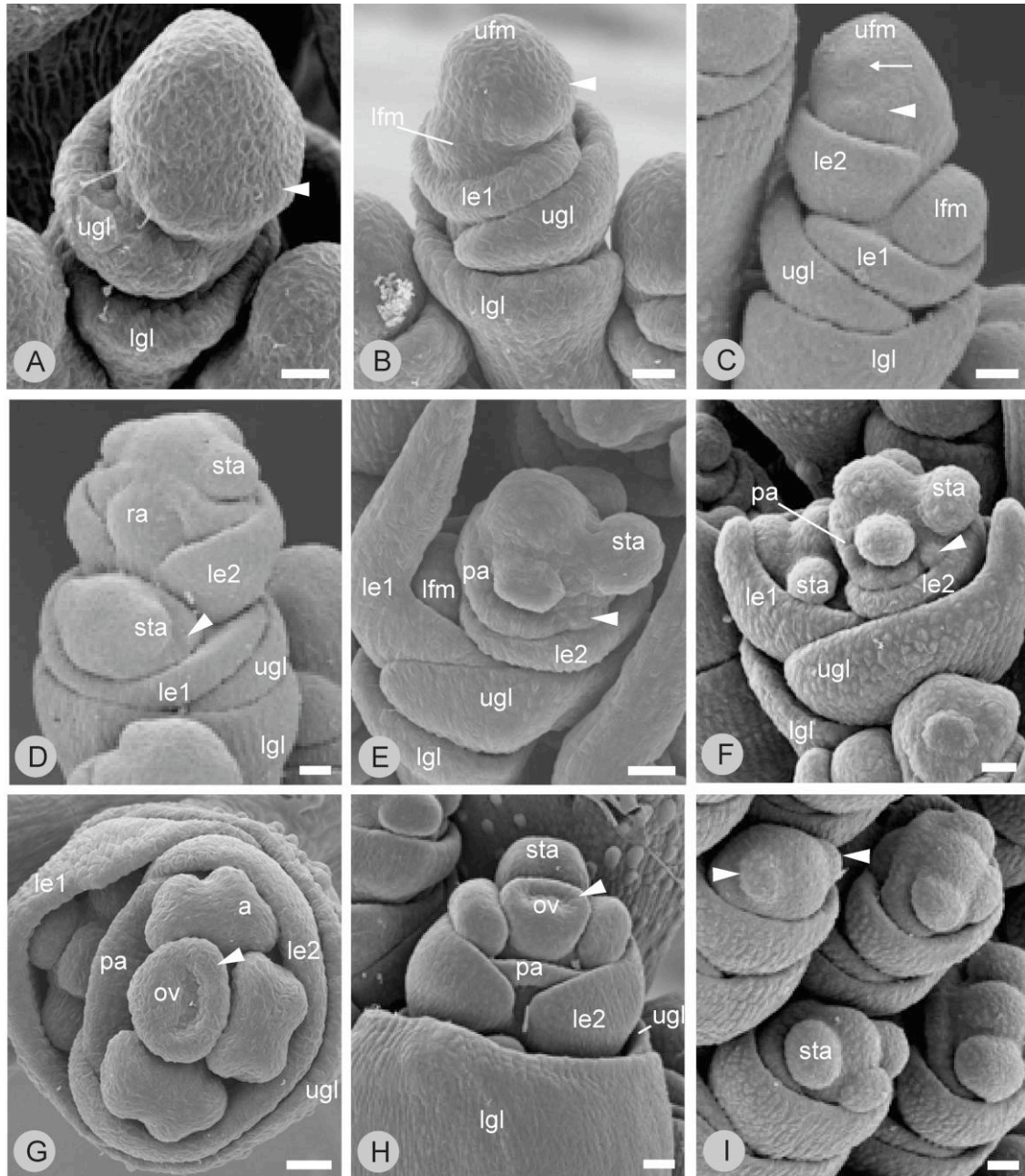
### Variations in Stamen Initiation

We observed differences during stamen initiation among species of the PCK clade. Stamens of the upper floret always develop first, stamens of the lower floret develop last (fig. 2C), and then the stamens of the lower floret initiate (fig. 2D). However, in *Melinis minutiflora* and *Urochloa distachya* stamens of the lower floret never develop (fig. 2E). In the upper and lower florets, two of the stamens develop at the flank of the floral meristem, and the third one is abaxial. In *Melinis*, *Megathyrsus maximus*, and most species of *Urochloa* (*U. bovonei*, *U. jubata*, *U. lorentziana*, *U. mollis*, *U. panicoides*, *U. paucispicata*, *U. plantaginea*, *U. platyphylla*, and *U. xantholeuca*), the abaxial stamen develops before the lateral stamens, the abaxial stamen being larger than the lateral stamens (fig. 2E). However, in *Eriochloa*, *Moorochloa eruciformis*, and some *Urochloa* species (*U. distachya*, *U. lata*, *U. leucacantha*, *U. mosambicensis*, and *U. ruziziensis*), the lateral stamens develop before the abaxial one and are larger (fig. 2I).

### Patterns of Differentiation of the Glumes and Lemmas

In order to study the differences in the rates of glume and lemma differentiation, we determined the elongation of these organs in three successive developmental stages: (1) when both floral meristems are delimited by lemmas, (2) when stamens of the upper floret are initiated, and (3) when carpels and anthers of the upper floret are initiated.

When the upper and lower floral meristems are first visible, glumes and lemmas of most species studied do not show any differentiation, and both floral meristems are uncovered (fig. 3A). However, in *Melinis* the lower lemma differentiated an awn early, although the floral meristems are still uncovered (fig. 3B). Another variation was found in *Eriochloa*, where the lower glume is reduced in comparison with the upper glume and lemmas (fig. 3C).



