Comparative development of fiber in wild and cultivated cotton

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SUMMARY One of the most striking examples of plant hairs is the single-celled epidermal seed trichome of cultivated cotton. The developmental morphology of these commercial “fibers” has been well-characterized in Gossypium hirsutum, but little is known about the pattern and tempo of fiber development in wild Gossypium species, all of which have short, agronomically inferior fiber. To identify developmental differences that account for variation in fiber length, and to place these differences in a phylogenetic context, we conducted SEM studies of ovules at and near the time of flowering, and generated growth curves for cultivated and wild diploid and tetraploid species. Trichome initiation was found to be similar in all taxa, with few notable differences in trichome density or early growth. Developmental profiles of the fibers of most wild species are similar, with fiber elongation terminating at about two weeks post-anthesis. In contrast, growth is extended to three weeks in the A- and F-genome diploids. This prolonged elongation period is diagnosed as a key evolutionary event in the origin of long fiber. A second evolutionary innovation is that absolute growth rate is higher in species with long fibers. Domestication of species is associated with a further prolongation of elongation at both the diploid and allopolyploid levels, suggesting the effects of parallel artificial selection. Comparative analysis of fiber growth curves lends developmental support to previous quantitative genetic suggestions that genes for fiber “improvement” in tetraploid cotton were contributed by the agronomically inferior D-genome diploid parent.

INTRODUCTION

The epidermal seed hairs of the cultivated cotton species provide a striking example of nearly unidirectional, single-celled growth. In the last several decades, ultrastructural analysis (reviewed in Ryser 1999) has generated a reasonably complete understanding of the morphological development of these trichomes (the cotton “fibers” of commerce). At the time of flowering, approximately one in four epidermal cells on each ovule differentiates into a single-celled trichome (Stewart 1975). Fiber initials appear on the day of anthesis as single-celled, spherical protrusions from the ovular surface (Stewart 1975; Ryser 1999). Differentiation begins near the chalazal end of the ovule, progressing later toward the micropylar end (Lang 1938; Stewart 1975). On the first and second days after anthesis, the spherical fiber initials begin to expand lengthwise, bending and growing toward the micropyle; by three days after anthesis, the fibers are long enough that they begin to clump together and their tips taper (Stewart 1975). Thereafter, the rate of fiber elongation in fertilized ovules increases steadily (Van’t Hof 1998), reaching a maximum around two weeks after anthesis (Schubert et al. 1973), and decreasing rapidly after three weeks post-anthesis. The length of the elongation period is highly dependent upon environment (Quisenberry and Kohel 1975), but generally lasts from 24 to 34 days. Secondary cell-wall deposition then strengthens the fiber; the thickness and evenness of this cellulose layer may affect the texture and conformation of the mature fiber (Hutchinson et al. 1945). Whereas the traditional view was that secondary cell-wall deposition began only after fiber elongation had ceased, studies of fiber length and weight over time and transmission electron microscopy have demonstrated clearly that the secondary cell-wall thickening phase overlaps with the elongation phase by up to 10 days (Schubert et al. 1973; Quisenberry and Kohel 1975). By 50 days after flowering, seeds of most cultivars have matured and the fruits dehisce.

The foregoing description is applicable to the most important of the four cultivated species of Gossypium, namely, G. hirsutum. Considerably less information exists on the other three cultivated species, and virtually no study has been made of fiber development among the numerous wild species in the genus, although the morphology of fibers from mature seeds is adequately described (Fryxell 1979). Inasmuch as these wild species contain the best modern representatives of the progenitors of the domesticated species, comparative analysis of fiber morphology may yield insights into the key evolutionary steps involved in the morphological and,
ultimately, the underlying genetic transformations that led to modern, agronomically improved cotton. The purpose of this article is to describe our initial findings in this analysis.

