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Initiation and early development of fibers in wild and cultivated cotton

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Initiation and early development of fibers in wild and cultivated cotton

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Signatures have been redacted for privacy

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter 1: General Introduction

The Genus *Gossypium*

Gossypium (Malvaceae) is a diverse genus best known for cultivated cotton. It includes about 50 species, 45 diploid and 5 allopolyploid, which occur in arid and semi-arid regions throughout the world (Vollesen, 1987; Fryxell, 1992). The diploids are divided into eight genome groups based on chromosome pairing and size, and fertility between species (Endrizzi, Turcotte, and Kohel, 1985). These groups comprise natural lineages within the genus and correspond to geographic locations: A, B, E, F - Africa and Arabia; C, G, K- Australia; and D- New World. Allopolyploid members are found in the New World and contain the A and D genomes (Wendel, 1995; Wendel et al., 1998; Brubaker, Bourland, and Wendel, 1999; Percival, Wendel, and Stewart, 1999; Cronn et al., 2002). This understanding of the evolutionary history of the genus allows many aspects of evolutionary differences in development and morphology to be studied in a phylogenetic context.

The Cotton Fiber

Small single-celled trichomes (<1 cm) originate from the epidermis of the seed coat of almost all members of the genus except those in the K genome (Fryxell, 1992). Four species, two diploid (*G. arboreum L.* and *G. herbaceum L.*) and two allopolyploid (*G. hirsutum L.* and *G. barbadense L.*) have been independently domesticated for fiber properties and have fibers (lint) up to six cm long (Ryser, Meier, and Holloway, 1983; Ryser and Holloway, 1985; Wendel, 1995; Wendel et al., 1998; Brubaker, Bourland, and Wendel, 1999; Percival, Wendel, and Stewart, 1999; Weis, Jacobsen, and Jernstedt, 1999; Kim and Triplett, 2001). Domesticated cottons also have a second, short type of fibers commonly called fuzz (Aiyangar, 1951; Joshi, Wadhwani, and Johri, 1967; Beasley, 1979). This observation leads to questions of homology.

If cultivated cottons have been domesticated independently four times from four different wild ancestors, each with only a single layer of "wild-type" fiber, then what do lint and fuzz represent? Is fuzz, for example, a fiber that initiates later than lint and has compositional properties of wild-type fiber or a novel morphological expression of the domestication process? The answer to these questions may lie in the development and structure of each fiber type.

Fiber developmental sequences come almost entirely from detailed studies of *G. hirsutum*, the most commonly cultivated species (Farr, 1931; Anderson and Kerr, 1938; Lang, 1938; Aiyangar, 1951; O'Kelley and Carr, 1953; Rollins, 1968; Quisenberry and Kohel, 1975; Stewart, 1975; DeLanghe, 1986; Graves and Stewart, 1988; Ryser and Holloway, 1999), although a few studies have compared specific aspects of wild and cultivated cotton (Hutchinson and Stephens, 1945; Fryxell, 1963, 1964; Ryser and Holloway, 1985; Applequist, Cronn, and Wendel, 2001). Fiber cells develop during a series of overlapping stages: initiation, elongation, secondary wall synthesis, and maturation (Wilkins and Jernstedt, 1999; Kim and Triplett, 2001). Initiation of both wild-type and lint fibers begins on the day of anthesis with the emergence of fiber primordia, which arise from epidermal cells that protrude above the seed coat (Farr, 1933; Stewart, 1975; Ramsey and Berlin, 1976; Ryser, 1977; Graves and Stewart, 1988; Applequist, Cronn, and Wendel, 2001). The primordia are initiated on the chalazal end of the ovule and progress toward the micropylar end in most species (Farr, 1933; Stewart, 1975; Ramsey and Berlin, 1976; Ryser, 1977; Graves and Stewart, 1988; Applequist, Cronn, and Wendel, 2001). Fuzz, however, is reported to initiate 6-9 days post anthesis (dpa) in *G. arboreum*; 10-12 dpa in *G. herbaceum*; 5-9 dpa in *G. hirsutum*; and 12 dpa in *G. barbadense*, and may only occur on certain regions of the ovule, depending on the species and variety (Lang, 1938; Joshi, Wadhwani, and Johri, 1967; Berlin, 1986). Lint elongation continues for the next 20-30 days in cultivated species (Schubert et al., 1973; Quisenberry and Kohel, 1975; Ryser, 1977; Applequist, Cronn, and Wendel, 2001) whereas elongation of

most wild-type fibers lasts about 15 days (Applequist, Cronn, and Wendel, 2001). The end of elongation coincides with the beginning of secondary wall synthesis (Anderson and Kerr, 1938; Meinert and Delmer, 1977), at which time a thick wall of cellulose is laid down. Finally, at maturity the capsule dehisces and the fibers dessicate, forming twisted ribbon-like masses that adhere together and hence are useful for spinning.

This study focuses on fiber development from the day of anthesis (0 dpa) to 5 days post anthesis (dpa). Comparisons of timing of initiation, timing of nucleus migration, timing of the vacuole becoming prominent, fiber size, fiber shape, and distance fibers cover the epidermis from the chalazal to micropylar ends of the ovule between cultivated and wild diploid and tetraploid species (Table 1.2) are made using a variety of landmark morphometric

Table 1.1: Comparisons of fiber initials per species and/or variety used in the study

Fiber type comparisons	Taxa Used
Cultivated diploid vs. Cultivated tetraploid	*G. herbaceum* var. Wagad vs. *G. hirsutum* var. Maxxa
Wild Tetraploid vs. Cultivated Tetraploid	*G. hirsutum* var. *yucatanense* vs. *G. hirsutum* var. Maxxa
Wild D-genome donor vs. Wild Tetraploid	*G. raimondii* vs. *G. hirsutum* var. *yucatanense*

and statistical techniques. A fiber developmental sequence is described for each species and variety studied. Significant differences are noted in the timing of events between species and varieties. This timing of events gives a framework from which to compare fiber types and to determine whether phylogeny or domestication is driving the observed morphological similarities.

Methods Used

Microscopy techniques. The most recent comparative growth study (Applequist, Cronn, and Wendel, 2001) used scanning electron microscopy (SEM) to study the initiation and development of fibers in a variety of wild and cultivated cotton species. SEM works well for studying initiation at 0 and 1 dpa; however, initiation events past 2 dpa are not visible

using this technique, as any later initials are hidden under the older fibers. Also, no internal characteristics may be observed, such as nucleus placement. To overcome these limitations in the present study, developing ovules were embedded in Spurr's Resin (described in detail in Chapter 2) and sectioned two μm thick, allowing the observation of fiber initials after 1 dpa, as well as observations of their internal cellular characteristics.

Morphometric shape analysis. The morphometric anaylsis employed in this study uses a set of homologous landmarks, rather than sets of linear distances, as the starting point of a shape anlaysis. From these, non-shape information (position, orientation, and size) is held constant, and corresponding landmarks are optimally aligned using a generalized Procrustes superimposition (Rohlf and Slice, 1990; Adams and Rohlf, 2000). For this analysis, size is calculated as centroid size, or the square-root of the sum of squared distances between all landmarks and the center of mass for each specimen (Bookstein, 1991). From the aligned coordinates, a set of shape variables can be generated from the thin-plate spline (Bookstein, 1991), which preserve the geometry of the structure being studied. The resulting shape variables can then be used in statistical analyses and used to create graphical representations of mean shapes (Adams and Funk, 1997; Caldecutt and Adams, 1998; Adams and Rohlf, 2000; Rüber and Adams, 2001; Kassam et al., 2003). For this study, ten landmarks are used to represent the outline of the fibers (Figure 1.1). Because this is a comparison of outline shapes, there are no distinct points, such as an eye or the intersection of two bones, which can be used as homologous points. To compensate for this difficulty, the

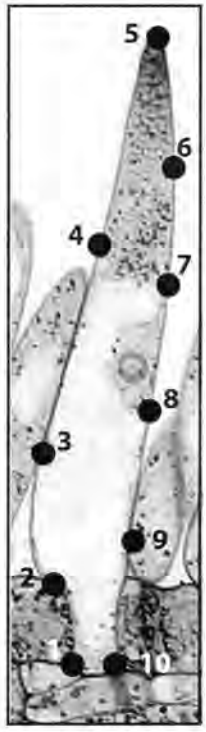

Figure 1.1. Diagram of the location of ten landmarks used to analyze the outline shape of the fibers.

Procurstes 'relaxation' method is employed (Bookstein, 1997). With this approach, a series of sliding semi-landmarks are defined around the perimeter of the object. These points are then allowed to slide in a direction around the perimeter of outline during Procrustes superimposition, thereby minimizing the overall differences between objects (Bookstein, 1997).

Thesis Organization

This thesis begins with an introduction (this chapter) to the genus *Gossypium* (Cotton) and the specific cells of interest, namely, cotton fibers. Chapter 2 follows, which represents a manuscript to be submitted to the International Journal of Plant Sciences. In this manuscript, patterns of fiber development are desribed within and between species and varieties of cotton. Chapter 3 includes a summary and general conclusions, and describes future directions that will yield additional insight into the questions posed.

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Chapter 2: Initiation and Early Development of Fibers in Wild and Cultivated Cotton

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Introduction

Cultivated cotton fiber has a long and complex history involving both natural evolutionary and human domestication processes. The genus *Gossypium* includes about 50 species, 45 diploids and 5 allopolyploids, collectively distributed in the arid and semi-arid tropics (Vollesen, 1987; Fryxell, 1992). The diploids are divided into eight genome groups based on chromosome pairing and size and fertility between species (Endrizzi, Turcotte, and Kohel, 1985). These groups comprise natural lineages in the genus and correspond to geographic locations: A, B, E, F - Africa; C, G, K- Australia; and D- New World. Allopolyploid members are found in the New World and contain A and D genomes (Wendel, 1995; Wendel et al., 1998; Brubaker, Bourland, and Wendel, 1999; Percival, Wendel, and Stewart, 1999). The genus is best known for four species, two A-genome diploids (*G. arboreum* L. and *G. herbaceum* L.) and two tetraploids (*G. hirsutum* L. and *G. barbadense* L.) that have been independently domesticated for fiber properties useful in textile spinning (Wendel, 1995; Wendel et al., 1998; Brubaker, Bourland, and Wendel, 1999; Percival, Wendel, and Stewart, 1999). The evolutionary history of the genus is well understood (Cronn et al., 2002), which facilitates comparative study of fiber development in a phylogenetic context.