**Fig. 2** General pattern of spikelet and floret development in species of the PCK (phosphoenolpyruvate carboxykinase) clade (scanning electron micrographs). *A–D*, Spikelet of *Urochloa lata* with developing florets. *A*, Initiation of the lower lemma (at arrowhead) after upper glume initiation. *B*, Initiation of the upper lemma (at arrowhead) after lower lemma initiation. *C*, After upper lemma initiation, the palea (at arrowhead) and stamen primordia (at arrow) of the upper floret are initiated. *D*, After the initiation of the stamens of the upper floret, the palea (at arrowhead) and stamens of the lower floret are initiated. Only a single stamen primordium is labeled in each floret. Two of the stamens develop at the flank of the floral meristem and the third one is abaxial. In addition, the sterile end of the spikelet rachilla is evident. *E*, Spikelet with developing florets of *Melinis minutiflora*, in which the lower floral meristem does not form any floral organ. In contrast, the upper floral meristem initiates stamen and lodicule (at arrowhead) primordia. The abaxial stamen initiates before the lateral stamens, being also larger in size (only one stamen is labeled). *F*, Spikelet of *Melinis repens* containing developing florets. Stamens initiation is followed by the initiation of the lodicule primordia (at arrowhead) of the upper florets. *G*, Spikelet of *Moorochloa eruciformis* with developing florets. Lodicule initiation is followed by carpel initiation (at arrowhead) that will expand and delimit the single ovule; meanwhile stamen primordia expand to form the anthers before the filament elongation (only one anther is labeled). At this moment, the lower floret is almost protected by the lower lemma. *H*, Spikelet of *Urochloa paucispicata* containing developing florets. During carpel development (at arrowhead) the lower floret is covered by the lower glume. *I*, Developing upper florets of *Eriochloa*, where the lateral stamens (at arrowheads) initiate before the abaxial one. In this case, lateral stamens are larger in size than the abaxial one. *a* = anther; *le1* = lower lemma; *le2* = upper lemma; *lfm* = lower floral meristem; *lgl* = lower glume; *ov* = ovule; *pa* = palea; *ra* = spikelet rachilla; *sta* = stamens; *ufm* = upper floral meristem; *ugl* = upper glume. Scale bars = 20  $\mu$ m.

When stamens of the upper floret are initiated, we find two different patterns in the PCK clade. In the first pattern, the lower meristem is covered by the elongation of the lower glume alone, as in *U. bovonei* and *U. plantaginea* (fig. 3D), or by the lower lemma, as in *M. minutiflora* and *U. distachya* (fig. 3E). In all other studied species, neither glumes nor lemmas cover the floret meristems (fig. 3F). Hence, in the latter group, glume and lemma differentiation occurs when carpels and anthers of the upper floret initiate. At this stage, we found three different patterns of glume and lemma differentiation: (1) the lower glume elongates and covers the lower floral meristem, as in *U. lorentziana*, *U. mosambicensis*, *U. paucispicata*, *U. platyphylla*, *U. ruziziensis*, and *U. mollis* (figs. 2H, 3G); (2) the lower lemma elongates and protects the lower floral meristem, as in *M. eruciformis*, *M. repens*, *U. lata*, and *U. panicoides* (fig. 3H); and (3) the lower and upper florets remain naked, as in *Eriochloa*, *Megathyrsus*, *U. jubata*, *U. leucacantha*, and *U. xantholeuca* (fig. 3I; see also Reinheimer et al. 2005). These are finally covered by the lower lemma only somewhat later.

#### Floral Organ Differentiation

We found differences in the pattern of floral organ differentiation between the lower and upper florets. The distal floret always initiates as a perfect floret (fig. 4A). When stamens and carpels are initiated, the anthers expand and elongate, and the carpels also expand surrounding the single ovule (fig. 4A). Meanwhile the glumes, lemma, and palea continue their differentiation gradually covering the upper floret (fig. 4A). Thus, the glumes, lemmas, and paleas have to be removed to study the differentiation of stamens, lodicules, and carpels. Ovule formation usually continues with the initiation of the integument when the carpel has already enclosed the ovule (fig. 4A, 4B). Later, the style elongates (fig. 4B), lodicules expand (fig. 4C), anthers continue their differentiation, and filaments begin to elongate (fig. 4B, 4C). The stigma begins to form when the style reaches the height of the connective tissue of the stamens (fig. 4D). The differentiation of the floral organs continues, and a perfect floret is formed (fig. 4E–4G). At the same time, glumes, lemmas, and paleas gradually adopt their final shapes. One variation to this general pattern is found in *U. mollis*, where the ovule integument initiates before it is enclosed by the carpel (fig. 4H, 4I).

In most species, the lower floret initiates as a perfect floret (fig. 5A) containing stamens and carpels (fig. 5B). Generally, stamens continue their development, while the gynoecium may abort (1) soon after carpel initiation, as in *U. panicoides* (fig. 5C); (2) soon after carpel differentiation, as in *M. eruciformis* and most of the *Urochloa* species (fig. 5D); or (3) before carpel initiation, as in *Megathyrsus* (Reinheimer et al. 2005). Alternatively, stamens, lodicules, and carpels all abort early in development, soon after the differentiation of the anthers, as in *U. plantaginea* (Reinheimer et al. 2005). In *Eriochloa*, stamens stop their development before anther formation, and lodicules and carpels never initiate (fig. 5E). Finally, in the extreme case, no floral organs form in the lower floret, as in *M. minutiflora* and *U. distachya* (fig. 5F).

In those species that follow the first three patterns of development, the lower floret is staminate at maturity; in those species that follow the last three patterns, the lower floret is

sterile at maturity. Table 1 summarizes variations observed during the floret development.

