

## INITIATION AND EARLY DEVELOPMENT OF FIBER IN WILD AND CULTIVATED COTTON

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Cultivated cotton fiber has undergone transformation from short, coarse fibers found in progenitor wild species to economically important, long, fine fibers grown globally. Morphological transformation requires understanding of development of wild fiber and developmental differences between wild and cultivated fiber. We examined early development of fibers, including abundance and placement on seed surface, nucleus position, presence of vacuoles, and fiber size and shape. Four species were studied using microscopic, morphometric, and statistical methods: *Gossypium raimondii* (wild D genome), *Gossypium herbaceum* (cultivated A genome), *Gossypium hirsutum* (wild tetraploid), and *Gossypium hirsutum* (cultivated tetraploid). Early fiber development is highly asynchronous in *G. raimondii* but more synchronous in other taxa. Significant changes associated with domestication include pronounced synchronization of fiber development in *G. hirsutum* relative to other taxa studied, implicating unconscious selection that shaped early molecular and cellular events, and a delay in some developmental features in fibers of *G. herbaceum*, including delayed vacuole formation and nuclear migration. Increased fiber cover and synchronized development selection in cultivated cotton may have facilitated both yield and uniformity of the crop. However, for the taxa and developmental timeframe studied, phylogeny is found to play a more important role than domestication in determining early fiber size and shape.

**Keywords:** cotton, domestication, early development, fibers, *Gossypium*, morphometrics, polyploidy.

### 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 semiarid tropics (Vollesen 1987; Fryxell 1992). The diploids are divided into eight genome groups based on chromosome pairing and size and fertility between species (Endrizzi et al. 1985). These groups comprise natural lineages in the genus and correspond to geographic locations: A, B, E, F in Africa; C, G, K in Australia; and D in the New World. Allopolyploid members are found in the New World and contain A and D genomes (Wendel and Cronn 2003). The genus is best known for four species, two A-genome diploids (*Gossypium arboreum* L. and *Gossypium herbaceum* L.), and two tetraploids (*Gossypium hirsutum* L. and *Gossypium barbadense* L.), that have been independently domesticated for fiber properties useful in textile spinning (Wendel 1995; Wendel et al. 1998; Brubaker et al. 1999; Percival et al. 1999). The evolutionary history of the genus is well understood (Cronn et al. 2002; Wendel and Cronn 2003), which facilitates comparative study of fiber development in a phylogenetic context.

At maturity, the cultivated cotton fiber is a single cell on the epidermis of the seed that initiates before anthesis and that may elongate to a final length of up to 6 cm (Ryser et al. 1983; Ryser and Holloway 1985; Weis et al. 1999; Kim and Triplett 2001). At maturity, the wild fiber is also a single cell, but unlike the cultivated fiber, it seldom exceeds 1 cm in length (Applequist et al. 2001). In addition to differing in fiber length, wild and cultivated species differ in the number of fiber types present on the seed coat at maturity. 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 et al. 1947; Stephens 1958; Fryxell 1963, 1964, 1992; Vollesen 1987).

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 before anthesis (Tiwari and Wilkins 1995; Wilkins and Jernstedt 1999; Kim and Triplett 2001) with the emergence of fiber initials, epidermal cells that protrude above the ovule coat epidermis (Farr 1933; Stewart 1975; Ramsey and Berlin 1976; Ryser 1977; Graves and Stewart 1988; Applequist et al. 2001). Initiation of these two types of fibers begins at the chalazal end of the ovule and progresses toward the micropylar end (Farr 1933; Stewart 1975; Ramsey and

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Berlin 1976; Ryser 1977; Graves and Stewart 1988; Applequist et al. 2001). Fuzz, however, is reported to initiate 6–9 d postanthesis (dpa) in *G. arboreum*, 10–12 dpa in *G. herbaceum*, 5–9 dpa in *G. hirsutum*, and 12 dpa in *G. barbadense*, and it may occur only on certain regions of the seed, depending on the species and/or variety (Lang 1938; Joshi et al. 1967; Berlin 1986). Lint elongation continues for the next 20–30 d in cultivated species (Schubert et al. 1973; Quisenberry and Kohel 1975; Ryser 1977; Applequist et al. 2001), whereas in most wild-type fibers elongation lasts about 15 d (Applequist et al. 2001). The end of elongation overlaps with the beginning of secondary wall synthesis (Meinert and Delmer 1977; Tiwari and Wilkins 1995; Wilkins and Jernstedt 1999), during which a thick wall of cellulose is laid down. Finally, the capsule dehisces and the fibers desiccate, forming twisted ribbonlike fiber cells that provide the world's most important natural fiber.

In this study, we describe fiber initiation patterns and morphological changes that occur from day of anthesis (0 dpa) through 5 d later (5 dpa) in two wild and two cultivated taxa. Four taxa representing three species were chosen to represent the main evolutionary lineages relevant to fiber cultivation (fig. 1; Cronn et al. 2002; Wendel and Cronn 2003): *G. raimondii*, the best living wild model of the D-genome donor to the tetraploid; *G. herbaceum* var. Wagad, an A-genome cultivated variety; *G. hirsutum* var. *yucatanense*, a wild accession of the most commonly cultivated tetraploid (AD) species; and *G. hirsutum* var. Maxxa, a cultivated variety of the tetraploid. 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 similarities and differences early in fiber development among species and to assess whether they relate to mature fiber morphology.

## Material and Methods

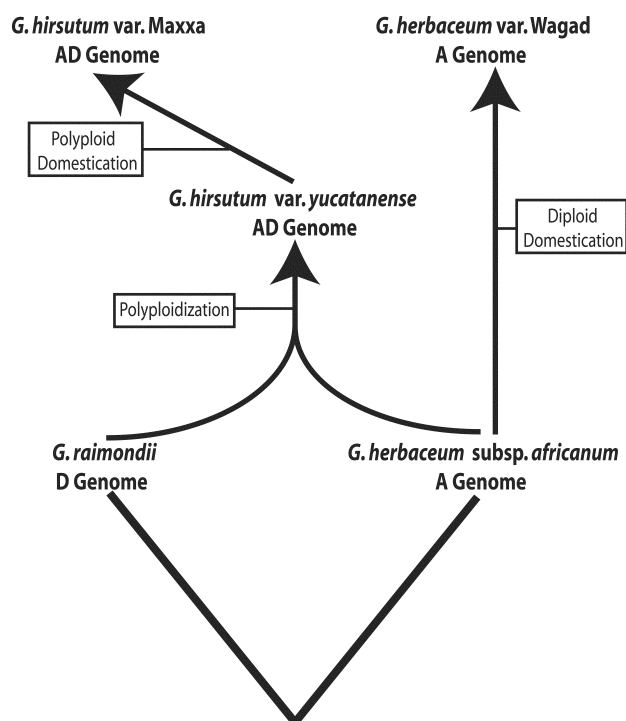
**Plants.** *Gossypium raimondii* Ulbrich (wild D genome), *Gossypium herbaceum* L. var. Wagad (cultivated A genome), *Gossypium hirsutum* L. var. *yucatanense* (wild tetraploid), and *G. hirsutum* L. var. Maxxa (cultivated tetraploid) were used in this study. *Gossypium herbaceum* var. *africanum* (wild A genome) was unavailable at the time of the study. All plants were grown at the same time in the Bessey Rooftop Greenhouse (Iowa State University) with a year-round average 14-h day length, and diurnal/nocturnal temperatures of 26.7°/15.6°C during winter 2001–2002 and spring 2003, and soil conditions described previously (Applequist et al. 2001). Flowers were hand-pollinated and tagged on the day of anthesis. They were then collected 0, 24, 48, 72, or 96 h after pollination for immediate fixation.