The cultivated cotton fiber is a single cell on the epidermis of the ovule that initiates prior to anthesis and which may elongate to a final length of up to six centimeters (Ryser, Meier, and Holloway, 1983; Ryser and Holloway, 1985; Weis, Jacobsen, and Jernstedt, 1999; Kim and Triplett, 2001). The wild fiber is also a single cell but, unlike the cultivated fiber, seldom exceeds one centimeter in length (Applequist, Cronn, and Wendel, 2001). In addition

to length differences, wild and cultivated species differ in the number of fiber types present on the seed coat. Wild species have one layer of short fibers that adhere to the seed coat, hereafter referred to as "wild-type" (Fryxell, 1992). Cultivated species are described as having two layers and types of fibers, one long and one short, referred to as lint and fuzz, respectively (Lang, 1938; Hutchinson, Silow, and Stephens, 1947; Stephens, 1958; Fryxell, 1963, 1964; Vollesen, 1987; Fryxell, 1992).

Previous work on fiber development has been based almost exclusively on lint fibers of cultivated varieties of *G. hirsutum*. Development takes place during four continuous stages: initiation, elongation, secondary wall synthesis and maturation (Wilkins and Jernstedt, 1999; Kim and Triplett, 2001). Initiation of both wild-type and lint fibers occurs prior to anthesis with the emergence of fiber initials, epidermal cells that protrude above the seed coat epidermis (Farr, 1933; Stewart, 1975; Ramsey and Berlin, 1976; Ryser, 1977; Graves and Stewart, 1988; Applequist, Cronn, and Wendel, 2001). Initiation of these two types of fibers begins on the chalazal end of the ovule, and progresses toward the micropylar end (Farr, 1933; Stewart, 1975; Ramsey and Berlin, 1976; Ryser, 1977; Graves and Stewart, 1988; Applequist, Cronn, and Wendel, 2001). Fuzz, however, is reported to initiate 6-9 days post anthesis (dpa) in *G. arboreum*; 10-12 dpa in *G. herbaceum*; 5-9 dpa in *G. hirsutum*; and 12 dpa in *G. barbadense*, and may only occur on certain regions of the ovule, depending on the species and/or variety (Lang, 1938; Joshi, Wadhwani, and Johri, 1967; Berlin, 1986). Lint elongation continues for the next 20-30 days in cultivated species (Schubert et al., 1973; Quisenberry and Kohel, 1975; Ryser, 1977; Applequist, Cronn, and Wendel, 2001) whereas in most wild-type fibers elongation lasts about 15 days (Applequist, Cronn, and Wendel, 2001). The end of elongation coincides with the beginning of secondary wall synthesis (Anderson and Kerr, 1938; Meinert and Delmer, 1977), wherein a thick wall of cellulose is laid down. Finally, the capsule dehisces and the fibers dessicate, forming twisted ribbon-like cells that provide the world's most important fiber plant.

These previous observations leave many unanswered questions about wild-type and fuzz fibers. If cultivated cottons have been domesticated independently four times from four different wild ancestors, each with only a single layer of "wild-type" fiber, then what do lint and fuzz represent? Is fuzz, for example, a fiber that initiates later than lint and has compositional properties of wild-type fiber or a novel morphological consequence of the domestication process? How different are early developmental pathways in fibers from cultivated and wild accessions? Answers to these and related questions require a more complete understanding of the development of each fiber type.

In the present study, we describe the initiation patterns and morphological changes that occur from the day of anthesis (0 dpa) through five days later (5 dpa) in two wild and two cultivated taxa. Three species were chosen to represent the main evolutionary lineages relevant to fiber cultivation (Fig. 2.1) (Cronn et al., 2002): the best living wild model of

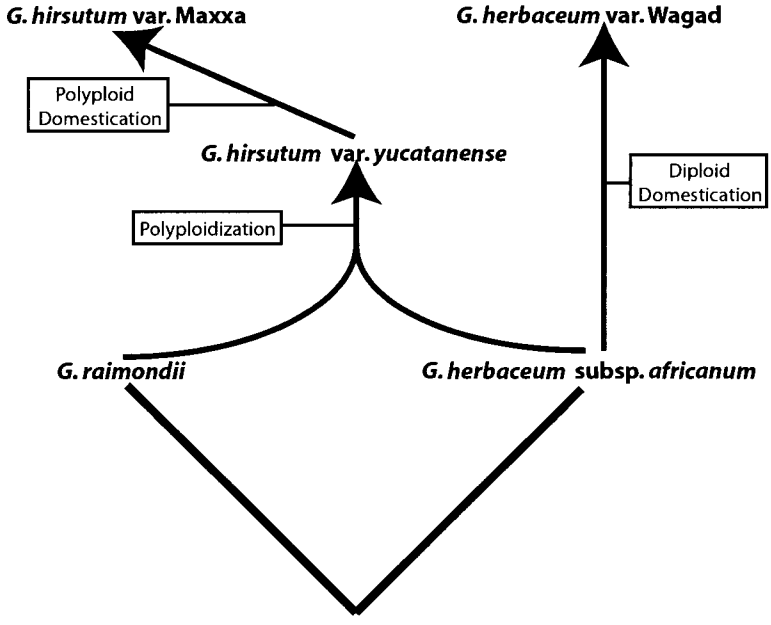


Figure 2.1. Phylogenetic history of the polyploidization and domestication of *Gossypium*.

the D-genome donor to the tetraploid - *G. raimondii;* an A-genome cultivated variety - *G. herbaceum* var. Wagad; a wild accession of the most commonly cultivated tetraploid (AD) species - *G. hirsutum* var. *yucatanense;* and a cultivated variety of the tetraploid - *G. hirsutum*

var. Maxxa. We studied the presence and location of fiber initials, nucleus position within each fiber, presence of a fiber vacuole, fiber length and centroid size, and fiber shape, all from the chalazal to the micropylar ends of the seed, in an effort to reveal fiber similarities and difference among species, and to assess whether they relate to mature fiber morphology.

Materials and Methods

Plant Materials. All plants were grown in the Bessey Greenhouse (Iowa State University) between Winter 2001-02 and Spring 2003 under greenhouse growing conditions previously described (Applequist, Cronn, and Wendel, 2001). Flowers were tagged on the day of anthesis and collected between zero and five days for immediate fixation.

Processing. Ovules were removed from the fruits and immediately fixed whole in 2% glutaraldehyde / 2% paraformaldehyde in 0.1 M cacodylate buffer pH 7.2 for 3-5 days. They were rinsed in 0.1 M cacodylate buffer and post-fixed for 4-12 hours using 1% osmium tetroxide in 0.1 M cacodylate buffer pH 7.2. Ovules were rinsed in distilled water and dehydrated through an ethanol series (25%, 50%, 70%, 95%, 100%) for two days at each step followed by 100% acetone. Finally, the material was infiltrated in a series of acetone:Spurr's resin steps, two days at each step, and embedded into pure Spurr's resin.

Section Preparation. 2μm thick longitudnal sections were taken from the middle of the ovule using glass knives on a Reichert Ultracut S ultramicrotome. Sections were stained using 1% Toluidine Blue 0 with 1% Borax in distilled water and mounted in Permount.

Image collection, processing and data collection. Images of sections were obtained using a Zeiss Axioplan 2 system fitted with a HRC Axiocam digital camera. Images were processed using Adobe® Photoshop® 7.0. Linear measurements of fiber length and seed coat cover were taken using Carnoy 2.0 (Schols and Smets, 2001). Ovule coat cover was determined by drawing a line from the location of the last fiber initials at the micropylar end of the ovule and measuring from the center of the chalazal end to the center of the

line. This distance was divided by 100 to determine the percent of cover. Fiber shape data was obtained using geometric morphometric methods (Bookstein, 1991; Rohlf and Marcus, 1993). These methods use a set of homologous landmarks, rather than sets of linear distances, as the starting point of a shape analysis. From these, non-shape information (position, orientation, and size) is held constant, and corresponding landmarks are optimally aligned using a generalized Procrustes superimposition (Rohlf and Slice, 1990; Adams and Rohlf, 2000). For this analysis, area is calculated as centroid size, or the square-root of the sum of squared distances between all landmarks and the center of mass for each specimen (Bookstein, 1991). Centroid size was measured using the same landmarks as the shape data in TPSRELW (Rohlf, 1999). From the aligned coordinates, a set of shape variables can be generated from the thin-plate spline (Bookstein, 1991), which preserve the geometry of the structure being studied. The resulting shape variables can then be used in statistical analyses and used to create graphical representations of mean shapes (Adams and Funk, 1997; Caldecutt and Adams, 1998; Adams and Rohlf, 2000; Rüber and Adams, 2001; Kassam et al., 2003). For this study, ten landmarks are used to represent the outline of the fibers (Figure 2.2). Because this is a comparison of outline shapes, there are no distinct points, such as an eye or the intersection of two bones, which can be used as homologous points. To compensate for this difficulty, the Procrustes 'relaxation' method was used (Bookstein, 1997). With this approach, a series of sliding semi-landmarks are defined around the perimeter of the object. These points

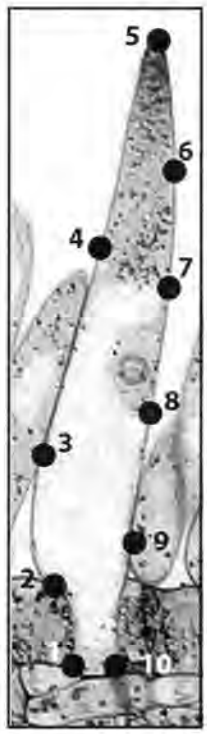


Figure 2.2. Diagram of the location of ten landmarks used to analyze the outline shape of the fibers.

are then allowed to slide in a direction along the perimeter of outline during Procrustes superimposition, thereby minimizing the overall differences between objects (Bookstein, 1997).

Statistical analysis. The percentage of migrating nuclei, percentage of fibers with a vacuole, and percentage of seed coat cover were calculated and graphed using Microsoft® Excel X. Pairwise means comparisons using the Tukey-Kramer HSD model were made of both fiber cell length and centroid size using JMP™ v. 5.0.1.2 and the correlation between length and centroid size was calculated using Microsoft® Excel X.

Shape data was analyzed using NTSYS-pc (Rohlf, 2000) and JMP™ v. 5.0.1.2. Six dimensions of shape were found to contain data, and were used in principal component analyses (PCA) for an initial exploration of the data, in a multivariate analysis of variance (MANOVA) and in pairwise comparisons to determine significant differences between species and days. Finally, mean shapes of each day of development for each species were generated using TPSRELW (Rohlf, 1999).