## Discussion

### General Aspects of Floret Development in the PCK Clade

The sequence of floral organ initiation of the PCK clade was previously described for *Eleusine* Gaertn., *Oryzopsis* Michx., *Panicum* L., *Pennisetum* Rich., and *Setaria* P. Beauv., but it differs from that of other grasses (Clifford 1987). For instance, in *Bromus* L., *Dactylis* L., and *Phalaris* L., the stamens are the first organs to be formed, followed by lodicules or the palea, depending on the species, while in other grasses (as in *Avena* L., *Bambusa* Schreb., *Eleusine*, *Hordeum* L., *Lolium* L., *Oryza* L., *Oryzopsis*, *Panicum*, *Pennisetum*, *Setaria*, *Triticum* L., *Zea* L., and *Zinania* L.), the palea is the first organ to be initiated, followed by lodicules or stamens. Similarly, carpels may be initiated immediately after stamen (as in *Triticum* and *Oryza*), lodicule (as in *Dactylis*, *Bromus*, *Eleusine*, *Oryzopsis*, *Panicum*, *Pennisetum*, and *Setaria*), or palea formation (as in *Phalaris* and some species of *Bromus*).

Several differences were found during floret development in the PCK clade. Such differences involve sequence of stamen development, sex of the floret, type of organ that protects the lower floral meristem, and the stage at which the floral meristem is covered.

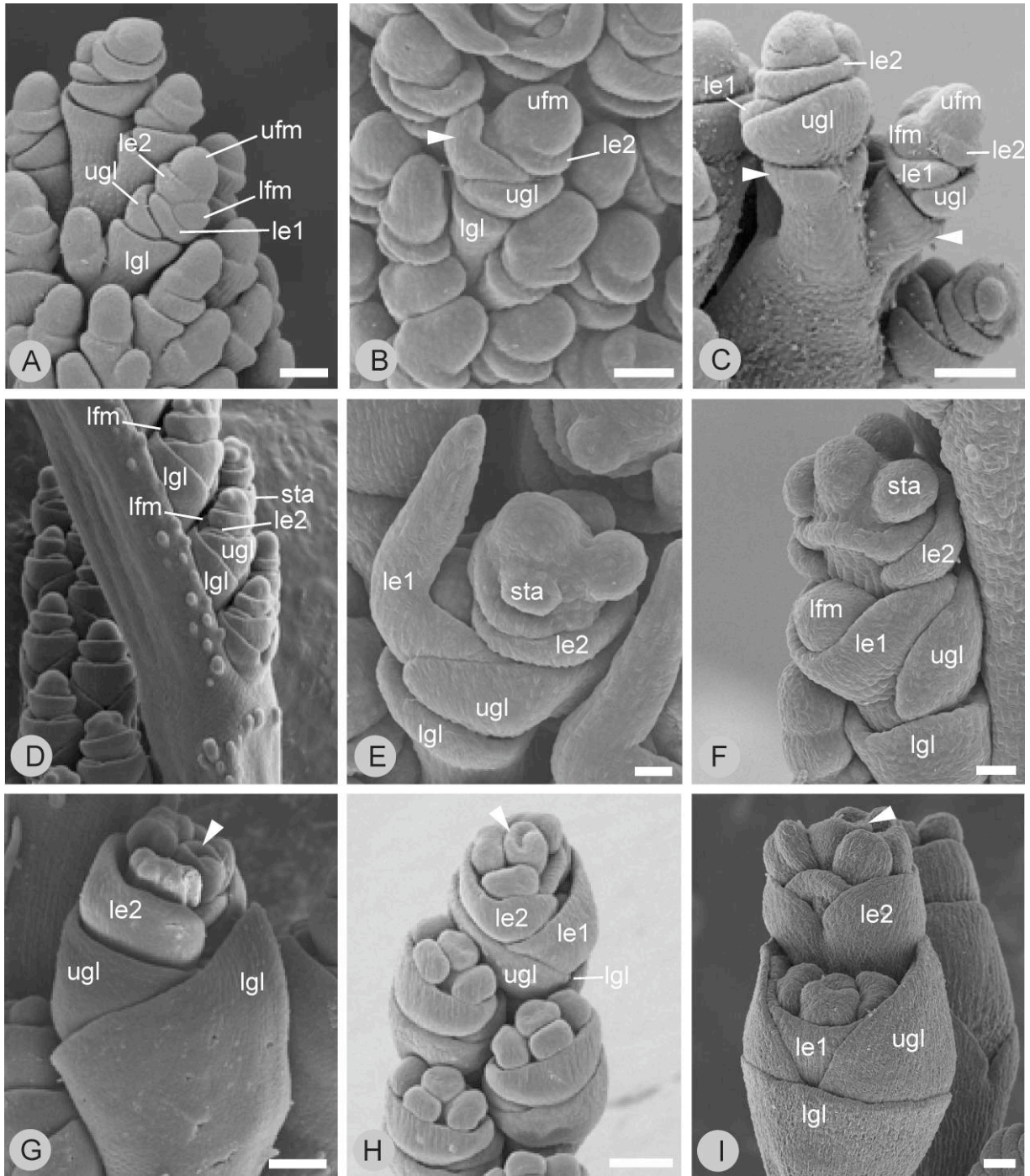
### Floral Organ Differentiation and Sex Determination

The transition between perfect and staminate or pistillate florets has occurred many times during grass diversification, even among very closely related species (e.g., *Bouteloua* Lag., Chloridoideae; Columbus 1999; Kinney et al. 2003; Malcomber and Kellogg 2006). The development of the upper floret is uniform across the PCK clade, and all floral organs continue normal development up to the formation of a perfect floret. One subtle difference was observed in *U. mollis*, in which the integument of the ovule starts to form before the carpel covers the ovule, similar to the gynoecium primordium of the male floret of *Zizania aquatica* (Ehrhartoideae; Zaitchik et al. 2000).