**Processing.** Ovules (0 dpa) and seeds (1–5 dpa) were removed from fruits and immediately fixed whole in 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2) for 3–5 d. They were rinsed in 0.1 M cacodylate buffer and postfixed for 4–12 h using 1% osmium tetroxide in the same buffer. Ovules and seeds were rinsed in deionized water and dehydrated through an ethanol series (25%, 50%, 70%, 95%, 100%) for 2 d at each step and followed by 100% acetone. Finally, materials were infiltrated in a series of acetone : Spurr's resin steps (3 : 1, 1 : 1, 1 : 3), 2 d at each step, and embedded into hard Spurr's resin. This prolonged fixation and dehydration process produced excellent preservation and sectioning.

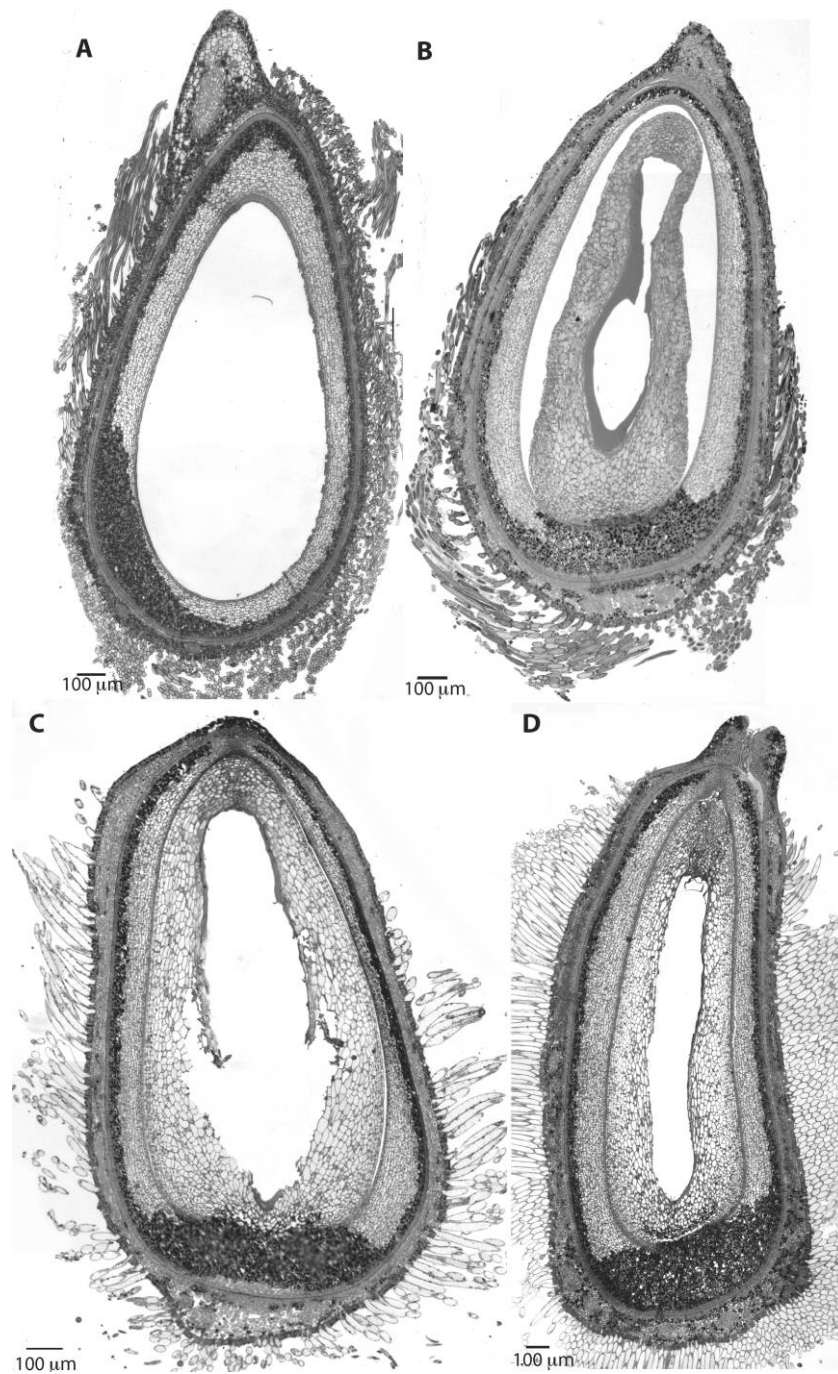
**Section preparation.** For each taxon, 2- $\mu$ m-thick longitudinal sections were taken from the middle of five ovules and seeds for each day of development (five median sections per species per day; fig. 2) using glass knives on a Reichert Ultracut S ultramicrotome. Sections were stained using 1% toluidine blue 0 with 1% Borax in deionized water and mounted in Permount.

**Image collection, processing and data collection.** Images of sections were obtained using a Zeiss Axioplan 2 system fitted with an HRC Axiocam digital camera. The magnification was calibrated so that exact bar scales were placed on each image to allow for linear measurements. 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). Five fiber lengths were measured from each of five median sections per day per species ( $5 \times 5 = 25$  measurements per day per species), with the exception of some 0- and 1-dpa fibers for which there was insufficient material. For all days, only fibers that had clearly defined tips were included in the measurements. Coat cover was determined by drawing a line from the location of the last fiber initials at the micropylar end of the seed and measuring from the center of the chalazal end to the center of the line. This distance was divided by the total distance from the chalazal to the micropylar end and multiplied by 100 to determine the percent of cover (fig. 2).

Fiber shape data was obtained using geometric morphometric methods (Bookstein 1991; Rohlf and Marcus 1993; Adams et al. 2004). These methods use a set of homologous landmarks



**Fig. 1** Phylogenetic history of polyploidization and domestication in *Gossypium*.



**Fig. 2** Median longitudinal sections of 5-d-postanthesis ovules illustrating the extent of fiber cover on the surface of the seed. A, *Gossypium raimondii*; B, *Gossypium hirsutum* var. *yucatanense*; C, *Gossypium herbaceum* var. *Wagad*; D, *G. hirsutum* var. *Maxxa*.

rather than sets of linear distances as the starting point of a shape analysis. From these, nonshape 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