The mechanism driving fiber similarity was tested using pairwise tests comparing the effects of phylogeny and domestication. If phylogeny (P) is the driving force behind fiber shape and size similarity, it is predicted that the observed morphological similarities of shape and size between the closely related taxa (*G. hirsutum* var. *yucatanense* and *G. hirsutum* var. Maxxa) will be more similar than the observed morphological similarities of shape and size between the cultivated taxa (*G. hirsutum* var. Maxxa and *G. herbaceum* var. Wagad). Alternatively, if domestication (D) is the driving force behind fiber shape and size similarity, it is predicted that the cultivars (*G. hirsutum* var. Maxxa and *G. herbaceum* var. Wagad) will be more similar than the closely related taxa (*G. hirsutum* var. *yucatanense* and *G. hirsutum* var. Maxxa). For fiber size, these predictions were tested by comparing the difference of mean fiber sizes for each dpa: P-D. If phylogeny is driving fiber size, then the result will be negative and if domestication is driving fiber size, the result will be positive. For fiber shape,

the difference between mean shape was tested by using the Malhalanobis distance (MD) used in the pairwise comparison of fiber shape described above. Again, the test for each dpa was MDP-MDD, with a negative result suggesting a phylogenetic mechanism driving shape similarity and a positive result suggesting a mechanism from domestication driving the shape.

To determine which hypothesis is more likely, a randomization test was performed, following the protocol of Adams and Rohlf (2000). In this procedure, the difference in the observed test values (P-D and MDP-MDD) are first calculated. Next, specimens were randomly assigned to groups, and P, D, MDP and MDD recalculated, as was the difference score. This was repeated 10,000 times and the proportion of randomly generated values more extreme than the observed was taken as the significance of the observed data.

Results

Location of fiber initials and fiber cover on the ovule epidermis. The most striking difference between the four taxa studied is the amount of fiber cover on the seed coat (Fig. 2.3). Fiber initials are found in all taxa at the chalazal end of the ovule on the day of anthesis and progressively toward the micropylar end each day thereafter. It should be noted, however,

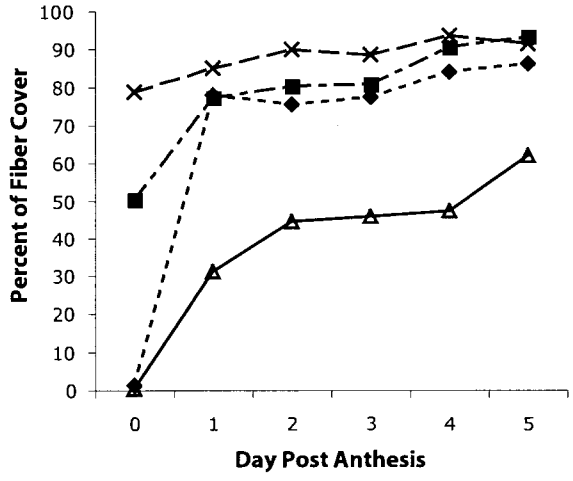


Figure 2.4. Percentage of fiber cover over the seed from the chalazal to the micropylar end. - - -◆- - - *G. herbaceum* var. Wagad; — -■- - *G. raimondii*; ——△—— *G. hirsutum* var. *yucatanense*; — -✕- - *G. hirsutum* var. Maxxa

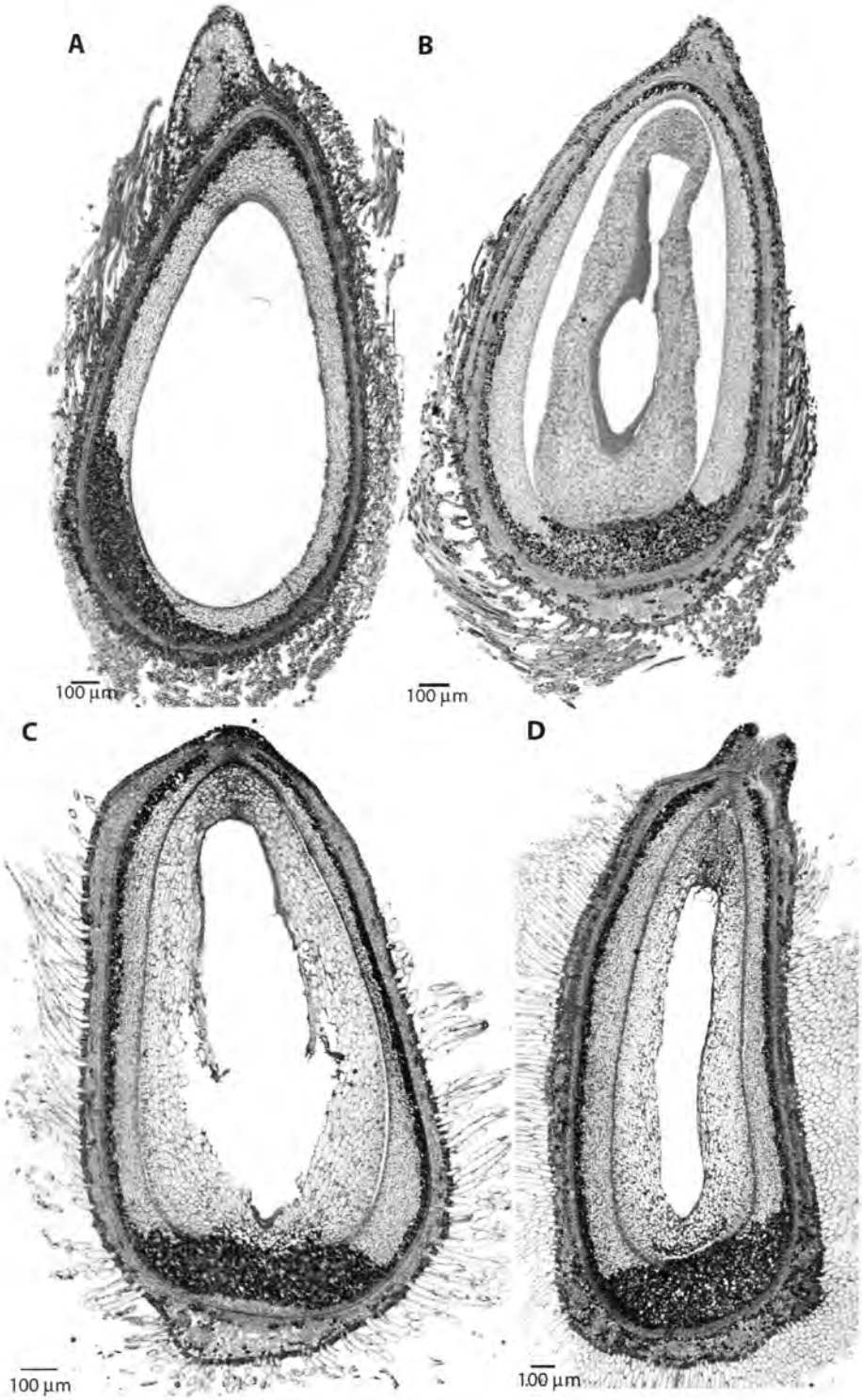


Figure 2.3. Cross sections of 5 dpa ovules illustrating the extent of cover over the surface of the ovule. A) *G. raimondii* B) *G. hirsutum* var. *yucatanense* C) *G. herbaceum* var. Wagad D) *G. hirsutum* var. Maxxa

that no new fiber initials were found after 1 dpa at the chalazal end of *G. herbaceum* var. Wagad, *G. hirsutum* var. *yucatanense*, or *G. hirsutum* var. Maxxa, but new fiber initials were observed at the chalazal end of *G. raimondii* under older fibers throughout the days studied. The percentage of the seed coat covered by fibers from the chalazal to the micropylar ends of the seed coat by 5 dpa differed among species (Fig. 2.4). Seeds of *G. raimondii* and *G. hirsutum* var. Maxxa are covered halfway or more at 0 dpa while at this same stage those of *G. hirsutum* var. *yucatanense* and *G. herbaceum* var. Wagad have almost no initials, with those present found at the extreme chalazal end. Both of these latter species have bursts of initiation between 0 and 1 dpa, with *G. herbaceum* var. Wagad eventually reaching a similar amount of coverage as *G. raimondii* and *G. hirsutum* var. Maxxa at 1 dpa, but *G. hirsutum* var. *yucatanense* never attains more than 60 percent fiber cover in the six days studied (0 – 5 dpa).

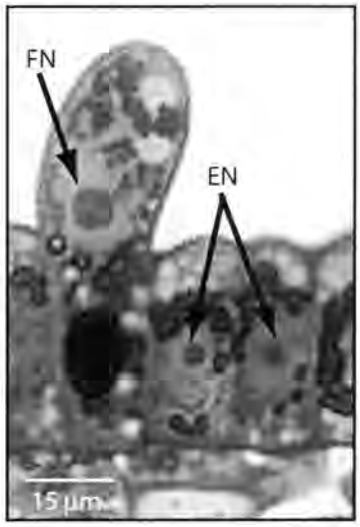


Figure 2.5. Diagram of fiber initial nucleus position in fiber cells. FN = fiber nucleus; EN- epidermal nucleus

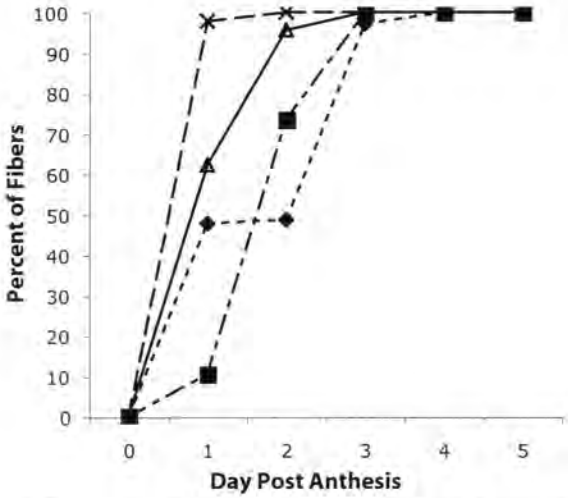


Figure 2.6. Percentage of fiber nuclei more distal to the epidermal nuclei.
G. herbaceum var. Wagad; *G. raimondii*; *G. hirsutum* var. *yucatanense*; *G. hirsutum* var. Maxxa

Fiber nucleus location. The position of the nucleus in fiber initials from the same position as in non-fiber epidermal cells to the middle of the elongating fiber cell (Fig. 2.5) begins 1 dpa in all species, but the change in position varies between species (Fig. 2.6). In the wild species *G. hirsutum* var. *yucatanense* and *G. raimondii*, there is an increasing number of cells with nuclei near to the center of the cell by 1 dpa and 2 dpa, with all fiber nuclei near the center by 3 dpa. Nucleus position in fibers of *G. herbaceum* var. Wagad is not complete until 4 dpa, whereas the nuclei in *G. hirsutum* var. Maxxa mostly central by 2 dpa.