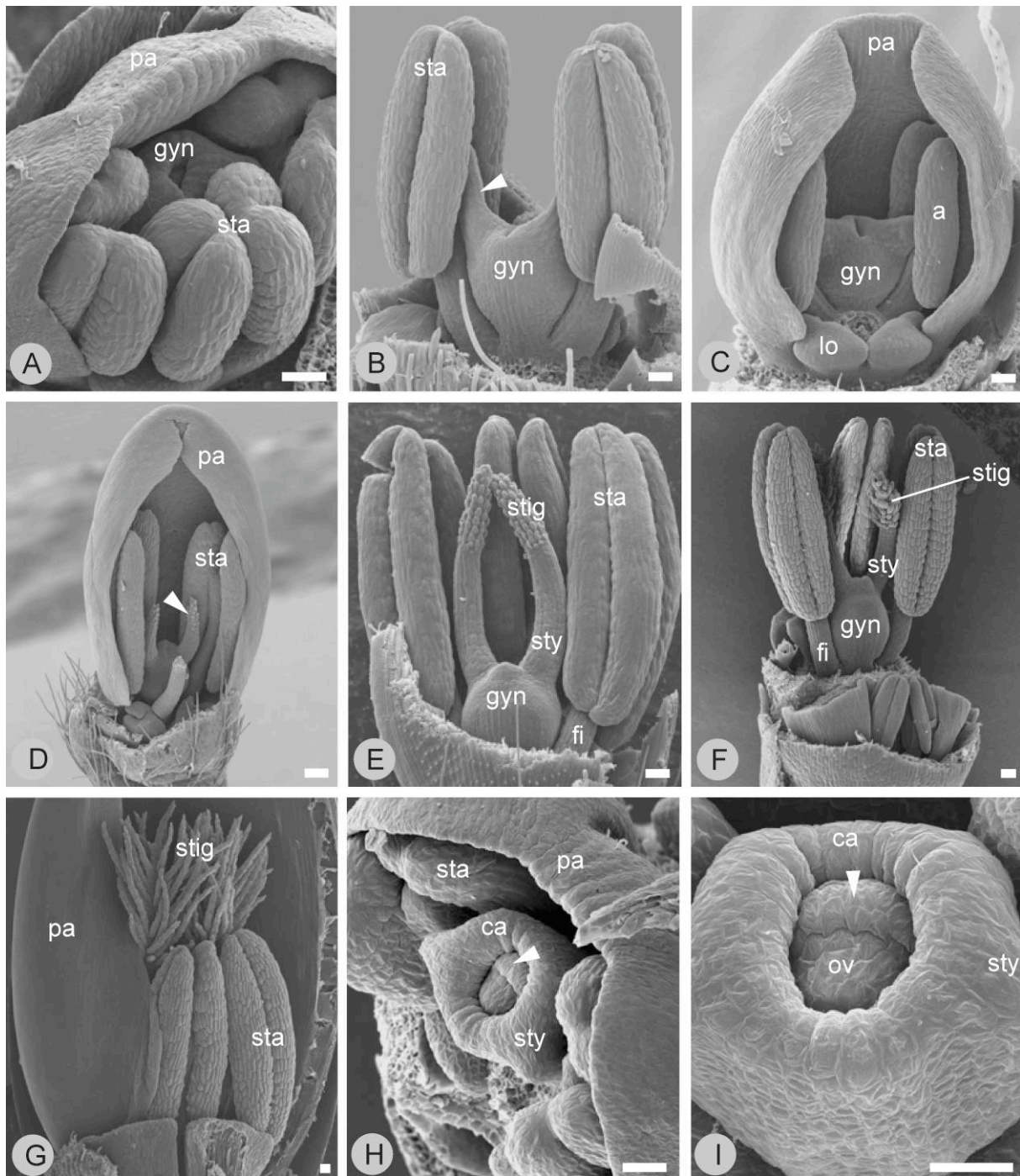
In contrast, we found several differences during the development of the lower floret in the PCK clade. In some species, the lower floret may be staminate. The staminate floret initiates as a perfect floret as observed in other grasses (Cheng et al. 1983; Le Roux and Kellogg 1999; Zaitchik et al. 2000). Somewhat late in development, the central gynoecium aborts while the stamens and lodicules proceed with normal development, as has been documented in staminate florets of several Andropogoneae, such as maize (Sundberg and Orr 1996), *Heteropogon contortus* (L.) Roem. & Schult. (Le Roux and Kellogg 1999), *Tripsacum dactyloides* L. (Orr et al. 2001), and in one Paniceae species, *Panicum repens* L. (Le Roux and Kellogg 1999).

Previous studies have suggested that the mechanism of gynoecium abortion varies among grasses and is associated with particular patterns of cell death. Some authors correlate the abortion of the gynoecium with the occurrence of cell death in which only the cell walls are preserved. Those cells are located in a subepidermal layer of the developing gynoecium in An-