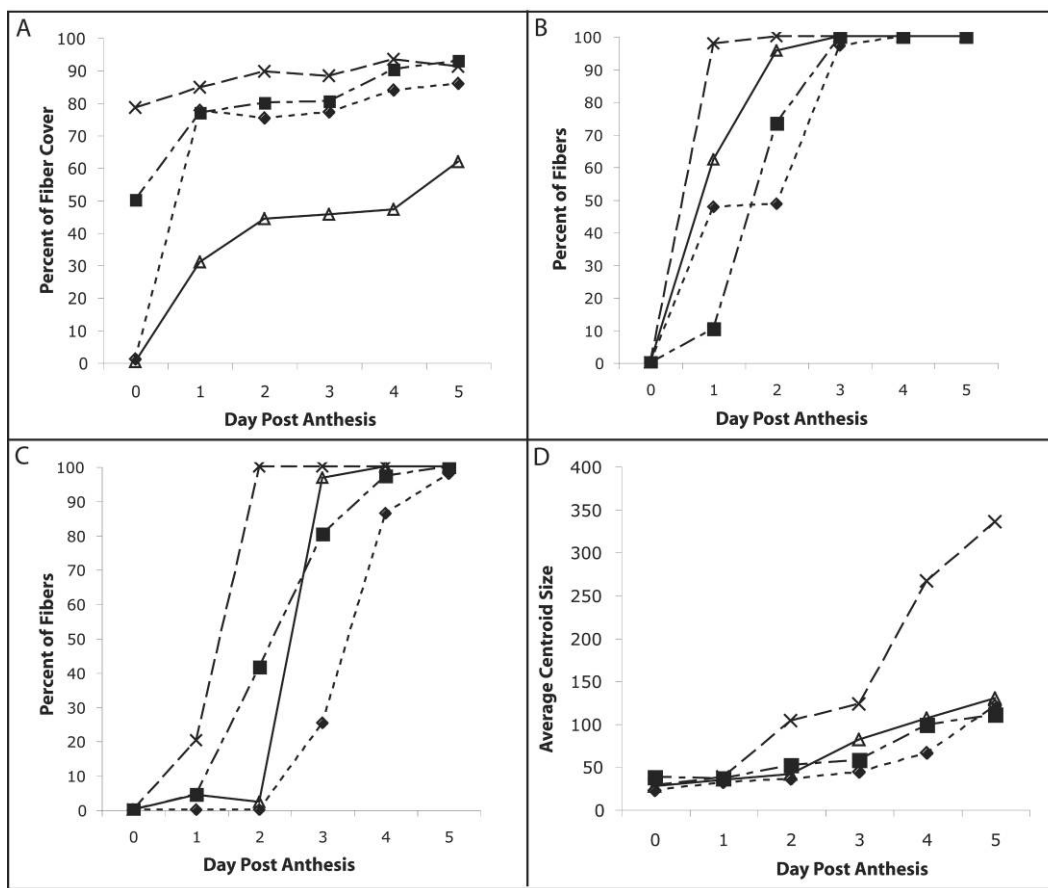
landmarks and the center of mass for each specimen (Bookstein 1991). Centroid size was measured using the same landmarks as the shape data in TPSRELW (Rohlf 1999). From the aligned coordinates, a set of shape variables can be generated from the thin-plate spline (Bookstein 1991), which preserve the geometry of the structure being studied. The resulting shape variables then can be used in statistical analyses and 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; Adams 2004). For this study, 10 landmarks are used to represent the outline of the fibers (fig. 3A). Because this is a comparison of outline shapes, there are no distinct points, such as an eye or the intersection of two bones, that 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 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; Bookstein et al. 1999). Nuclei and vacuole positions also were determined (fig. 3B, 3C).

**Statistical analyses.** 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 version 5.0.1.2 (SAS 2003), and the correlation between length and centroid size was calculated using Microsoft Excel X.

Shape data were analyzed using NTSYS-pc (Rohlf 2000) and JMP version 5.0.1.2. Six dimensions of shape were found to contain nontrivial shape information and were used in subsequent analyses, which included principal component analyses (PCA) for an initial exploration of the data, multivariate ANOVA (MANOVA) for group comparisons, and pairwise tests to determine which species and days differed in shape. Finally, graphical depictions of mean shapes for 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 most important factor underlying 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) has been more important than phylogeny in determining 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 fi-



**Fig. 3** A, Percent fiber cover on the seed; B, percent fiber nuclei more distal than those of other epidermal nuclei; C, percent fibers with a vacuole; D, average fiber centroid size each day of development. Diamond = *Gossypium herbaceum* var. Wagad; triangle = *Gossypium hirsutum* var. *yucatanense*; X = *G. hirsutum* var. Maxxa; square = *Gossypium raimondii*.

ber size, these predictions were tested by comparing the difference of mean trichome 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 Mahalanobis distance (MD) used in the pairwise comparison of fiber shape described previously. 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.

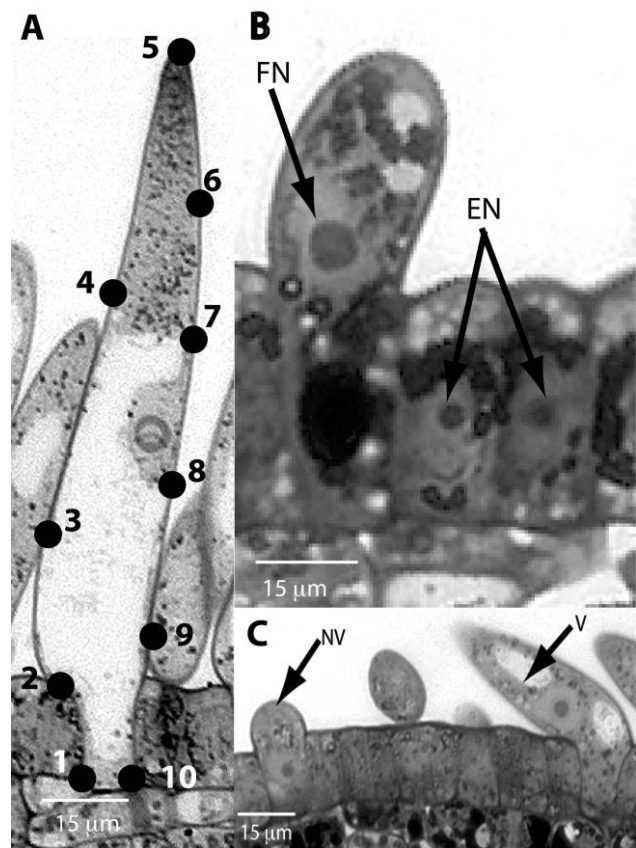
To determine which hypothesis is more likely, a randomization test was performed, following the protocol of Adams and Rohlf (2000). In this procedure, the difference in the observed test values (P-D and MDP-MDD) was first calculated. Next, specimens were randomly assigned to groups, and P, D, MDP, and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

Finally, studies vary in their use of the term fiber or trichome. In our study, we will consistently use fiber from the earliest stage of initiation to later stages of development.

## Results

**Location of fiber initials and fiber cover on the seed epidermis.** The most striking difference between the four taxa studied was the amount of fiber cover on the seed coat (fig. 2). Fiber initials (epidermal cells visible above epidermal surface) were 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, that no new fiber initials were found after 1 dpa at the chalazal end of *Gossypium herbaceum* var. Wagad, *Gossypium hirsutum* var. *yucatanense*, or *G. hirsutum* var. Maxxa, but new fiber initials were observed at the chalazal end of *Gossypium 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. 3A). Seeds of *Gossypium raimondii* and *G. hirsutum* var. Maxxa were covered halfway or more at anthesis, while at this same stage, those of *G. hirsutum* var. *yucatanense* and *Gossypium herbaceum* var. Wagad had almost no initials, with those present found at the extreme chalazal end. Both of these latter species had 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 attained more than 60% fiber cover in the 6 d studied (0–5 dpa).

**Fiber nucleus location.** The position of the nucleus in fiber initials changes during development from the same position as in nonfiber epidermal cells to the middle of the elongating fiber cell (fig. 4B). This change became evident at about 1 dpa in all species, but subsequent migration patterns varied somewhat among species (fig. 3B). The movement of the nucleus to the central position in fibers of *G. herbaceum* var. Wagad was not complete until 4 dpa, whereas the nuclei in *G. hirsutum* var. Maxxa were mostly central by 2 dpa. Nuclei in fibers of the two wild entities, *G. raimondii* and *G. hirsutum* var. *yucatanense*, followed differ-