Figure 2.7. Diagram of vacuolate (V) and non-vacuolate (NV) cotton fibers.

Fiber vacuolation. The emergence of the vacuole in fiber initials (Fig. 2.7) is more varied among species than is the central nucleus (Fig. 2.8). The vacuole is first observed

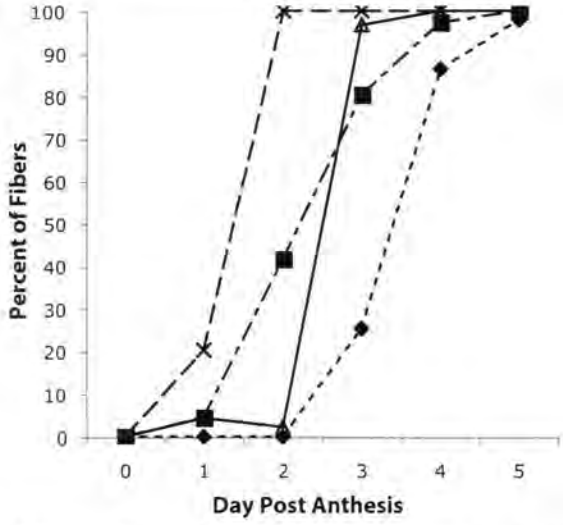
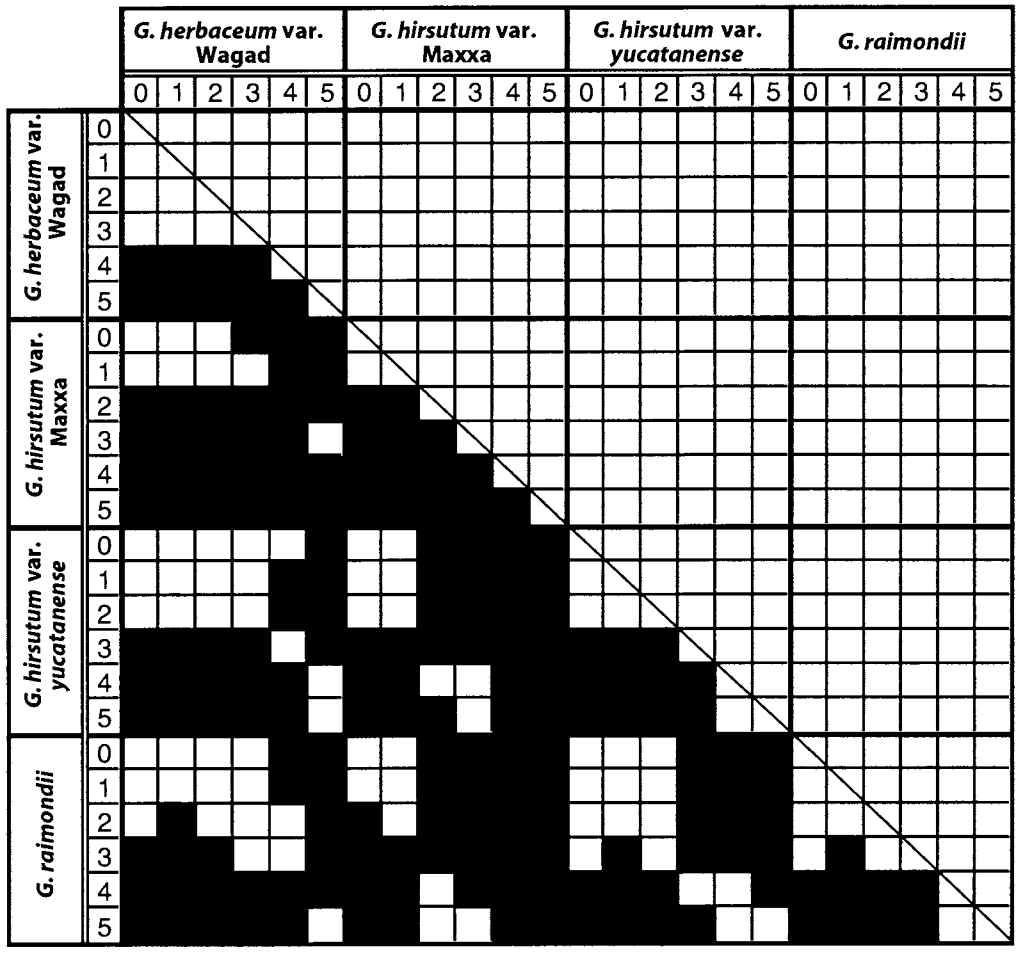


Figure 2.8. Percent of fibers with a vacuole. - - -◆- - - *G. herbaceum* var. Wagad; — ■ — *G. raimondii;* —△— *G. hirsutum* var. *yucatanense;* —×— *G. hirsutum* var. Maxxa

in *G. hirsutum* var. *yucatanense* and *G. raimondii* at 1 dpa, and is observed in all fibers by 4 dpa in *G. hirsutum* var. *yucatanense* and by 5 dpa in *G. raimondii*. In *G. herbaceum* var. Wagad, vacuoles are not observed until 3 dpa and have not developed in some fibers by 5

Table 2.1. Pairwise comparisons of centroid size using Tukey-Kramer HSD. A ■ indicates a significant difference with $\alpha = 0.05$.



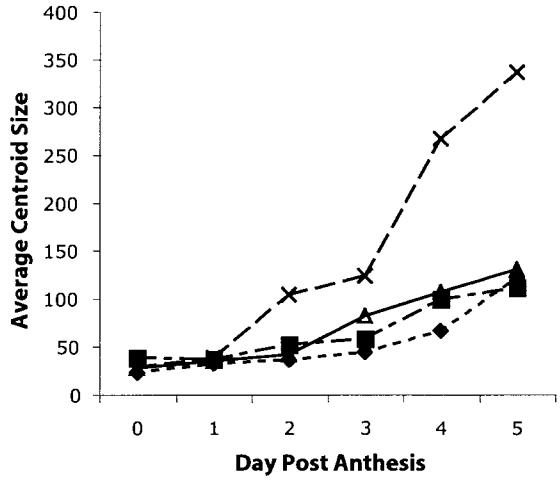


Figure 2.9. Average fiber centroid size each day of development. - - -◆- - - *G. herbaceum* var. Wagad; - - ■ - - *G. raimondii;* —△— *G. hirsutum* var. *yucatanense;* — ✕ - - *G. hirsutum* var. Maxxa

dpa, suggesting that vacuole formation continues beyond 5 dpa in this species. Vacuoles are initially present in fibers of *G. hirsutum* var. Maxxa at 1 dpa and all fibers are vacuolate by 2 dpa.

Centroid size and fiber length. Length and centroid size are highly correlated (r=.968). Fiber growth (Table 2.1) in *G. raimondii, G. herbaceum* var. Wagad and *G. hirsutum* var. *yucatanense* is similar initially (0-1 dpa) as is the length achieved (104 μm to 116 μm; Fig. 2.9) by 5 dpa. One difference in growth between these species, however, is the timing of the elongation burst (≥40 μm in one day), which occurs 3-4 dpa, 4-5 dpa and 2-3 dpa,

Table 2.2. MANOVA results of fiber shape.

	Test	DF1	DF2	F	Prob>F
Species	Wilks' Lambda	15	2974	31.985	<.0001
Day	Wilks' Lambda	25	4002	52.806	<.0001
Species by Day	Wilks' Lambda	75	5163	9.5038	<.0001

respectively, in *G. raimondii, G. herbaceum* var. Wagad and *G. hirsutum* var. *yucatanense.* Fibers from *G. hirsutum* var. Maxxa increase in length on average 70 μm a day between 1-2, 3-4 and 4-5 dpa, achieving an average size of 322 μm by 5 dpa.