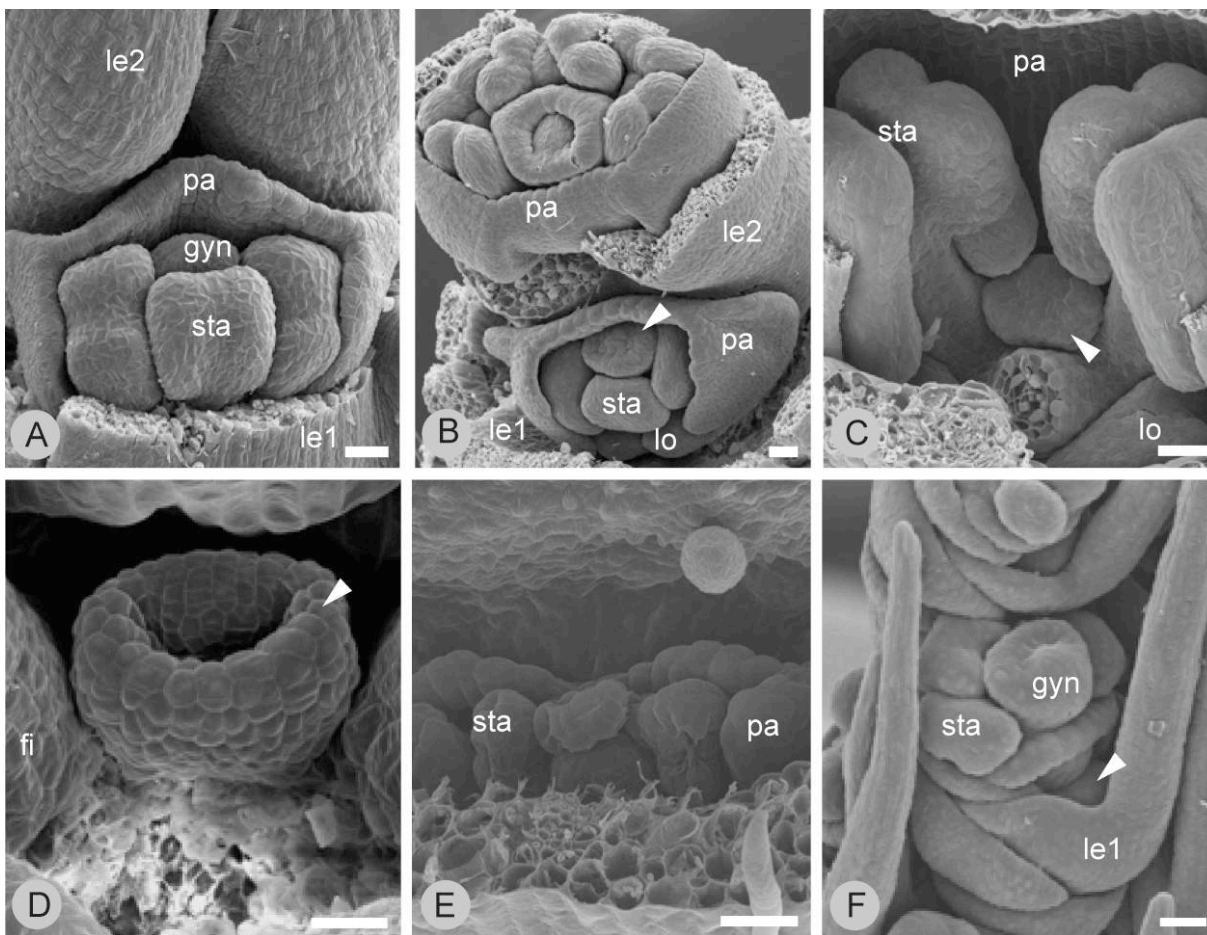


**Fig. 3** Differentiation of the lower glume and lemma during different stages of floret development (scanning electron micrographs). A–C, Comparison of the rate of glume and lemma differentiation when both floral meristems are visible. A, Spikelet of *Eriochloa montevidensis* with developing florets. Glumes and lemmas do not show any differentiation, and in consequence both floral meristems are uncovered. B, Spikelet of *Melinis minutiflora* with developing florets. Although both floral meristems are uncovered, the lower lemma has already differentiated the awn (at arrowhead). C, Spikelet of *E. montevidensis* with developing florets where the lower glume is reduced (at arrowhead). D–F, Comparison of the rate of glume and lemma differentiation when stamens of the upper floret are initiating. D, Spikelet of *Urochloa bovonei* with developing florets. The lower glume covers the lower floret. E, Spikelet of *M. minutiflora* with developing florets. The lower lemma covers the lower floret. F, Spikelet of *Moorochloa eruciformis* with developing florets. None of the floral meristems are protected by the glumes or lemmas. G–I, Comparison of the rate of glume and lemma differentiation when carpels (at arrowhead) of the upper floret are initiated. G, Spikelet of *Urochloa lorentziana* with developing florets. The lower glume covers the lower floret. H, Spikelet of *Moorochloa eruciformis* with developing florets. The lower lemma covers the lower floret. I, Spikelet of *Megathyrsus maximus* with developing florets. Both florets are uncovered by the glumes or lemmas. *le1* = lower lemma; *le2* = upper lemma; *lfm* = lower floral meristem; *lgl* = lower glume; *sta* = stamens; *ufm* = upper floral meristem; *ugl* = upper glume. Scale bars = 20  $\mu\text{m}$ .



**Fig. 4** Upper floret development in species of the PCK (phosphoenolpyruvate carboxykinase) clade (scanning electron micrographs). A–G, Upper floret of *Morochloa eruciformis*. A, The upper floret initiates as a perfect floret primordium. The carpel surrounds the single ovule; meanwhile, anthers expand and elongate, and connective tissue is initiated. B, Elongation of the style. C, Lodicule differentiation. D, The stigma (at arrowhead) begins to form at the tip of the style when the style reaches the height of the connective tissue of the stamens. E, Floret development continues with the differentiation of the stigmas, and the elongation of styles and the stamen filaments. F, Style, stigmas, and stamens filaments continue to elongate. G, The differentiation of the distal floral organs continues to form a perfect floret at maturity. H, I, Upper floret of *Urochloa mollis*. H, Carpel differentiation, initiation of the style, and initiation of the integument of the ovule (at arrowhead). I, Close-up of the gynoecium showing the initiation of the integument of the ovule (at arrowhead) before carpels cover the developing ovule. a = anther; ca = carpel; fi = stamen filament; gyn = gynoecium; lo = lodicules; ov = ovule; pa = palea; sta = stamens; sty = style; stig = stigma. Scale bars = 20  $\mu$ m.