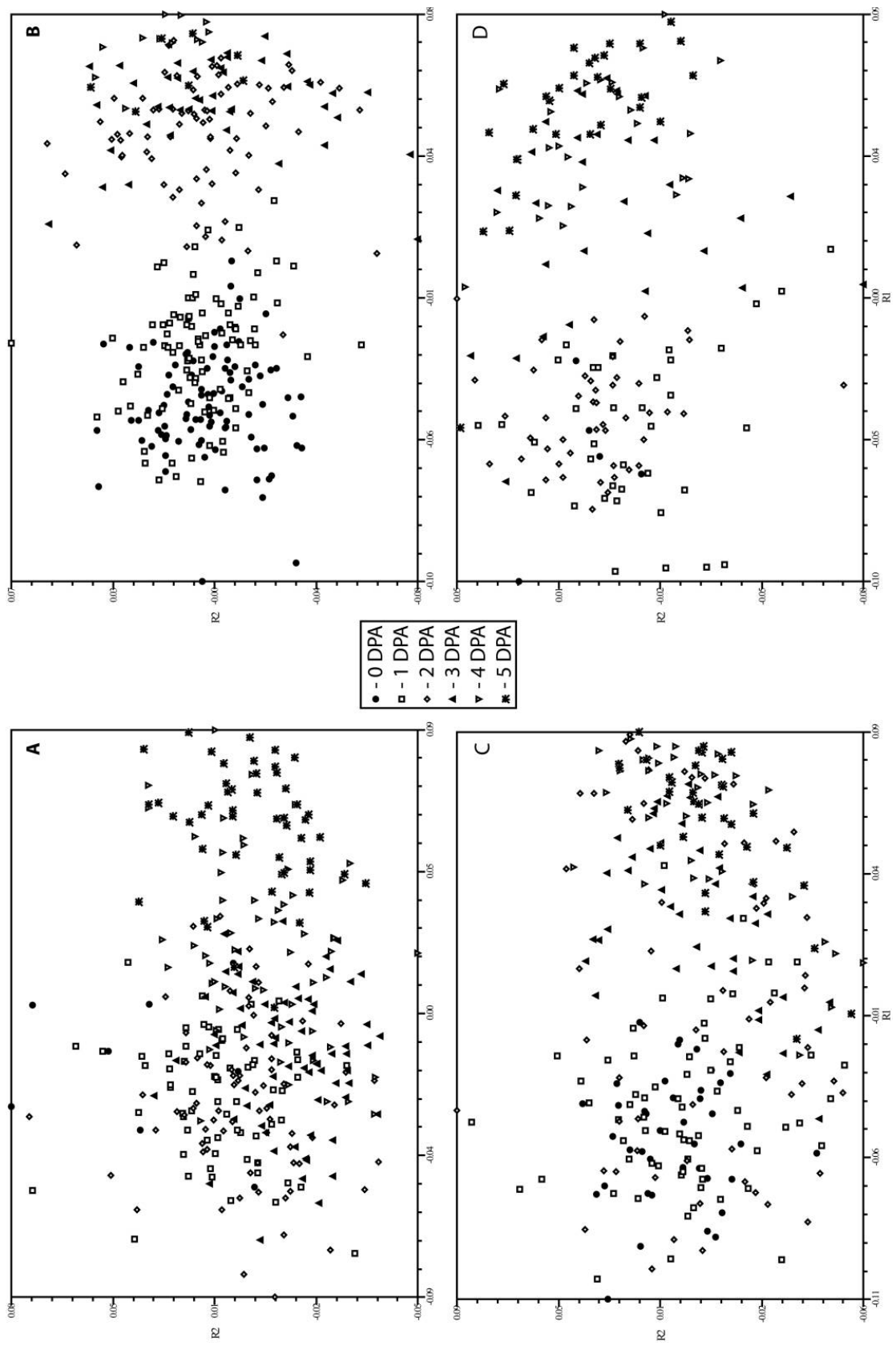


**Fig. 4** A, Locations of 10 fiber landmarks (dots) used to analyze the outline shape of the fibers. B, Diagram of fiber initial nucleus position in fiber cells; FN = fiber nucleus; EN = epidermal nucleus. C, Vacuolate (V) and nonvacuolate (NV) cotton fibers.

ent developmental trajectories with respect to nuclear migration, but nearly all cells had centrally located nuclei by 3 dpa.

**Fiber vacuolation.** The emergence of the vacuole in fiber initials (fig. 3C) was more variable among species than was the location of the nucleus. The vacuole was first observed in fibers of *G. hirsutum* var. *yucatanense* and *G. raimondii* at 1 dpa and was observed in all fibers by 4 and 5 dpa in these two species, respectively. In *G. herbaceum* var. Wagad, vacuoles were not observed until 3 dpa and had not developed in some fibers by 5 dpa, suggesting that vacuole formation continues beyond 5 dpa in this species. Vacuoles were initially present in fibers of *G. hirsutum* var. Maxxa at 1 dpa and in all fibers by 2 dpa.

**Centroid size and fiber length.** Length and centroid size were highly correlated ( $r = .968$ ), which is to be expected from a cell that is expanding in length and presumably in overall size. Fiber growth in *G. raimondii*, *G. herbaceum* var. Wagad, and *G. hirsutum* var. *yucatanense* followed similar trajectories and final lengths achieved (104–116 μm) by 5 dpa (fig. 3D). One difference in growth between these species, however, was the timing of the elongation burst (40 μm in 1 d), which occurred 3–4, 4–5, and 2–3 dpa, respectively, in *G. raimondii*, *G. herbaceum* var. Wagad and *G. hirsutum* var. *yucatanense*. Fibers from *G. hirsutum* var. Maxxa increased in length an average of 70 μm



**Fig. 5** Principal component analysis of the six axes of shape information. A, *Gossypium herbaceum* var. Wagad; B, *Gossypium hirsutum* var. Maxxa; C, *Gossypium raimondii*; D, *G. hirsutum* var. *yucatanense*.

**Table 1**

**MANOVA and MANCOVA Tests of Fiber Shape and Size**

	df1	df2	F	Pr > F
MANOVA:				
Species	15	2974	31.985	<.0001
Day	25	4002	52.806	<.0001
Species × day	75	5163	9.5038	<.0001
MANCOVA:				
Species	15	3018	29.81	<.0001
Day	5	1093	373.11	<.0001
Species × day	15	3018	24.45	<.0001

Note. The Wilks's λ test was used for all fiber data sets.

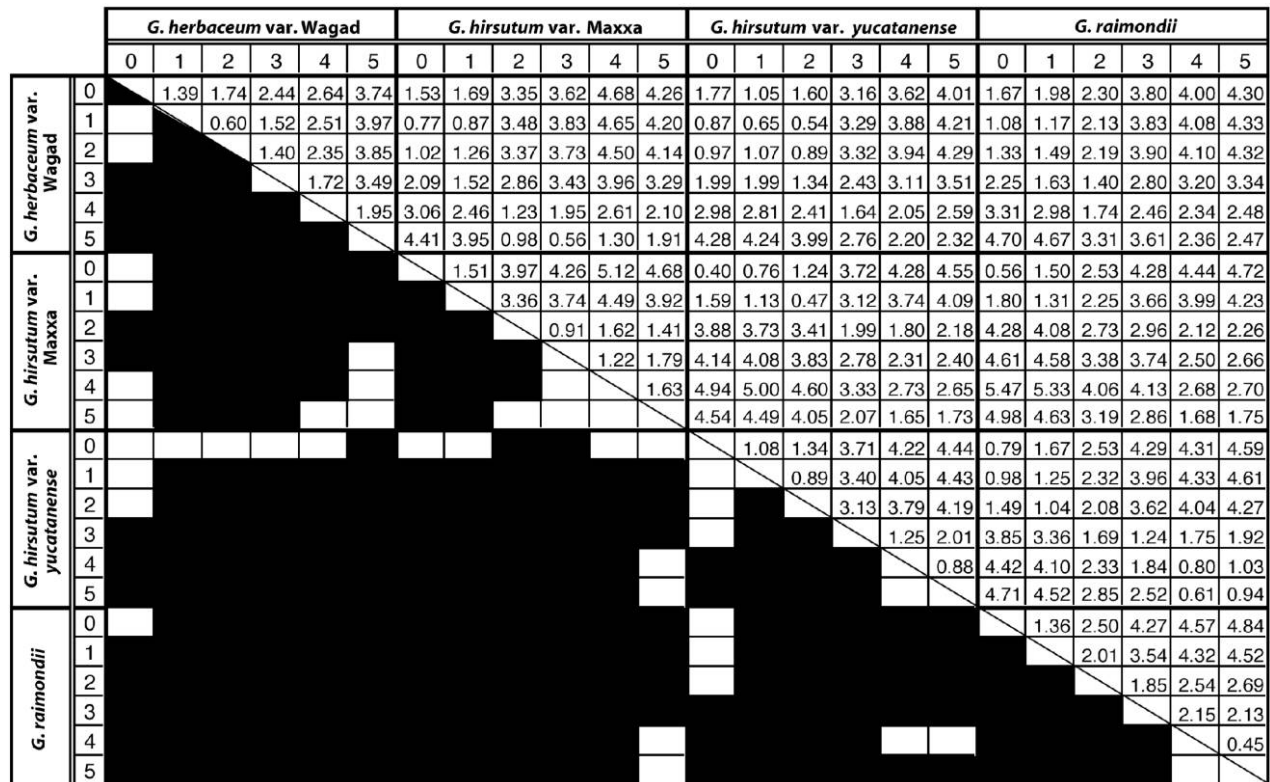
each day between 1–2, 3–4, and 4–5 dpa, achieving an average length of 322 μm by 5 dpa.