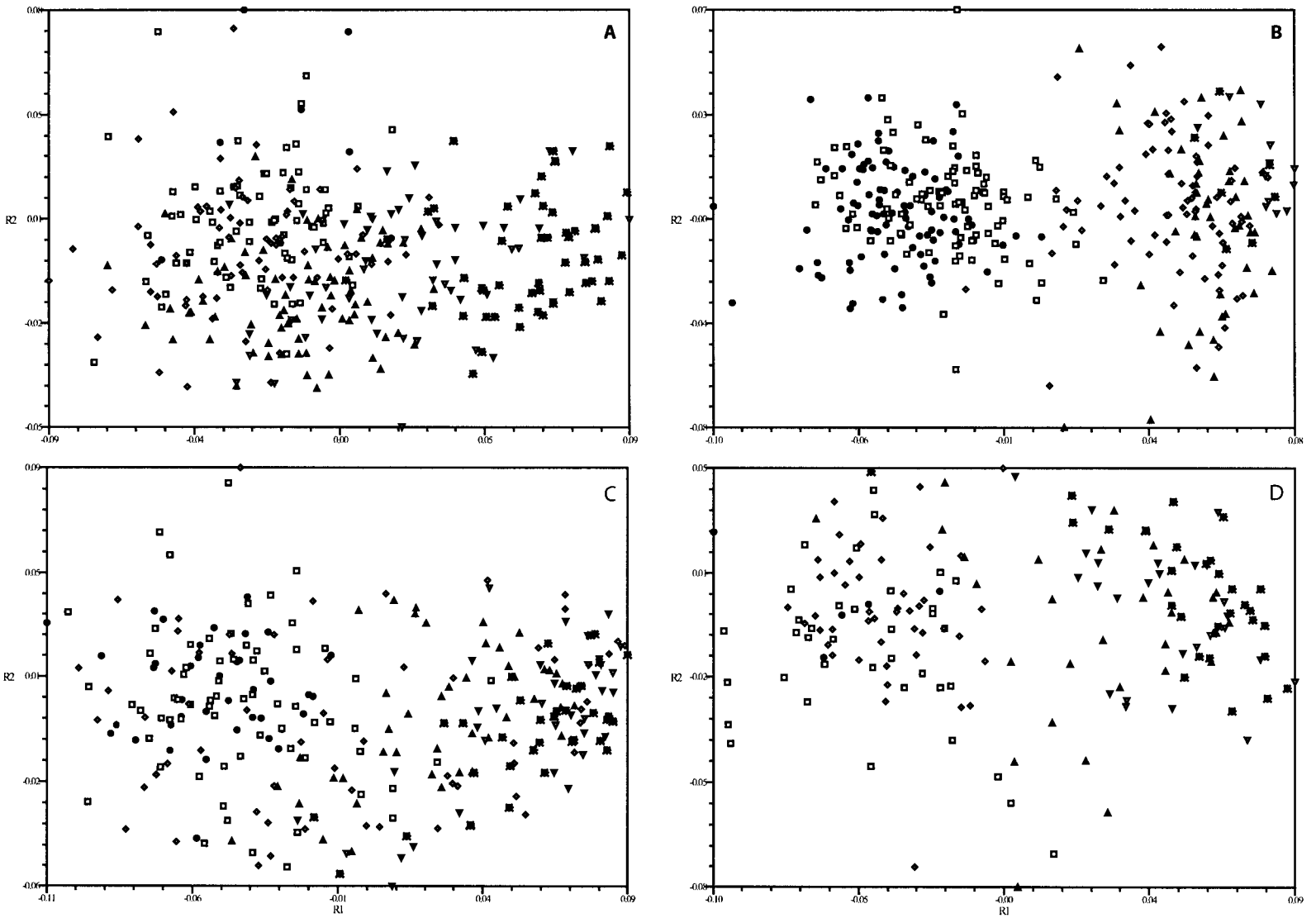


Figure 2.10 Principal component analysis of the six axes of shape information. A) *G. herbaceum* var. Wagad B) *G. hirsutum* var. Maxxa C) *G. raimondii* D) *G. hirsutum* var. *yucatanense* ●- 0 DPA ▫- 1 DPA ♦- 2 DPA ▲- 3 DPA ▼- 4 DPA ■- 5 DPA

Table 2.3. Pairwise comparisons of fiber shape. Numbers indicate the generalized distance between shapes. The ■ indicates a significant difference in shape.

		G. herbaceum var. Wagad						*G. hirsutum* var. Maxxa						*G. hirsutum* var. *yucatanense*						*G. raimondii*					
		0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
G. herbaceum var. Wagad	0		1.39	1.74	2.44	2.64	3.74	1.53	1.69	3.35	3.62	4.68	4.26	1.77	1.05	1.60	3.16	3.62	4.01	1.67	1.98	2.30	3.80	4.00	4.30
	1			0.60	1.52	2.51	3.97	0.77	0.87	3.48	3.83	4.65	4.20	0.87	0.65	0.54	3.29	3.88	4.21	1.08	1.17	2.13	3.83	4.08	4.33
	2				1.40	2.35	3.85	1.02	1.26	3.37	3.73	4.50	4.14	0.97	1.07	0.89	3.32	3.94	4.29	1.33	1.49	2.19	3.90	4.10	4.32
	3					1.72	3.49	2.09	1.52	2.86	3.43	3.96	3.29	1.99	1.99	1.34	2.43	3.11	3.51	2.25	1.63	1.40	2.80	3.20	3.34
	4						1.95	3.06	2.46	1.23	1.95	2.61	2.10	2.98	2.81	2.41	1.64	2.05	2.59	3.31	2.98	1.74	2.46	2.34	2.48
	5							4.41	3.95	0.98	0.56	1.30	1.91	4.28	4.24	3.99	2.76	2.20	2.32	4.70	4.67	3.31	3.61	2.36	2.47
G. hirsutum var. Maxxa	0								1.51	3.97	4.26	5.12	4.68	0.40	0.76	1.24	3.72	4.28	4.55	0.56	1.50	2.53	4.28	4.44	4.72
	1									3.36	3.74	4.49	3.92	1.59	1.13	0.47	3.12	3.74	4.09	1.80	1.31	2.25	3.66	3.99	4.23
	2										0.91	1.62	1.41	3.88	3.73	3.41	1.99	1.80	2.18	4.28	4.08	2.73	2.96	2.12	2.26
	3											1.22	1.79	4.14	4.08	3.83	2.78	2.31	2.40	4.61	4.58	3.38	3.74	2.50	2.66
	4												1.63	4.94	5.00	4.60	3.33	2.73	2.65	5.47	5.33	4.06	4.13	2.68	2.70
	5													4.54	4.49	4.05	2.07	1.65	1.73	4.98	4.63	3.19	2.86	1.68	1.75
G. hirsutum var. *yucatanense*	0														1.08	1.34	3.71	4.22	4.44	0.79	1.67	2.53	4.29	4.31	4.59
	1															0.89	3.40	4.05	4.43	0.98	1.25	2.32	3.96	4.33	4.61
	2																3.13	3.79	4.19	1.49	1.04	2.08	3.62	4.04	4.27
	3																	1.25	2.01	3.85	3.36	1.69	1.24	1.75	1.92
	4																		0.88	4.42	4.10	2.33	1.84	0.80	1.03
	5																			4.71	4.52	2.85	2.52	0.61	0.94
G. raimondii	0																				1.36	2.50	4.27	4.57	4.84
	1																					2.01	3.54	4.32	4.52
	2																						1.85	2.54	2.69
	3																							2.15	2.13
	4																								0.45
	5																								

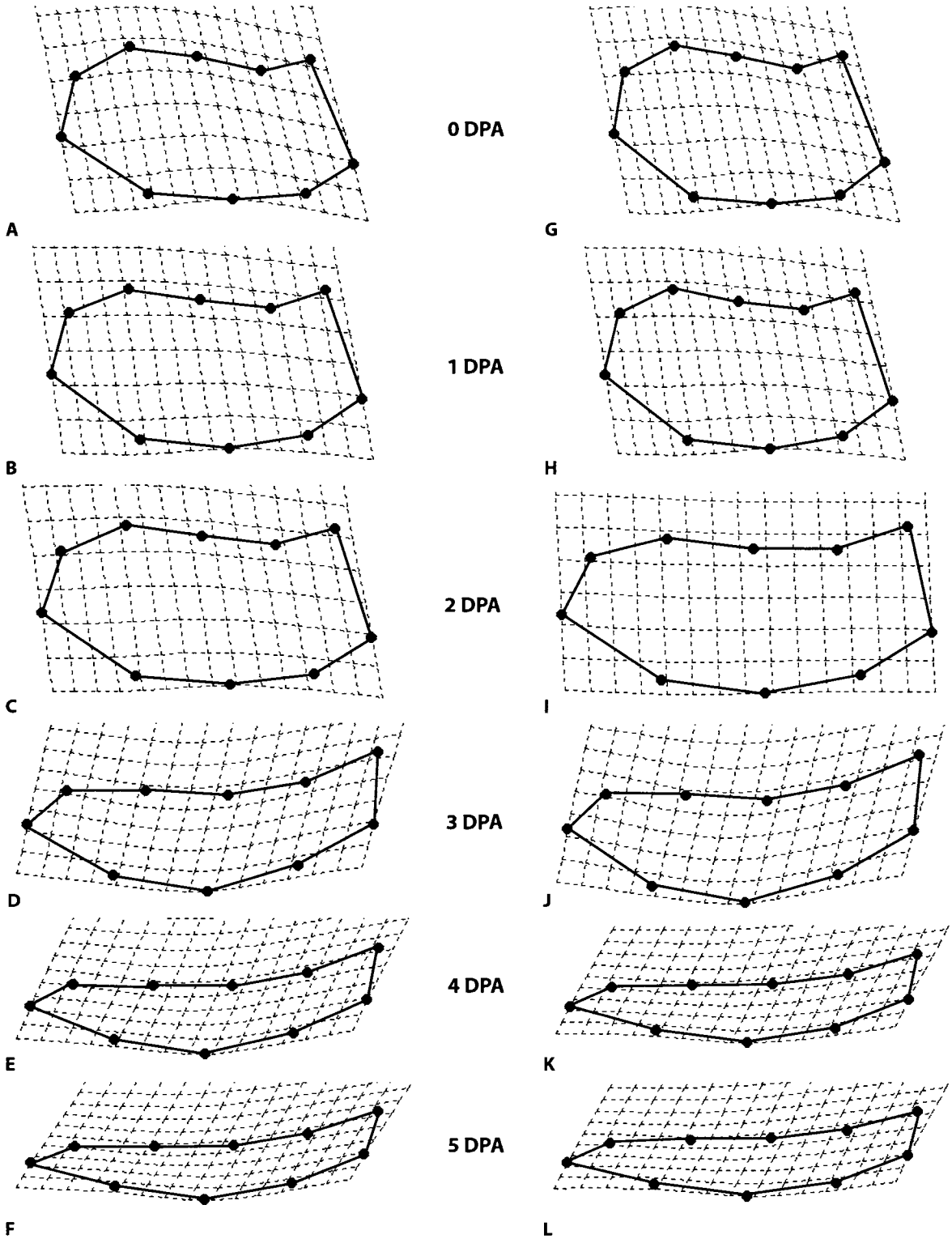


Figure 2.11. Warp grids illustrating shape change over time in wild species *G. hirsutum* var. *yucatanense* (A-F), *G. raimondii* (G-L), *G. herbaceum* var. Wagad (M-R) and *G. hirsutum* var. Maxxa (S-X).