**Fig. 5** Development of the lower floret in species of the PCK (phosphoenolpyruvate carboxykinase) clade (scanning electron micrographs). A, Lower floret of *Urochloa lata*. The lower floret initiates as a perfect floret primordium. B, Lower floret of *Urochloa mollis*. The primordium of lower floret, in general, has lodicules, stamens, and carpel (at arrowhead) already initiated. C, Lower floret of *Urochloa panicoides* where the gynoecium stops developing just after carpel initiation (at arrowhead); meanwhile, lodicules and stamens proceed with normal development. D, Lower floret of *Urochloa ruziziensis* where the gynoecium aborts its development during carpel differentiation (at arrowheads); meanwhile, lodicules and stamens continue normal development. E, Lower floret of *Eriochloa montevidensis* where stamens stop developing before anther formation (and lodicules and carpels never initiate). F, Florets of *Melinis minutiflora* where no floral organs initiate from the lower floral meristem (at arrowhead). fi = stamen filament; gyn = gynoecium; le1 = lower lemma; le2 = upper lemma; lo = lodicules; pa = palea; sta = stamens. Scale bars = 20  $\mu$ m.

dropogoneae but not in maize (Le Roux and Kellogg 1999). In maize the abortion of the gynoecium of staminate florets was correlated with the presence of dead cells in the center of the aborted gynoecium that are highly vacuolated and without ribosomes and organelles (Cheng et al. 1983). In addition, in *P. repens*, dead cells occur in a ring of epidermal cells at the base of the gynoecium (Le Roux and Kellogg 1999). In *Z. aquatica*, the gynoecium stops developing later than in other grasses. The gynoecium aborts as the style, stigma, and ovule are differentiating, and this is correlated with the deposition of a dark substance in the cell walls of the aborted gynoecium (Zaitchik et al. 2000).

Similarly, there are differences in the timing of gynoecium abortion among members of the PCK clade. In *Megathyrus maximus* the gynoecium aborts before carpel initiation, which was correlated with cell death in a transversal subapical layer in the developing gynoecium (Reinheimer et al.

2005). The dead cells retain only their cell walls, similarly to what was documented for Andropogoneae and *P. repens* (Le Roux and Kellogg 1999). Among *Urochloa* species we also found differences in the timing of gynoecium abortion. In *Urochloa panicoides* the gynoecium stops developing just after carpel initiation, while in other species of *Urochloa* (*U. lata*, *U. mollis*, and *U. ruziziensis*), development stops during the differentiation of the carpels.

We also found differences in the development of florets that will be sterile at maturity. In *Urochloa plantaginea* the lower floret initiates stamens and a gynoecium that will later abort, forming a sterile floret (Reinheimer et al. 2005). In *Eriochloa* we observed that on the lower floret, the palea and the stamen primordia abort much earlier than in *U. plantaginea*; the gynoecium never forms. Finally, in *Melinis minutiflora* and some species of *Urochloa* (*U. distachya*) the floral meristem does not initiate any floral organs at all. In this

**Table 1**  
**Differences Observed during Floret Development in Members of the**  
**PCK (Phosphoenolpyruvate Carboxykinase) Clade**

Species	Sex of the lower floret	Stamens first to be initiated	Organ that protects the lower floret when carpels of the upper floret are initiating
<i>Moorochloa eruciformis</i>	Staminate	Lateral	Le1
<i>Eriochloa montevidensis</i>	Sterile	Lateral	Le1
<i>Eriochloa punctata</i>	Sterile	Lateral	Le1
<i>Megathyrsus maximus</i>	Staminate	Abaxial	Le1
<i>Melinis minutiflora</i>	Sterile	Abaxial	Le1
<i>Melinis repens</i>	Staminate	Abaxial	Le1
<i>Urochloa bovonei</i>	Staminate	Abaxial	Lgl
<i>Urochloa distachya</i>	Sterile	Lateral	Le1
<i>Urochloa jubata</i>	Staminate	Abaxial	Le1
<i>Urochloa lata</i>	Sterile	Lateral	Le1
<i>Urochloa leucacrantha</i>	Sterile	Lateral	Le1
<i>Urochloa lorentziana</i>	Sterile	Abaxial	Lgl
<i>Urochloa mollis</i>	Staminate	Abaxial	Lgl
<i>Urochloa mosambicensis</i>	Staminate	Lateral	Lgl
<i>Urochloa panicoides</i>	Staminate	Abaxial	Le1
<i>Urochloa paucispicata</i>	Sterile	Abaxial	Lgl
<i>Urochloa plantaginea</i>	Sterile	Abaxial	Lgl
<i>Urochloa platyphylla</i>	Staminate	Abaxial	Lgl
<i>Urochloa ruziziensis</i>	Staminate	Lateral	Lgl
<i>Urochloa xantholeuca</i>	Sterile	Abaxial	Le1

Note. Lgl = lower glume; Le1 = lower lemma.

case, the control of sex determination may be at the meristem level rather than during floral organ initiation.

Information regarding the regulatory network that controls the transition between bisexual, unisexual, and sterile florets in grasses is rare and is focused mainly on maize. The diversity in patterns of floret development described here for the PCK adds more morphological variation and differs from the pattern described for maize. Future studies should investigate the histology as well as the genetics of floret development in the PCK clade in order to understand whether such diversity is due to different regulatory networks of floret development.

#### *Lower Glume and Lower Lemma Differentiation May Be Correlated with the Timing of Organ Abortion but Not with the Sex of the Lower Floret in the PCK Clade*

Irish and Nelson (1993) and Irish et al. (1994) found a correlation between the differentiation of the glumes and the sex of the floret in maize. In staminate florets, the glumes cover the floret before gynoecium abortion, while in pistillate florets, glumes never cover the young floret. Therefore, these authors suggested that genes that control sex determination may be also linked with the control of glume differentiation. Similar results were observed by Le Roux and Kellogg (1999) in several Andropogoneae.