*Fiber shape and size.* PCA analysis of fiber shape indicated that within species, fibers from any single day had shape characteristics that largely clustered together relative to fibers from other days (fig. 5). Further analysis using MANOVA (table 1) indicated that shape differed significantly between species and between days. Pairwise multiple comparisons of shape between all days and species revealed that nearly all day-species groups were significantly different from one another in shape (fig. 6). Those comparisons that were not significant were primarily found for combinations in which one

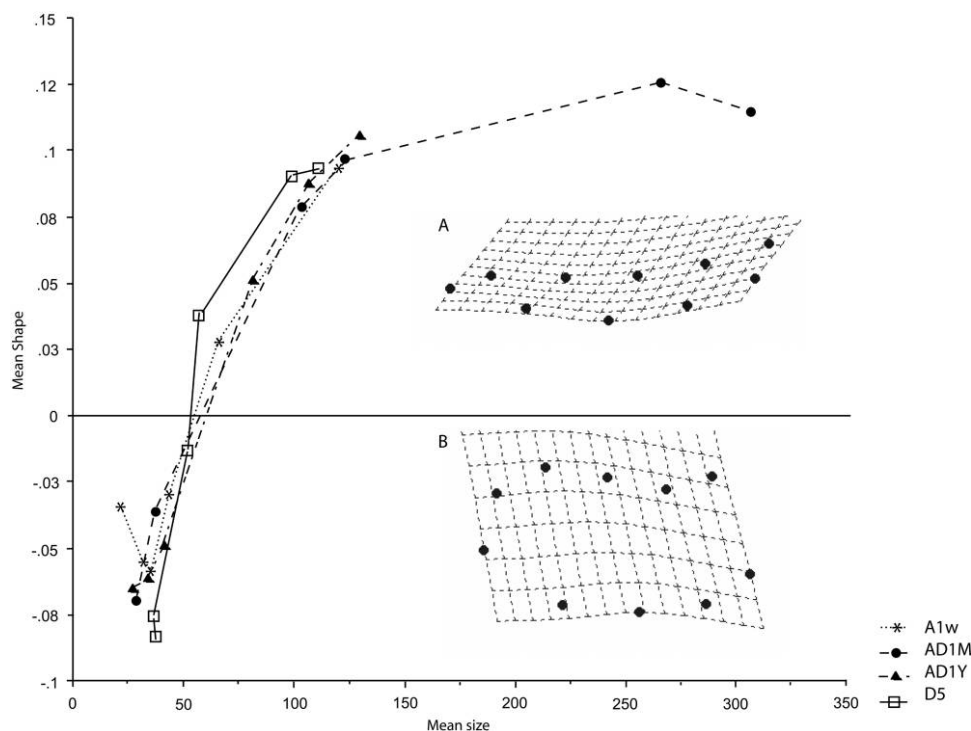
of the two species/days being compared had a small sample size (<5 fibers/d).

In general, differences in fiber shape followed the expected pattern: fibers began as relatively short and round epidermal initials, and as they developed, they became longer and pointed (fig. 7). While this pattern was consistent for all species, there was some variation in the developmental profile. Fibers of *G. raimondii* were short with rounded tips at 0 and 1 dpa and became long and pointed 4 and 5 dpa but were rather variable in these features between 2 and 3 dpa. During this time, the population of fiber cells included a combination of short and round as well as long and pointed fibers. Fibers of *G. hirsutum* var. *yucatanense* began to taper at 3 dpa, with the point becoming obvious by 4 dpa, whereas the fibers of *G. herbaceum* var. *Wagad* began to taper at 4 dpa, and *G. hirsutum* var. *Wagad* fibers were distinctly pointed by 3 dpa. An analysis using MANCOVA of fiber shape and size revealed that shape and size trends were highly significant (table 1). As fibers increased in size, the shape changed from round to pointed. The timing of both varied by species, as described previously.

*Process test.* To address whether shared ancestry or shared domestication pressure has been more influential in shaping fiber development and morphology, randomization tests were performed as described. These tests demonstrated that phylogenetic history played a more important role for fiber size and shape during most of the developmental period under



**Fig. 6** Pairwise comparisons of fiber shape. Numbers indicate the generalized distance between shapes. The solid boxes indicate a significant difference in fiber shape.



**Fig. 7** Relationship between mean fiber shape and mean fiber size. Warp grids (A, B) show the average shape of fibers on the given day postanthesis. Fibers above a mean size of 0 are pointed (A) while those with a mean shape of less than 0 are rounded (B). A key to taxa is provided in the lower right portion of the figure, where A1w denotes *Gossypium herbaceum* var. Wagad, AD1M and AD1Y refer to *Gossypium hirsutum* var. Maxxa and *Gossypium hirsutum* var. *yucatanense*, respectively, and D5 designates *Gossypium raimondii*.

study (table 2). At 5 dpa, however, there was weak (nonsignificant) support for domestication being more influential in shape.

**Fiber development in *G. raimondii*.** *Gossypium raimondii* had the most variable fiber development among the four taxa studied. On the day of anthesis, fiber initials as well as longer, older fibers were present (fig. 8A). These findings were consistent with those reported by Applequist et al. (2001). The day after anthesis (1 dpa, fig. 8B), nuclei were near the center of the elongated fiber cell, the vacuole becomes obvious, and the fibers have retained their short, round shape. At 2 dpa there was a mixture of short and elongated fibers with nuclei in various positions (fig. 8C). By 3 dpa (fig. 8D), the fibers undergo a burst of elongation as all fibers have begun to taper. Elongation continues for the next 2 d (fig. 8E, 8F). In addition, new fiber initials similar to those found at 0 dpa are present 2–5 dpa (fig. 8C–8E) throughout the coat of the seed, unlike the other taxa which only have fiber initials toward the micropylar end. This suggests a continued presence of fibers at different stages of development. Thus, early fiber development in this species is highly asynchronous.