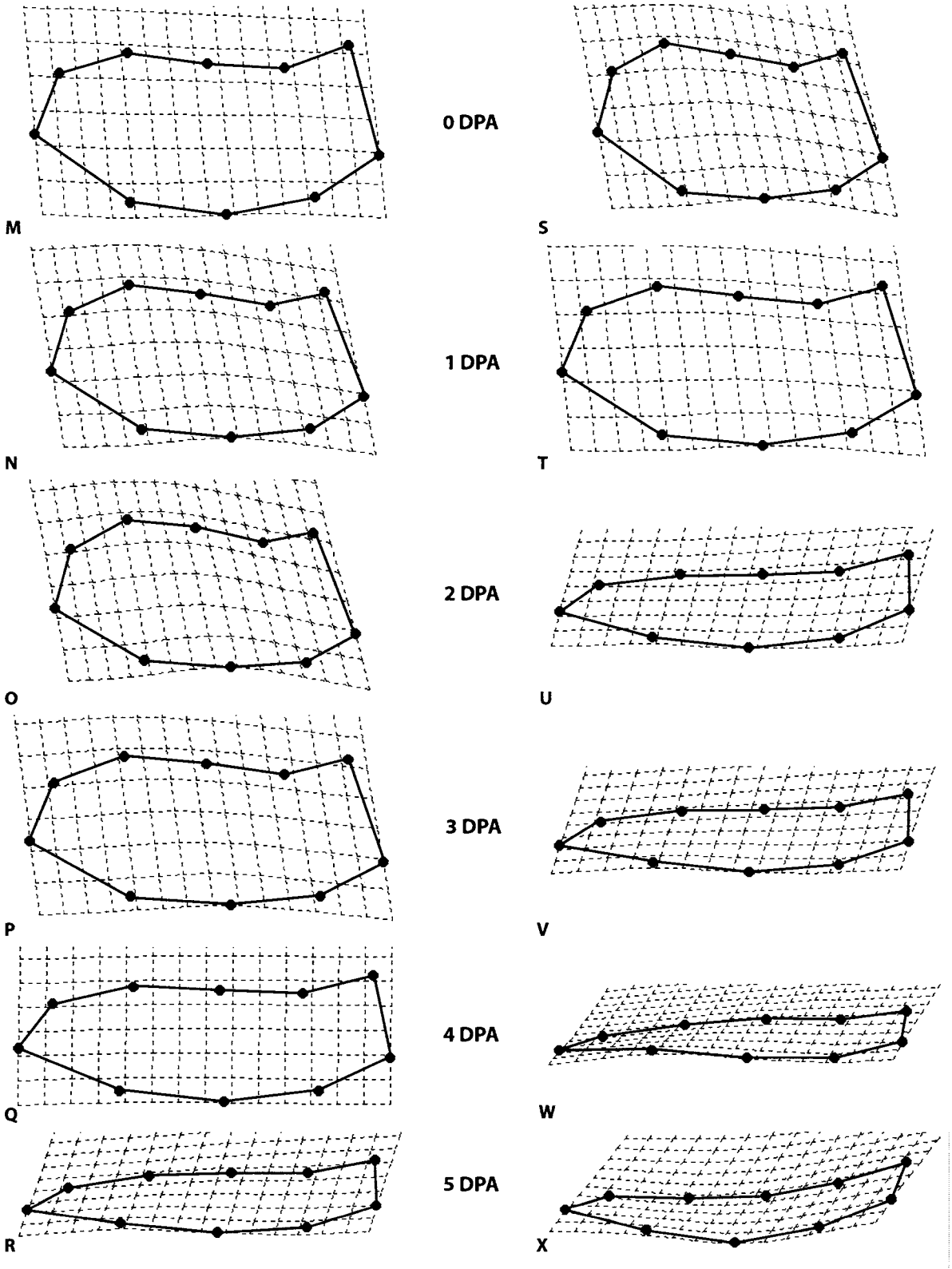


Figure 2.11. (Cont.)

Fiber shape. An initial PCA analysis of fiber shape indicates that within each species, fibers from any single day have shape characteristics that largely cluster together relative to fibers from other days (Fig 2.10). Further analysis using MANOVA (Table 2.2) indicates that these shape trends are significantly different between species and days. Pairwise distances between all days and species (Table 2.3) showed that almost all shapes were significantly different from each other with p-values <0.05. Those comparisons that were not significant were primarily found for combinations in which one of the two species/days being compared had a small sample size.

In general, differences in fiber shape follow the expected pattern: fibers begin as relatively short and round epidermal initial, and as they age they become relatively long and pointed (Fig 2.11). While this pattern is consistent for all species, the timing of this change differs among them. Fibers of *G raimondii* are short with rounded tips 0 and 1 dpa and long and pointed 4 and 5 dpa, but unlike in the other taxa that do not have more than one fiber shape each day, the fibers 2 and 3 dpa are a combination of short and round and long and pointed. Fibers and *G. hirsutum* var. *yucatanense* begin to taper at 3 dpa, with the point becoming very obvious by 4 dpa, whereas the fibers of *G. herbaceum* var. Wagad begin to taper at 4 dpa and *G. hirsutum* var. Maxxa fibers are distinctly pointed by 3 dpa.

Table 2.4. Randomization test results based on the number of times random data was more extreme than the observed value. A negative observed P-D 0r MDP-MDD value indicates support for the phylogenetic hypotheses. A positive observed P-D or MDP-MDD value indicates support for the domestication hypothesis. Significance was determined at the 0.05 level.

	Fiber size		Fiber Shape	
DPA	**Observed P-D**	**p-value**	**Observed MDP-MDD**	**p-value**
0	-4.292794934	0.0003	-1.636431075	0.0001
1	-2.678492565	0.0008	0.197959466	0.2725
2	-5.217913113	0.1144	-1.118167061	0.0001
3	-37.71687989	0.0001	-0.940925277	0.0002
4	-40.20934798	0.0012	0.183630559	0.3269
5	-8.960803156	0.2711	0.549466107	0.0563

Phylogeny vs. Domestication test. To address whether shared ancestry or shared domestication pressures have been more influential in shaping fiber development and morphology, randomization tests were performed as described. These tests demonstrate that phylogenetic history plays a dominant role for fiber size for all days examined except for 2 dpa, whereas for fiber shape, similar results were obtained (Table 2.4) for 0-4 dpa however, at 5 dpa there was weak support for domestication being influential in shape development.

Fiber development in G. raimondii. *Gossypium raimondii* has the most varied fiber development of the taxa studied. On the day of anthesis, fiber initials as well as longer, older fibers are present (Fig. 2.12). These findings are consistent with those reported in Applequist et. al. (2000). The day after anthesis (1 dpa), nuclei are near the center of the elongated fiber cell, the vacuole becomes obvious, and the fibers have retained their short, round shape. By 2 dpa there is a mixture of short and elongated fibers with nuclei in various positions (Fig. 2.12). By 3 dpa the fibers undergo a burst of elongation and all the fibers have begun to

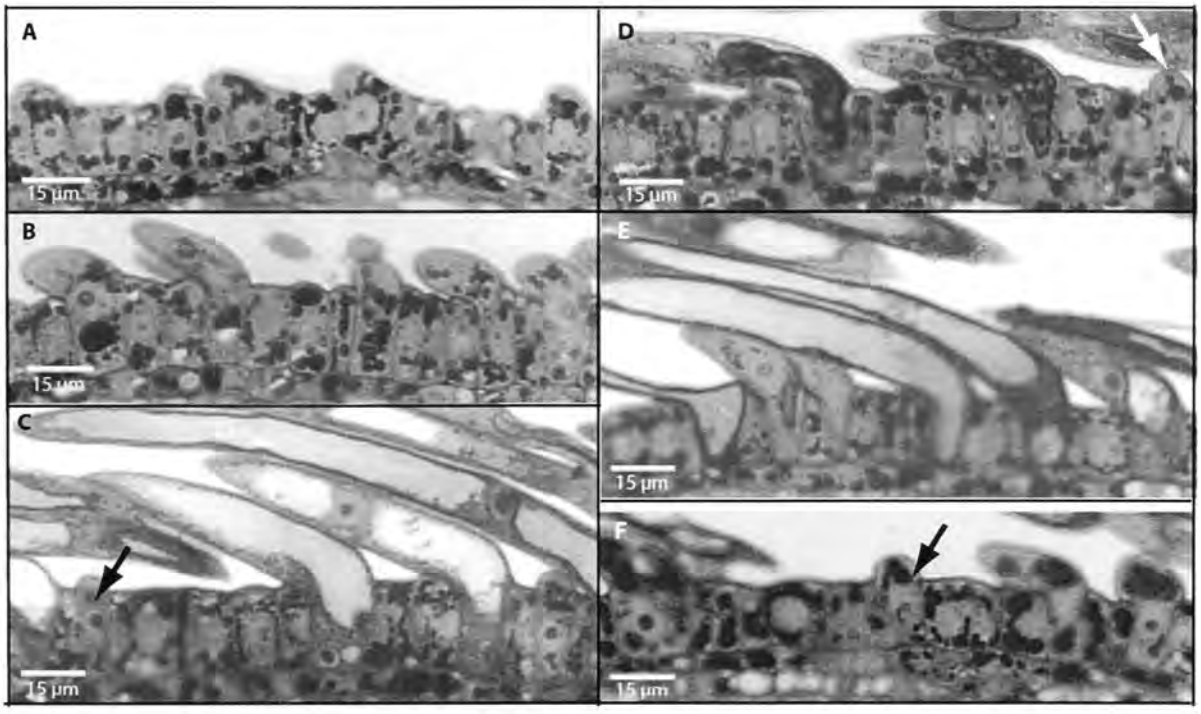


Figure 2.12. Fiber development in *G. raimondii*. A) 0 DPA; B) 1 DPA; C) 2 DPA; D) 3 DPA; E) 4 DPA; F) 5 DPA Arrows indicate fiber initials.