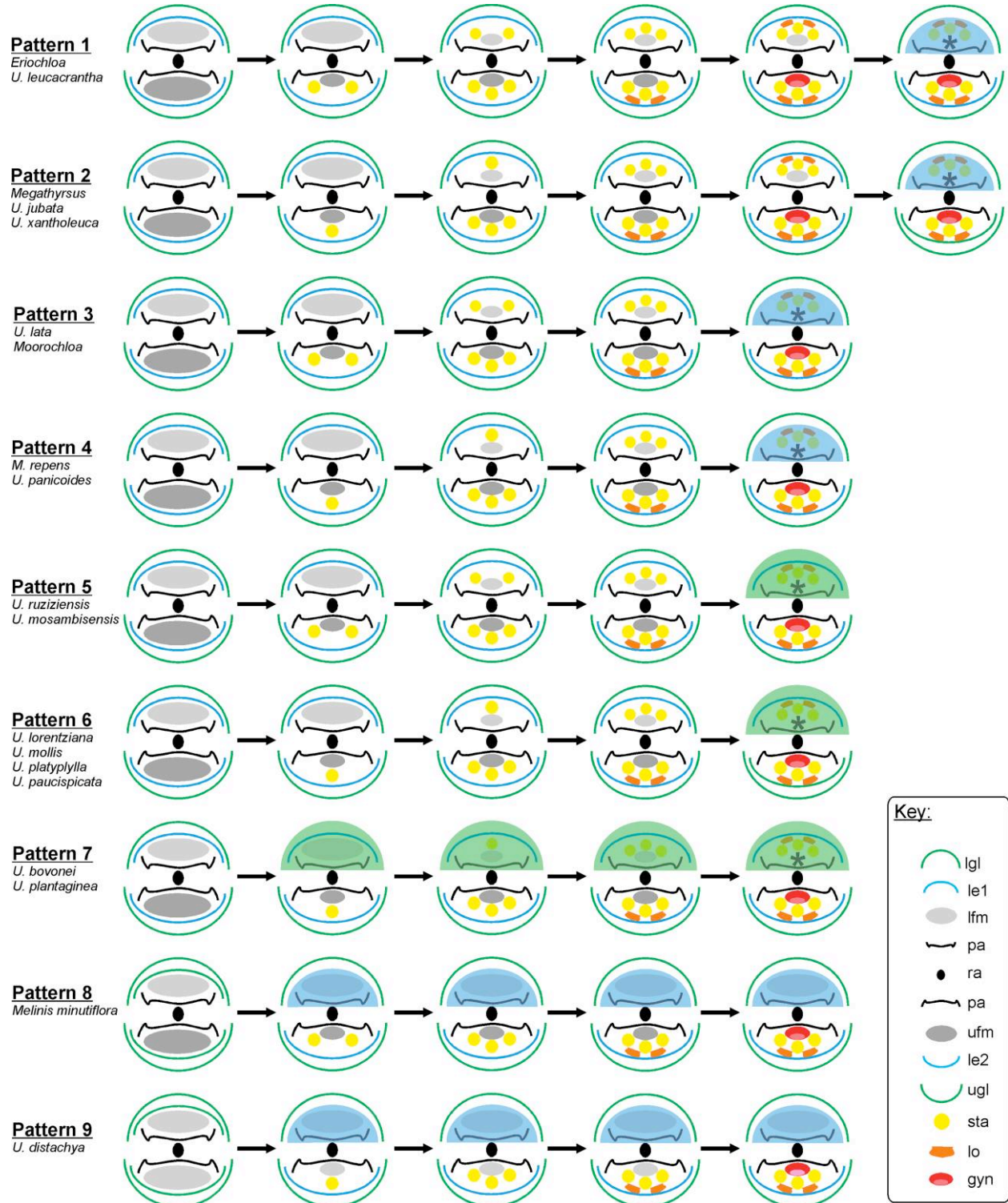
Similarly, in members of the PCK clade, the lower glume may cover the lower floret before gynoecium abortion (Reinheimer et al. 2005; this article). However, we found that in some species the lower floret is covered by the lower lemma rather than the lower glume. In addition, we found differences in the rate of lower glume or lemma elongation. That

is, in some species, the lower floret is covered by the lower glume or lemma early in development, before carpel initiation of the upper floret, while in other species the lower floret is covered by the lower glume or lemma later in development, after carpel initiation in the upper floret. These differences are not correlated with the sex of the lower floret in contrast to maize (Irish and Nelson 1993). In the PCK clade, species with staminate or sterile florets can be covered by lower glume/lemma before or after the carpel initiation of the upper floret. However, stamen and gynoecium differentiation and abortion occur when the lower floret is completely covered by glumes or lemmas. Our findings indicate that the pattern of glume or lemma differentiation is not necessarily correlated with the sex of the floret; however, differentiation of glumes or lemmas may be associated with the moment at which the sex of the floret begins to be established.

#### *Nine Different Floral Development Pathways Were Found in the PCK Clade*

We were able to identify nine different floral developmental pathways in the PCK clade (fig. 6). These are based on different rates of glume and lemma elongation and the time at which the lower floret is covered by one of these, in combination with the sex of the floret and the sequence of stamen development. The lower floret of some species is covered by the lower glume (downward green arch in fig. 6) or lower lemma (downward blue arch in fig. 6) during initiation (patterns 1 and 2) or the differentiation (patterns 3 to 6) of the carpel of the upper floret, but others are covered very early in development before the formation of floral organ of the lower floret (patterns 7 to 9). The differentiation of the lower





**Fig. 6** Schematic drawings of the nine different patterns of floral development found among species of the PCK (phosphoenolpyruvate carboxykinase) clade. *gyn* = gynoecium (red-pink oval), *lfm* = lower floral meristem (light gray oval), *lgl* = lower glume (downward green arch), *le1* = lower lemma (downward blue arch), *le2* = upper lemma (upward blue arch), *lo* = lodicules (orange pentagon), *pa* = palea (black line), *ra* = spikelet rachilla (black oval), *sta* = stamens (yellow circle), *ufm* = upper floral meristem (dark gray oval), and *ugl* = upper glume (upward green arch). Asterisk indicates the aborted gynoecium of the lower floret. Genera: *M.* = *Melinis*, and *U.* = *Urochloa*.

glume or lemma has not shown any correlation with the sequence of stamen initiation and the sex of the lower floret. Interestingly, none of these patterns coincide with the current delimitation of the genera in the clade. More importantly, the heterochrony observed as to when the lower floret is covered by the lower glume or lemma perhaps indicates that the moment when the sex of the floret is established may be not the same across the clade. This idea is also supported by the differences observed in the timing of gynoecium abortion in staminate florets or the differences observed when the floral organs of the sterile florets stop developing.