**Fiber development in *G. hirsutum* var. *yucatanense*.** In this wild form of the species that gave rise to modern Upland cotton, only a few short, round-tipped fiber initials were present at the chalazal end of the ovule on the day of anthesis (fig. 9A). The next day revealed an increased number of initials, a change in position of the nucleus, and the appearance of the vacuole (fig. 9B). By 2 dpa (fig. 9C), fibers began to elongate,

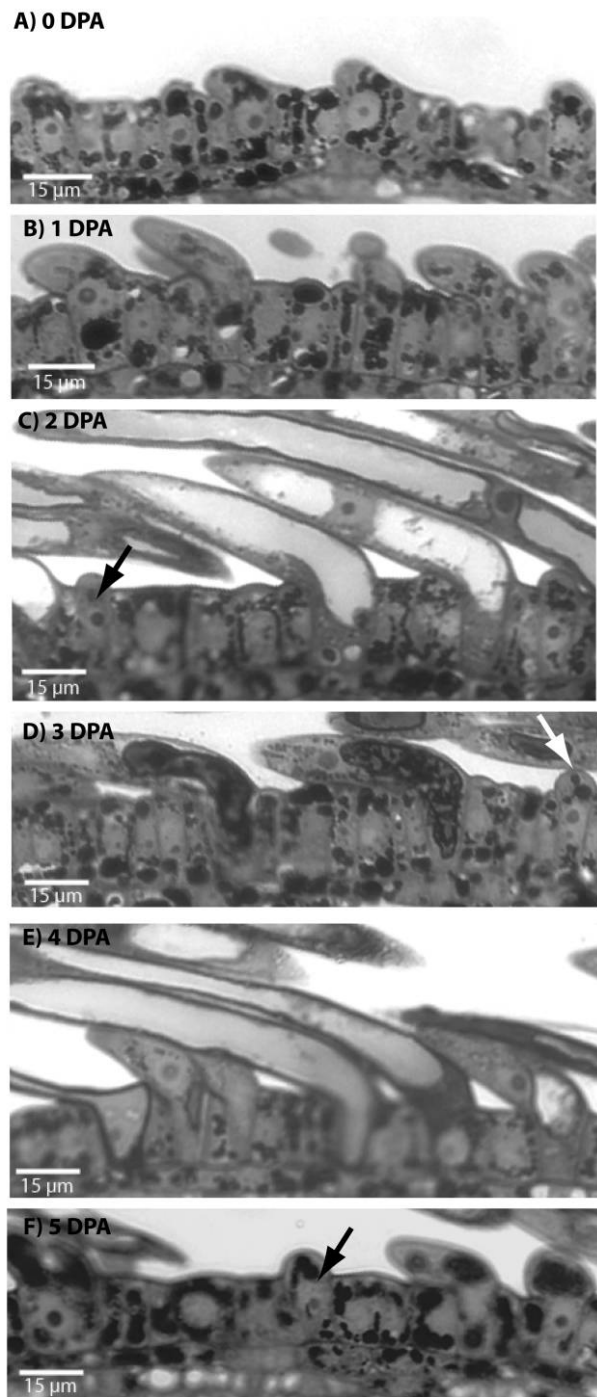
and by 3 dpa (fig. 9D), the fibers started to taper, becoming pointed during 4 and 5 dpa (fig. 9E, 9F). The presence of fiber initials after 2 dpa was observed solely in the cells toward the micropylar end of the seed.

**Fiber development in *G. herbaceum* var. *Wagad*.** As with *G. hirsutum* var. *yucatanense*, only a few initials were apparent on the day of anthesis at the chalazal end of the ovule (fig. 10A). The next day (fig. 10B), the nuclei changed posi-

**Table 2**

Days postanthesis	Fiber size		Fiber shape	
	Observed P-D	P value	Observed MDP-MDD	P value
0	-4.293	.0003	-1.636	.0001
1	-2.678	.0008	.198	.2725
2	-5.218	.1144	-1.118	.0001
3	-37.717	.0001	-.941	.0002
4	-40.209	.0012	.184	.3269
5	-8.961	.2711	.549	.0563

Note. P = phylogeny; D = domestication; MD = Malhalanobis distance. A negative observed P-D or MDP-MDD value indicates support for the hypothesis that shared history (phylogeny [P]) plays a more important role than does shared domestication (D). A positive observed P-D or MDP-MDD value indicates support for the converse, namely, that domestication pressures have played a more important role in determining either fiber size or shape.



**Fig. 8** Fiber development in *Gossypium raimondii*. Arrows indicate fiber initials. DPA = days postanthesis.

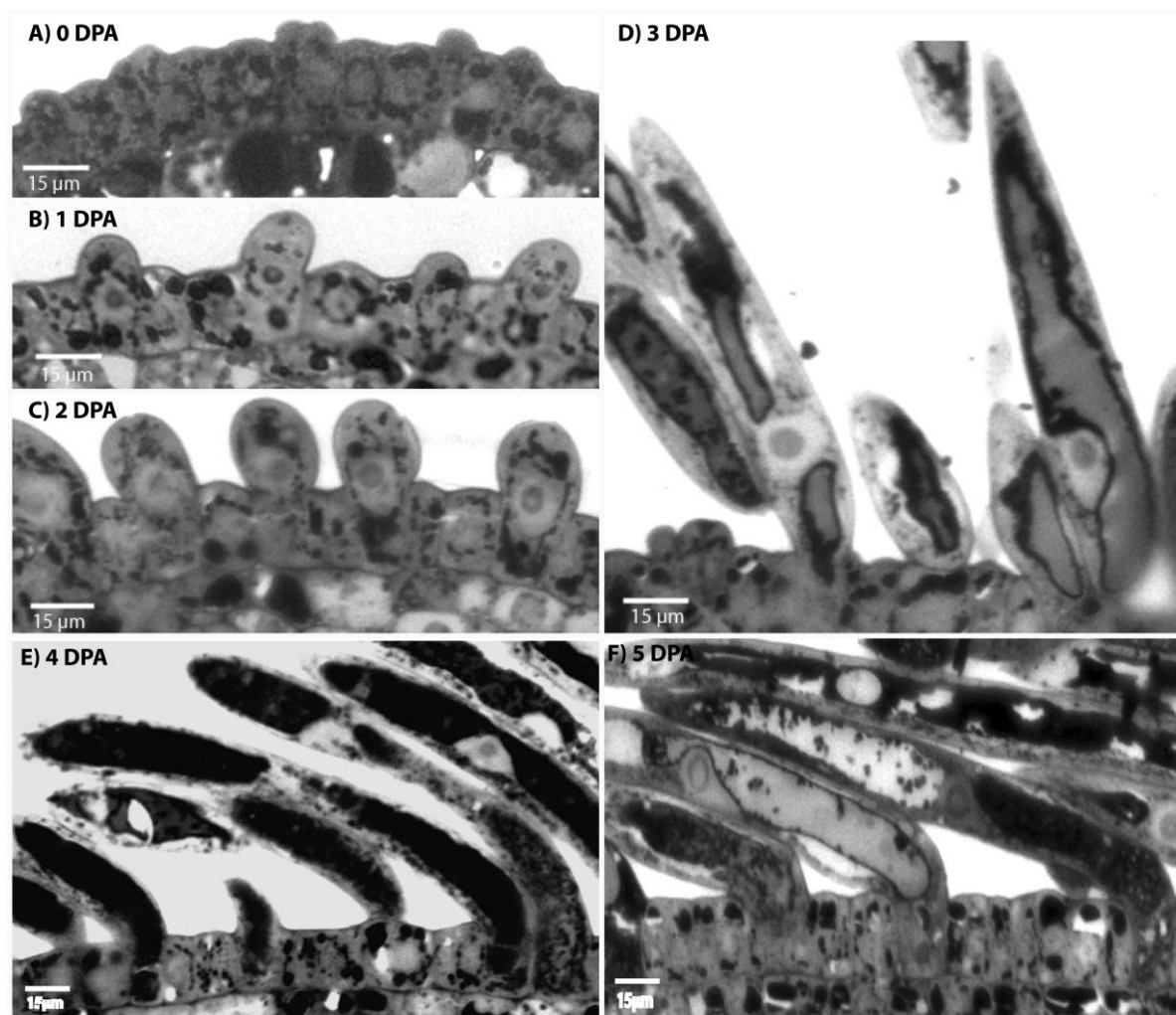
tion and the fibers slowly elongated (fig. 10C, 10D), remaining short and round-tipped until 4 dpa (fig. 10E), when they suddenly increased in size and became distinctly tapered (fig. 10F). There was no evidence of initials at the chalazal end after 1 dpa, although they continued to be found every day toward the micropylar end of the seed.