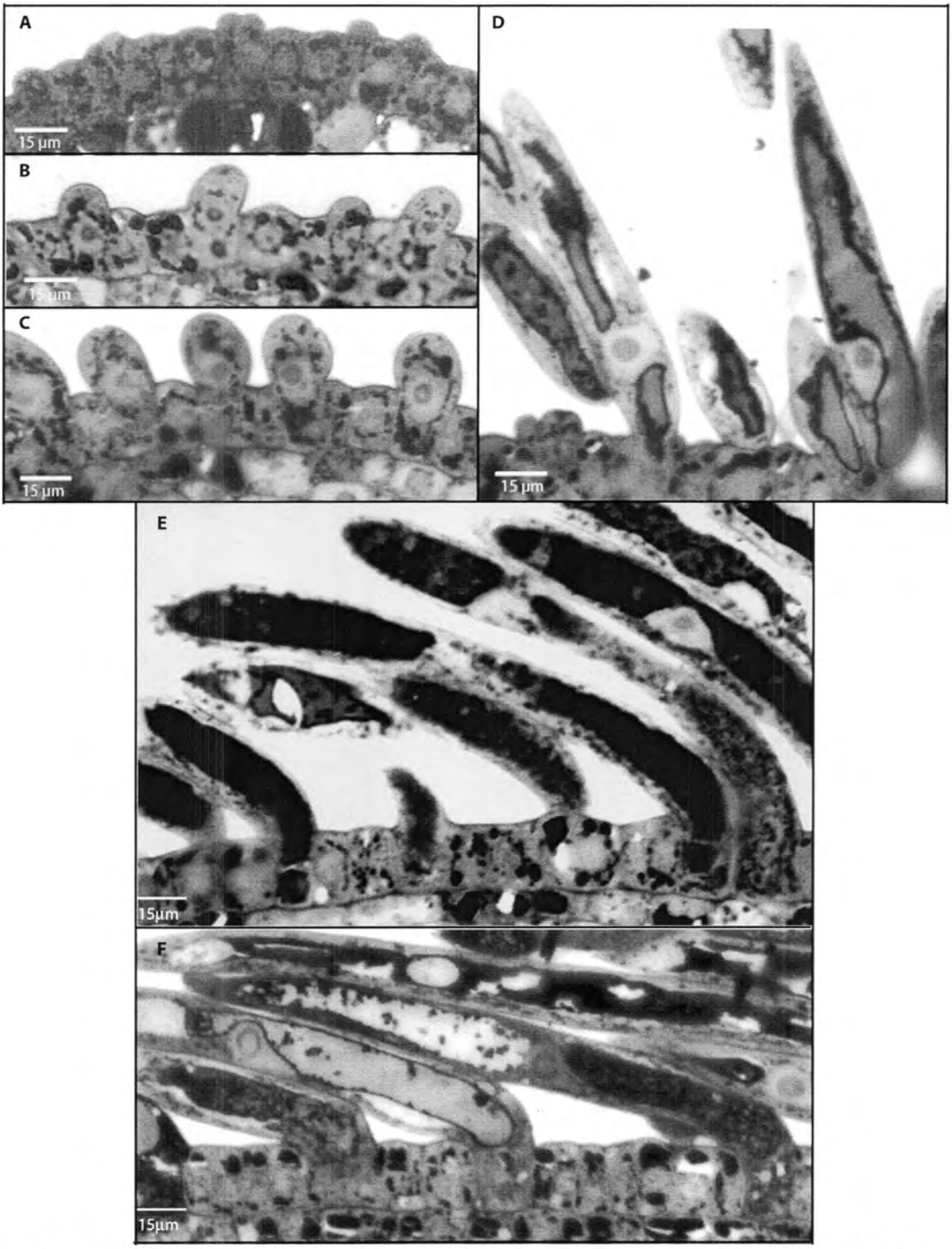


Figure 2.13. Fiber development in *G. hirsutum* var. *yucatanense.* A) 0 DPA; B) 1 DPA; C) 2 DPA; D) 3 DPA; E) 4 DPA; F) 5 DPA

taper. Elongation continues for the next two days. In addition, new fiber initials similar to those found at 0 dpa are present 2-5 dpa (Fig. 2.12 c-e), suggesting a continued presence of fibers at different stages of development. Thus, early fiber development in this species is not synchronous.

Fiber development in G. hirsutum var. yucatanense. On the day of anthesis in the other wild species studied, *G. hirsutum* var. *yucatanense*, a few short, round-tipped fiber

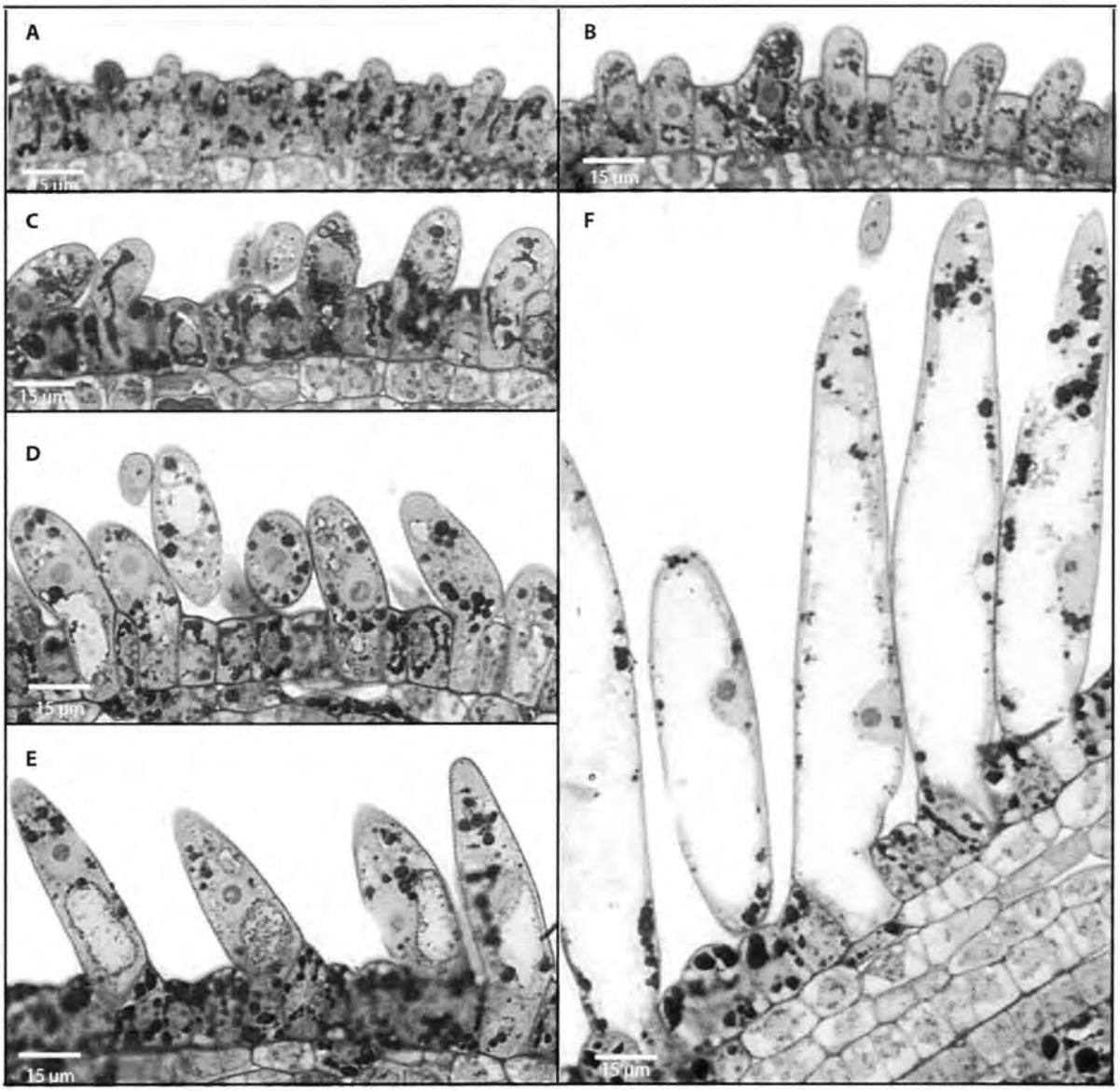


Figure 2.14. Fiber development in *G. herbaceum* var. Wagad. A) 0 DPA; B) 1 DPA; C) 2 DPA; D) 3 DPA; E) 4 DPA; F) 5 DPA

initials are present at the chalazal end of the ovule epidermis (Fig 2.13). The next day shows an increased number of initials, the change in position of the nucleus, and the appearance of the vacuole. By 2 dpa fibers are beginning to elongate and by 3 dpa, the fibers begin to taper, becoming pointed by 5 dpa. The presence of fiber initials after 2 dpa is observed solely in the cells toward the micropylar end of the ovule.

Fiber development in G. herbaceum var. Wagad. Like *G. hirsutum* var. *yucatanense*, *G. herbaceum* var. Wagad has few initials located on the extreme chalazal end of the ovule epidermis on the day of anthesis (Fig. 2.14). The next day the nuclei change position, and the fibers slowly elongate, remaining short and round-tipped until 4 dpa when they suddenly increase in size and become distinctly tapered. This variety also has no evidence of initials at the chalazal end after 1 dpa, although they continue to be found every day towards the micropylar end of the ovule.

Fiber development in G. hirsutum var. Maxxa. Development of fibers in *G. hirsutum* var. Maxxa (Fig. 2.15) is noticeably synchronized over the entire ovule surface. On the day of anthesis, almost 85% of the seed coat is covered with fiber initials, and the initials arising after 0 dpa are located at the micropylar end of the ovule. At 1 dpa the nucleus in each fiber cell changes position from near the base of the cell to near the center of the cell, and the vacuole becomes prominent. Between 1 and 2 dpa the first of three days of growth of over 70 μm a

Table 2.5. Timing of developmental events in cotton fibers.

	***G. herbaceum* var. Wagad**	***G. raimondii***	***G. hirsutum* var. *yucatanense***	***G. hirsutum* var. Maxxa**
Vacuole appears	3 DPA	1 DPA	1 DPA	1 DPA
Fiber nuclei change position	1 DPA	1 DPA	1 DPA	1 DPA
Largest change in centroid size (≥40)	4-5 DPA	3-4 DPA	2-3 DPA	1-2 DPA
Appearance of pointed fiber tip	4 DPA	3 DPA	3 DPA	2 DPA
Amount of fiber coat cover	85.9%	92.9%	61.7%	91.1%