In agreement with our expectations, we were able to identify more variation in patterns of floret development that may correlate with the sex determination process. Our observations in the PCK clade support the idea that patterns de-

scribed for maize and closely related species may not be easily extrapolated to other grasses.

### Acknowledgments

We thank G. H. Rua, J. F. Pensiero, O. Morrone, J. de D. Muñoz, and CIAT for providing plant material. We are grateful to Rosa Ortiz-Gentry, Felipe Zapata, Peter Stevens, Jimmy Triplett, and two anonymous reviewers for critical reading of the manuscript. We also thank F. Tricarico for assistance with SEM photography. Funding was provided in part by PIP-CONICET 2935/00 to A. Vegetti; ANPCyT 11793, 13374, and 12065 to O. Morrone; PIP-CONICET 2131 to O. Morrone; and UNL-CAID+D 2006 to R. Reinheimer.

### Literature Cited

- Aliscioni SS, LM Giussani, FO Zuloaga, EA Kellogg 2003 A molecular phylogeny of *Panicum* (Poaceae: Paniceae): test of monophyly and phylogenetic placement within the Panicoideae. *Am J Bot* 90: 796–821.
- Cheng PC, RI Greyson, DB Walden 1983 Organ initiation and the development of unisexual flowers in the tassel and ear of *Zea mays*. *Am J Bot* 70:450–462.
- Clayton WD, SA Renvoize 1986 Genera graminum: grasses of the world. *Kew Bull* 13:1–389.
- Clifford HT 1987 Spikelet and floral morphology. Pages 21–30 in TR Soderstrom, KW Hilu, CS Campbell, ME Barkworth, eds. *Grass systematics and evolution*. Smithsonian Institution, Washington, DC.
- Columbus JT 1999 An expanded circumscription of *Bouteloua* (Gramineae: Chloridoideae): new combinations and names. *Aliso* 18:61–65.
- Duvall MR, JD Noll, AH Minn 2001 Phylogenetics of Paniceae (Poaceae). *Am J Bot* 88:1988–1992.
- Frank L 1998 Análisis del sistema de ramificación del complejo *Brachiaria-Urochloa* (Poaceae-Paniceae). BSc diss. Universidad de Buenos Aires.
- Giussani LM, JH Cota-Sánchez, FO Zuloaga, EA Kellogg 2001 A molecular phylogeny of the grass subfamily Panicoideae (Poaceae) shows multiple origins of C4 photosynthesis. *Am J Bot* 88:1935–1944.
- Gómez-Martínez R, A Culham 2000. Phylogeny of the subfamily Panicoideae with emphasis on the tribe Paniceae: evidence from the TRNL-F CPDNa region. Pages 136–140 in SWL Jacobs, L Everett, eds. *Grasses: systematics and evolution*. CSIRO, Melbourne.
- Irish EE, JA Langdale, TM Nelson 1994 Interactions between tassel seed genes and other sex determining genes in maize. *Dev Genet* 15: 155–171.
- Irish EE, TM Nelson 1993 Development of tassel seed 2 inflorescences in maize. *Am J Bot* 80:292–299.
- Kinney MS, JT Columbus, EA Friar Kingston 2003 Molecular evolution of the maize sex-determining gene TASSELSEED2 in *Bouteloua* (Poaceae). *Mol Phylogenet Evol* 29:519–528.
- Le Roux LG, EA Kellogg 1999 Floral development and the formation of unisexual spikelets in the Andropogoneae (Poaceae). *Am J Botany* 86:354–366.
- Malcomber ST, EA Kellogg 2006 Evolution of unisexual flowers in grasses (Poaceae) and the putative sex-determination gene, TASSELSEED2 (TS2). *New Phytol* 170:885–899.
- Orr AR, R Kaparathi, CL Dewald, MD Sundberg 2001 Analysis of inflorescence organogenesis in eastern gamagrass, *Tripsacum dactyloides* (Poaceae): the wild type and the gynomonoeicous *gsf1* mutant. *Am J Bot* 88:363–381.
- Reinheimer R, R Pozner, AC Vegetti 2005 Inflorescence, spikelet and floral development in *Panicum maximum* and *Urochloa plantaginea* (Poaceae). *Am J Bot* 92:565–575.
- Sundberg MD, AR Orr 1996 Early inflorescence and floral development in *Zea mays* land race chapalote (Poaceae). *Am J Bot* 83:1255–1265.
- Torres González AM, CM Morton 2005 Molecular and morphological phylogenetic analysis of *Brachiaria* and *Urochloa* (Poaceae). *Plant Syst Evol* 37:36–44.
- Watson L, MJ Dallwitz 1992 The grass genera of the world. CABI, Wallingford.
- Zaitchik BF, LG Le Roux, EA Kellogg 2000 Development of male flowers in *Zizania aquatica* (North American wild-rice; Gramineae). *Int J Plant Sci* 161:345–351.
- Zuloaga FO, O Morrone, LM Giussani 2000 A cladistic analysis of the Paniceae: a preliminary approach. Pages 123–135 in WL Surrey, J Everett, eds. *Grass, systematics and evolution*. CSIRO, Collingwood.