*Fiber development in G. hirsutum var. Maxxa.* Development of fibers in *G. hirsutum* var. Maxxa (fig. 11) was noticeably synchronous over the entire seed surface. On the day of anthesis (fig. 11A), almost 85% of the ovule coat was covered with fiber initials, and the initials arising after 0 dpa were located at the micropylar end of the ovule. At 1 dpa (fig. 11B), the nucleus in each fiber cell changed position from near the base of the cell to near the center of the cell, and the vacuole became prominent. The first of 3 d of growth, between 1 and 2 dpa, occurred at a rate of more than 70  $\mu\text{m}/\text{d}$ . At 2 dpa (fig. 11C), the fibers began to taper with fibers becoming distinctly pointed by 3 dpa (fig. 11D). Growth continued at a rapid rate for the next 3 d (fig. 11E, 11F). The only initials present after 0 dpa were located at the micropylar end of the seed epidermis.

## Discussion

*Early stages of fiber development in wild cottons.* Comparisons between fibers from the wild D-genome species, *Gossypium raimondii*, and those of the wild tetraploid species *Gossypium hirsutum* var. *yucatanense* reveal several differences (table 3). These include the timing of the growth spurt, which occurs a day earlier in the tetraploid (fig. 3D); the greater asynchrony in fiber development and higher level of secondary fiber development in *G. raimondii* than in *G. hirsutum* var. *yucatanense* (figs. 8, 9); and the amount of seed epidermis covered by developing fibers, which is higher in the diploid than in the tetraploid (fig. 2). It is unclear whether the lower fiber cover in *G. hirsutum* var. *yucatanense* is due to a delay in the formation of new initials toward the micropylar end or whether instead there are simply fewer fibers produced. Given the limitations in our sampling, the data presented here suggest that in the wild tetraploid, which contains both an A genome and a D genome (fig. 1), the D-genome genetic program for early high seed coverage is suppressed. Evaluation of this possibility would at the minimum require a comparable examination of early fiber development in wild A-genome cotton (*Gossypium herbaceum* var. *africanum*), as well as in other accessions and temperature regimes (Xie et al. 1993).

*Early stages of fiber development in cultivated diploid and cultivated tetraploid cottons.* The cultivated varieties *G. herbaceum* var. Wagad (diploid) and *G. hirsutum* var. Maxxa (tetraploid) have several notable differences in development (table 3). Fibers of *G. hirsutum* var. Maxxa exhibit the greatest degree of developmental synchrony among the accessions studied, with respect to nuclear position, vacuole appearance, growth trajectories, and shape changes (figs. 3, 4, 7). These observations suggest that many if not all of these fiber properties have been unconsciously selected during the domestication process and that they contribute to making upland cotton the taxon of choice for world cotton commerce. In contrast, the cultivated but agronomically inferior Old World diploid cotton, *Gossypium herbaceum* var. Wagad, has trichomes that develop more slowly than those of even the wild species *G. raimondii*; the vacuole is not evident until 3 dpa, and the change in shape and large length increase do not occur until 4 dpa (fig. 3). This suggests a later period of maximal elongation rate resulting in a shorter elongation period



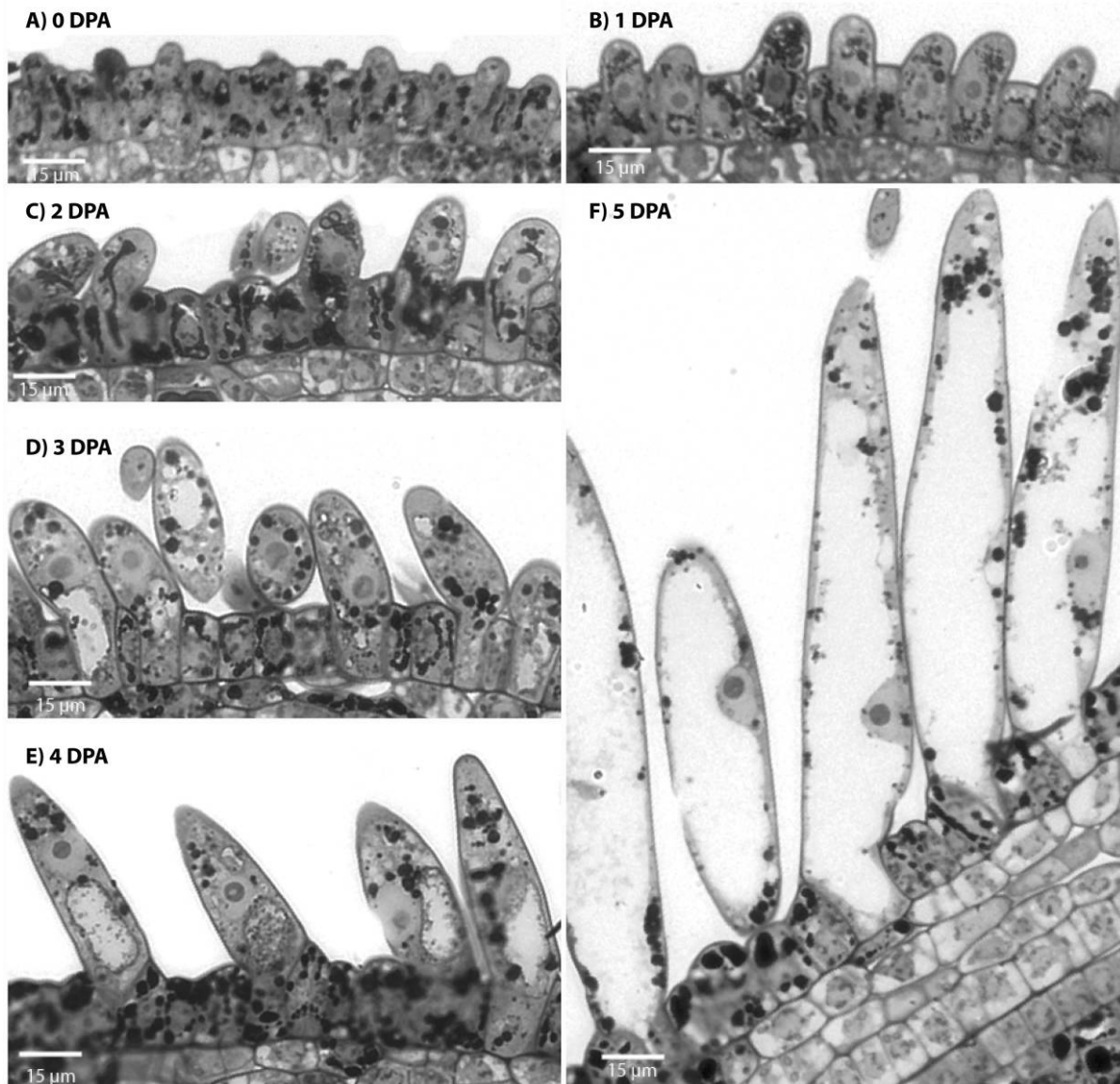
**Fig. 9** Fiber development in *Gossypium hirsutum* var. *yucatanense*. DPA = days postanthesis.

for the cultivated diploid than tetraploid cotton, although study of additional cultivars and growth conditions are required to verify this observation.