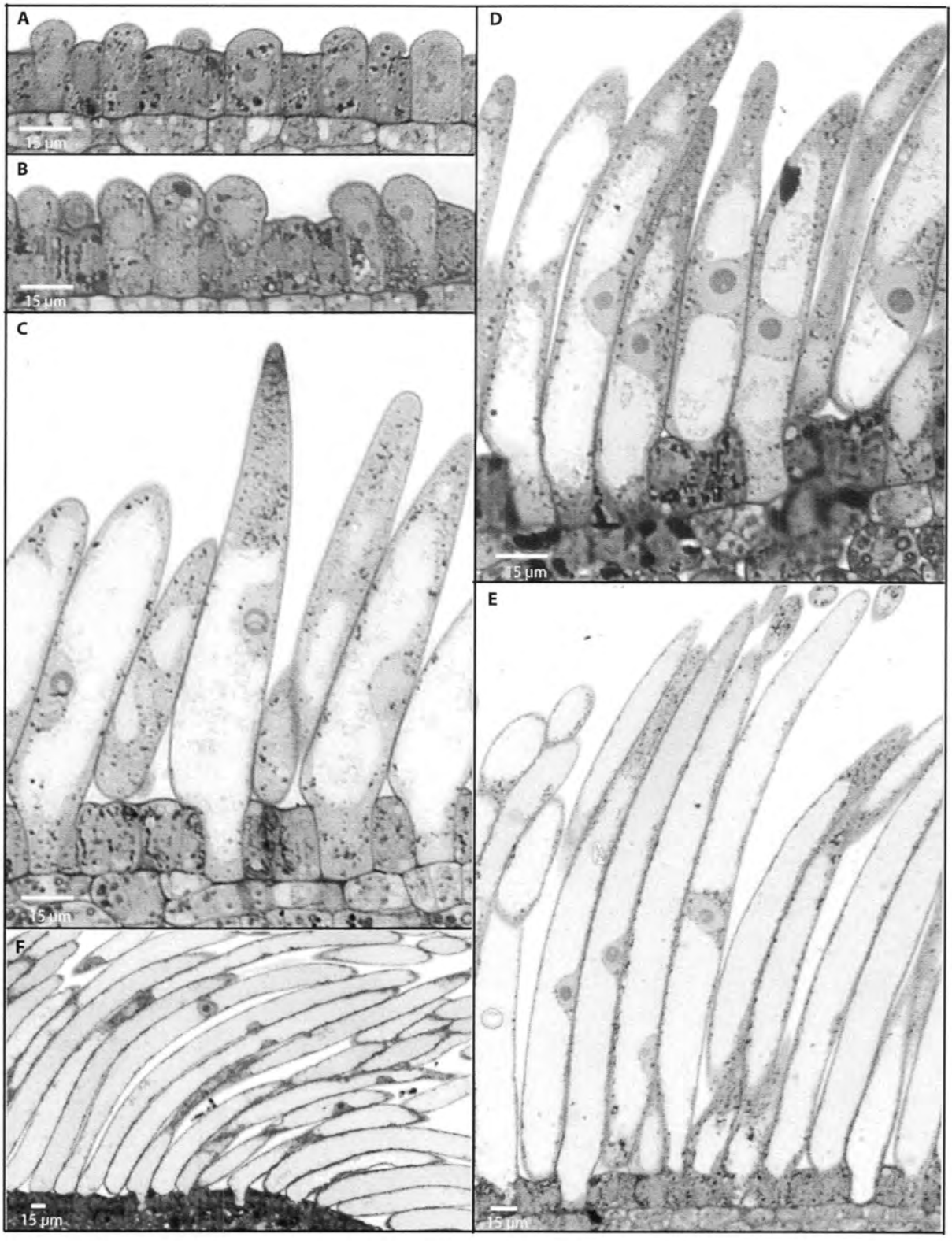


Figure 2.15. Fiber development in *G. hirsutum* var. Maxxa. A) 0 DPA; B) 1 DPA; C) 2 DPA; D) 3 DPA; E) 4 DPA; F) 5 DPA

day occurs. At 2 dpa, the fibers begin to taper with fibers becoming distinctly pointed by 3 dpa. Growth continues at a rapid rate for the next three days. The only initials present after 0 dpa are located at the micropylar end of the ovule epidermis.

Discussion

Wild D-genome diploid vs. wild tetraploid growth. Comparisons between fibers from the wild D-genome species *G. raimondii* and those of the wild tetraploid species *G. hirsutum* var. *yucatanense* show few differences except for the timing of the growth spurt, which occurs a day earlier in the tetraploid, and the amount of ovule epidermis cover, which is higher in the diploid than in the tetraploid (Table 2.5). It is unclear whether the lower amount of fiber cover in *G. hirsutum* var. *yucatanense* is due to a delay in the formation of new initials toward the micropylar end of the seed or whether there are simply fewer fibers produced in this variety. It does appear that in the tetraploid, the D-genome genetic controls for initial formation are being suppressed at these stages of development. To evaluate this possibility would at the minimum require a comparable examination of early fiber development in wild A-genome cotton (*G. herbaceum* var. *africanum*).

Cultivated diploid vs. cultivated tetraploid growth. The cultivated varieties *G. herbaceum* var. Wagad (diploid) and *G. hirsutum* var. Maxxa (tetraploid) have several notable differences in development (Table 2.5). Fibers of *G. hirsutum* var. Maxxa exhibit the greatest degree of developmental synchrony among the species studied, with nuclear position, vacuole appearance, growth, and shape changes all occurring in nearly all fibers by 2 dpa. These observations suggest that some of the fiber properties that make this species the taxon of choice for world cotton commerce are already reflected at this early developmental stage. In contrast, the cultivated but agronomically inferior Old World diploid cotton, *G. herbaceum* var. Wagad, has fibers that develop more slowly than even the wild species: the vacuole appears at 3 dpa, and the change in shape and first large length increase do not occur until 4

dpa. This suggests a later period of maximal elongation rate for the cultivated diploid than in tetraploid cotton, although study of additional cultivars is required to verify this suggestion.

Domestication of tetraploid cotton. A comparison of wild and cultivated tetraploids may lead to insights into the effects of the domestication process on early fiber development. In this respect, two fiber characteristics are revealed as different between the wild tetraploid, *G. hirsutum* var. *yucatanense*, and the cultivated tetraploid, *G. hirsutum* var. Maxxa. First, the wild accession shows a low amount of fiber cover relative to cultivated cotton (Table 2.5). Second, there are significantly fewer fiber initials on the day of anthesis in the wild tetraploid. The amount of fiber cover may, as mentioned earlier, be due to a delay in onset of fibers toward the micropylar end of the wild variety, or alternatively, perhaps plants producing a fuller cover of fibers were selected during the domestication process. In addition, as fiber development progresses, cultivated cotton exhibits highly synchronized development, which may assist in the production of large amounts of more uniform, spinnable fiber.

Phylogeny vs. domestication. By including two different cultivated species this study provides the opportunity to identify whether recent shared ancestry or domestication pressues have been most responsible for driving the present developmental patterns and morphology. For both fiber shape and size the data suggest that the morphology of the early stages is most strongly influenced by phylogeny, although by 5 dpa size differences between the two were not significant, and shape differences indicating a influence from domestication were weakly significant as determined by randomization tests. The idea that emerges is that similarities in developmental programs resulting from shared phylogenetic history play an important role in shaping early development of cotton fiber, but that developmental alterations that resulted from human-mediated selection during the 5,000-year old history of cotton domestication play an increasingly important role during later developmental stages. This idea is reasonable given the convergence of fiber properties and morphology between the Asiatic diploid cultigen *G. herbaceum* and the American tetraploid cultigen *G. hirsutum*. A

more precise test of this idea will require study of later developmental stages and the inclusion of wild A-genome diploid antecedents of cultivated *G. herbaceum*, so that the factor of ploidy level is removed as a confounding variable. In this respect it has been shown (Kelman, Oram, and Hayes, 1999; Kadota and Niimi, 2002) that polyploidy often results in size differences.

Future directions. These descriptions of fiber development in wild and cultivated cottons form the groundwork for future studies looking at the origin of a second type of fibers found on cultivated cottons called fuzz fibers. Additional work to identify fuzz will require a broader sampling of taxa as well as extending the present study to encompass a longer developmental time frame. For example, extending the study period to 10 dpa might be revealing, because by this time a later wave of "initials" should have formed which may be fuzz initials(Lang, 1938; Joshi, Wadhwani, and Johri, 1967; Berlin, 1986). In addition, the inclusion of a wild A-genome plant (*G. herbaceum* subsp. *africanum*), the F-genome species (*G. longicalyx*, phylogenetically sister to the A-genome), and additional tetraploid species and cultivars would add valuable information relevant to this question. Future work should also include additional cultivars of both diploid and tetraploid cottons, because cultivars may have different growth patterns (Quisenberry and Kohel, 1975).

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Chapter 3: General Discussion

To gain an understanding of the origin of the fuzz layer of fibers in cultivated cotton and to begin to appreciate the genesis of the differences in mature fiber morphology among species, it is first necessary to describe and compare the early stages of fiber development. A summary of some of the main findings of the present study is shown in Table 3.1. The

Table 3.1. Timing of developmental events in cotton fibers.

	G. herbaceum var. Wagad	*G. raimondii*	*G. hirsutum* var. *yucatanense*	*G. hirsutum* var. Maxxa
Vacuole appears	3 DPA	1 DPA	1 DPA	1 DPA
Fiber nuclei change position	1 DPA	1 DPA	1 DPA	1 DPA
Largest change in centroid size (≥40)	4-5 DPA	3-4 DPA	2-3 DPA	1-2 DPA
Appearance of pointed fiber tip	4 DPA	3 DPA	3 DPA	2 DPA
Amount of fiber coat cover	85.9%	92.9%	61.7%	91.1%

most noticeable difference among the species studied is a distinct synchronization of fiber development in the Upland cotton, *G. hirsutum* var. Maxxa. Unlike the other three taxa studied, including the cultivated diploid variety, the fibers of this variety has display nearly complete central nuclei and vacuoles the day after anthesis. In addition, the fibers begin to take on their characteristic pointed shape and begin rapid growth by this time, which is characteristic of cultivated tetraploid cotton fibers (Anderson and Kerr, 1938; Ramsey and Berlin, 1976; DeLanghe, 1986; Ryser and Holloway, 1999; Applequist, Cronn, and Wendel, 2001). The cultivated diploid variety, *G. herbaceum* var. Wagad, however, does not exhibit a synchronization of fiber development. Instead, it exhibits delayed vacuolation, appearance of the pointed shape, and growth spurt with respect to the other taxa studied. The two wild species have developmental timing that is intermediate to these two cultivated varieties.

These results represent a better understanding of the early development of both wild and cultivated cotton fibers. Future work would benefit from additional sampling of

both cultivated varieties as well as other species, including: 1) the wild A-genome diploid, *G. herbaceum* subsp. *africanum*; 2) the wild F-genome species, *G. longicalyx*, which has been shown (Applequist, Cronn, and Wendel, 2001) to have an extended growth period similar to that of the A-genome; 3) the wild tetraploid *G. tomentosum* which is phylogenetically sister to *G. hirsutum;* and 4) additional varieties of both the diploid and tetraploid cultivated species, *G. herbaceum* and *G. hirsutum.*

Future studies focusing on the origin of fuzz should include developmental stages beyond 5 dpa, especially in the cultivated varieties, because initials have been reported as late as 12 dpa in some varieties (Lang, 1938; Joshi, Wadhwani, and Johri, 1967; Berlin, 1986). This may be problematic in the tetraploid varieties. Preliminary work beyond 5 dpa (Butterworth, unpublished) found that the number of large fibers masked the presence of any initials in the resin sections by crowding and distorting remaining non-fiber epidermal cells. It is possible that alternative methods of visualization, such as cutting or fracturing ovules in half and processing for imaging with the scanning electron microscope (SEM) may prove more useful than sectioned material for this purpose. Other characters suggested to be important in previous work with fuzz (Fryxell, 1964; Berlin, 1986; Kosmidou-Dimitropoulou, 1986), such as nucleus size and timing and size of secondary wall synthesis should also be considered in further studies. These additions should provide a solid foundation for inferring characters selected during the domestication of cotton, and should help inform the search for genes involved in the process.

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