Comparison of wild and cultivated tetraploid cottons may help provide insights into the effects of domestication on early fiber development, as noted above for the pronounced synchrony in fiber development in *G. hirsutum* var. Maxxa. In this respect, we note that the wild accession has a reduced fiber cover relative to cultivated upland cotton (table 3), and that there are significantly fewer fiber initials on the day of anthesis in the wild tetraploid. This may reflect a delay in the developmental program of wild cotton, or alternatively, perhaps in cultivated plants, the domestication process resulted in selection for a fuller cover of fibers. Together, increased fiber cover and synchronized development in cultivated versus wild cotton is suggested here to have facilitated both yield and uniformity of the crop.

*Inference of evolutionary process.* By including two different cultivated species as well as two wild species, this study provides the opportunity to identify whether recent shared ancestry or domestication pressures have been more responsi-

ble for influencing present developmental patterns and morphology. In contrast to the data for fiber cover (fig. 3A) and synchronization of developmental features at the cellular and subcellular levels (table 3), where domestication is inferred to have played a large role at least in tetraploid cotton, fiber shape and size are most strongly influenced by shared ancestry (fig. 6), particularly in the first few days postanthesis. 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 fibers. However, it seems that developmental alterations that resulted from human-mediated selection during the 5000-yr history of cotton domestication also have played 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* (table 3). 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

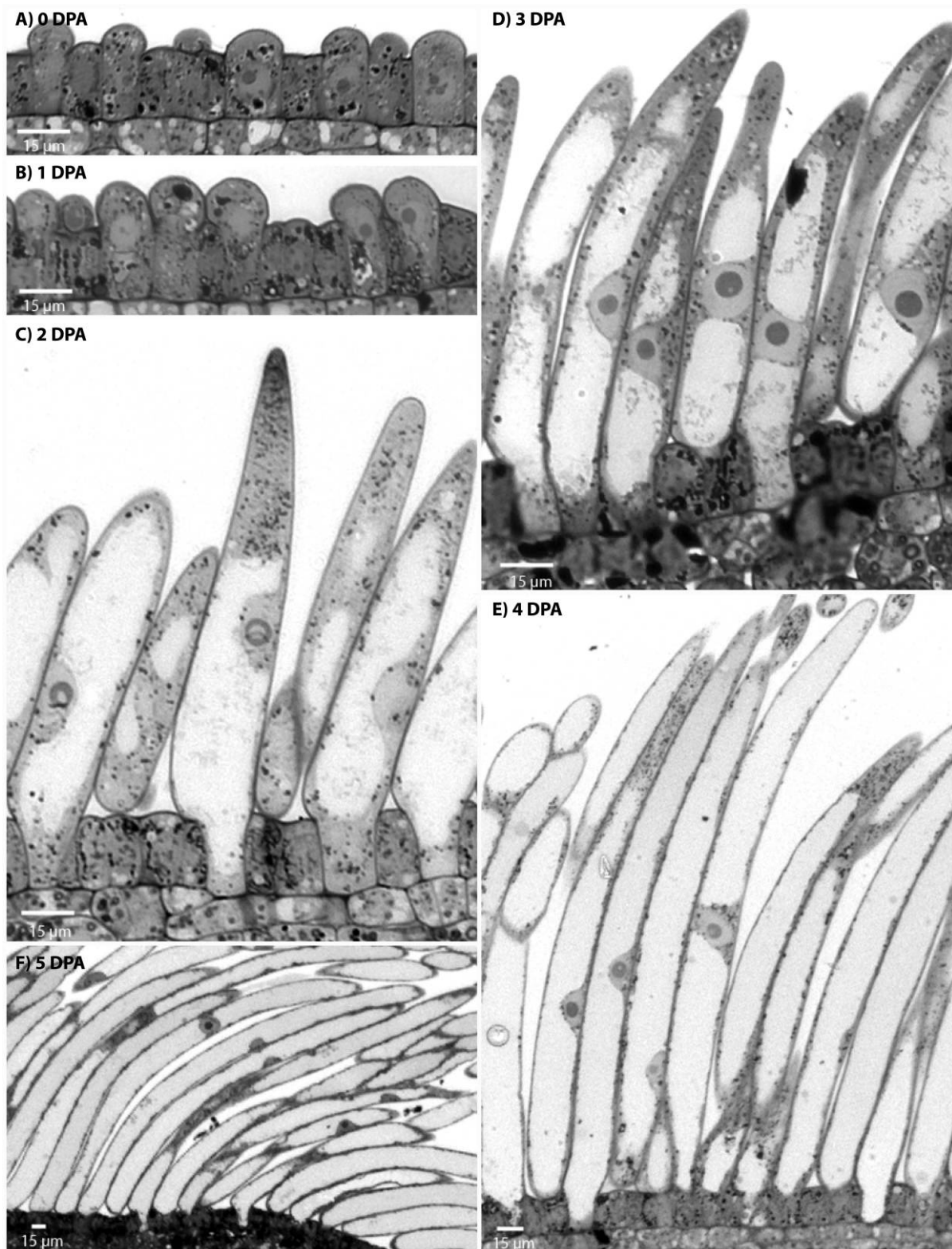


**Fig. 10** Fiber development in *Gossypium herbaceum* var. Wagad. DPA = days postanthesis.

ploidy level is removed as a confounding variable. In this respect, polyploidy is known to often result in size differences of various cells (Stebbins 1971; Melaragno et al. 1993; Master-son 1994; Kelman et al. 1999; Kadota and Niimi 2002; Sugiyama 2005). In addition, it would be useful to examine additional accessions of the species studied here, both under the same environmental conditions we used as well as under altered temperature regimes. The latter might be particularly informative given the effects of temperature on early cotton fiber growth.

**Future directions.** The descriptions provided here of early fiber development in wild and cultivated cottons help build a foundation for future studies aimed at understanding the origin of the extraordinary diversity of epidermal seed fibers found in *Gossypium*. One key question concerns the nature of a second

type of fiber, which is found on cultivated cottons and is called “fuzz fiber.” Understanding the genesis of this layer requires a broader sampling of taxa and accessions as well as extending this 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 that may be fuzz initials (Lang 1938; Joshi et al. 1967; Berlin 1986). In addition, the inclusion of a wild A-genome plant (*G. herbaceum* subsp. *africanum*), the F-genome species *Gossypium longicalyx* (which is 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).



**Fig. 11** Fiber development in *Gossypium hirsutum* var. Maxxa. DPA = days postanthesis.

**Table 3**  
**Timing (Days Postanthesis [dpa]) of Developmental Cellular Events in Cotton Fibers of Different *Glossynium* Species**

dpa	<i>G. herbaceum</i> var. Wagad	<i>G. raimondii</i>	<i>G. hirsutum</i> var. <i>yucatanense</i>	<i>G. hirsutum</i> var. Maxxa
1		Vacuole appears Nuclei migrate	Vacuole appears Nuclei migrate	Vacuole appears Nuclei migrate
2	Nuclei migrate			Largest centroid size jump ( $\geq 40$ ) Pointed tip appears
3	Vacuole appears	Pointed tip appears	Largest centroid size jump ( $\geq 40$ ) Pointed tip appears	
4	Pointed tip appears	Largest centroid size jump ( $\geq 40$ )		
5	Largest centroid size jump ( $\geq 40$ )			
Percent fiber cover	85.9	92.9	61.7	91.1
Fiber length (mm) at 5 dpa <sup>a</sup>	4 <sup>b</sup>	3	1 <sup>c</sup>	2 <sup>d</sup>

<sup>a</sup> Length data from Applequist et al. (2001).

<sup>b</sup> *G. herbaceum* A<sub>1</sub>73.

<sup>c</sup> *G. hirsutum* TX2094.

<sup>d</sup> *G. hirsutum* TM1.

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