The organismal context for this study is shown in Fig. 1. Gossypium is one of many genera of the Malvaceae in which seed trichomes are present. Following its origin perhaps 20 Myr BP (Wendel and Albert 1992; Seelanan et al. 1997), Gossypium diversified into approximately 50 species in tropical and semitropical arid to seasonally arid environments. This global radiation was accompanied by cytogenetic differentiation, such that different “genome groups” (A through G, and K) are now recognized based on variation in chromosome size and meiotic pairing behavior in interspecific hybrids (Endrizzi et al. 1985; Stewart 1995). As illustrated, each diploid (all n = 13) clade, as inferred from phylogenetic analysis of multiple molecular data sets (Wendel and Albert 1992; Seelanan et al. 1997), is congruent with the classically recognized genome groups. The wild diploids have varying degrees of seed pubescence, but these short (generally 1–10 mm) hairs are tightly adherent to the seed in most species (Fig. 2). Exceptions include G. australae and G. nelsonii, in which the fibers are straight and grow radially outward from the seed surface. Four different Gossypium species have been domesticated and transformed into fiber and seed oil plants (Wendel 1995). Two of these (G. arboreum and G. herbaceum) are A-genome diploids from the Old World, while the other two (G. hirsutum and G. barbadense) are AD-genome allotetraploids from the New World. Spinnable lint evolved in the ancestors of the modern A-genome diploids, representing the first stage in cotton fiber development from a wild ancestor. A second level of morphological innovation was precipitated by polyploid formation: following trans-oceanic dispersal of an A-genome species to the New World, hybridization between the immigrant A-genome species and a D-genome species (Endrizzi et al. 1985; Wendel et al. 1995) led to the evolution of the New World allotetraploids, the AD-genome species. Recent evidence (Jiang et al. 1998) shows that this biological recombination event is the molecular transformation that established the New World A-D allotetraploids. The taxa considered most similar to the progenitors of the allopolyploids are G. herbaceum (A-genome) and G. raimondii (D-genome), although there are other proposals (Endrizzi et al. 1985; Wendel et al. 1995). Subsequent to allopolyploid formation, the allopolyploids radiated into five modern species, including the commercially important species G. hirsutum (Upland cotton) and G. barbadense (Pima or Egyptian cotton), which now dominate world cotton commerce. DNA sequence divergence data suggest that the A-genome and D-genome groups diverged from a common ancestor 5 to 10 Myr BP, and that the two diverged diploid genomes became reunited via polyploidization 1 to 2 Myr BP (Fryxell 1979; Wendel 1989; Wendel and Albert 1992; Seelanan et al. 1997; Small et al. 1998). Each genome group represents a monophyletic assemblage of cytogenetically differentiated species. Branch lengths are arbitrary.

This organismal framework for wild and cultivated cotton species offers an opportunity to use a phylogenetically informed approach to discover the key evolutionary steps involved in the morphological transformations that led to modern cultivated cotton. In principle, interspecific differences in final fiber length may be due to several factors, including the duration of the growth period, itself possibly affected by date of fiber initiation or growth cessation, and the rate of fiber growth during the elongation phase. Here, we report ultrastructural and developmental results for seed trichomes for representative wild and cultivated cotton species, focusing on members of the A-, D-, and AD-genome groups (Figs. 1 and 2), but also including more distant diploid relatives. Our hope is that once an understanding is achieved of the interspecific differences in developmental profiles, the primary genes responsible for these growth differences may be inferred through the use of novel technologies (such as comparative analysis of microarray expression data), thereby providing insight into the genetic basis of morphological novelty. In addition, this research may have agronomic significance, in that insight into the mechanisms by which fiber quality has been improved in cultivated, relative to wild, cottons may offer clues to means of further crop improvement.
MATERIALS AND METHODS

Plant materials

Taxa examined in this study are shown in Table 1. These include three AD-genome allopolyploids (G. hirsutum TM1, the cytotgenetic and genetic experimental “standard” stock; G. hirsutum TX 2094, a wild accession of race yucatanense; and G. tomentosum, a wild Hawaiian Islands endemic); four A-genome diploids (two putatively wild [Wendel et al. 1989] accessions of G. herbaceum subsp. africam and two cultivated accessions of G. arboreum, a species known only as a domesticate); two D-genome representatives (G. raimondii, selected because it is the best model D-genome donor to allopolyploid cotton, and G. davidsonii, a species with seeds that appear nearly glabrous at maturity); and a single representative each of the B-genome (G. anomalum), C-genome (G. sturtianum), and F-genome (G. longicalyx) diploids. Plants were grown in the rooftop greenhouses of Bessey Hall, Iowa State University. Since the optimal greenhouse conditions for some of these species are unknown, a single set of growing conditions (14-h day length; diurnal/nocturnal temperatures of 80/60°F) was chosen based upon prior successful cultivation of several species under this regime.

Ultrastructural analysis

Ovules at various stages were dissected from immature ovaries, fixed in 3% glutaraldehyde, and stored in 100% ethanol under refrigeration until processing for SEM analysis or light microscopy. Ovules of each accession at 0 and 2 dpa (days post-anthesis) were examined using SEM, as were pre-anthesis ovules from several species. SEM specimens were prepared by critical point drying, followed by mounting on stubs, sputter coating, and SEM observation on a JEOL 5800 SEM at the Bessey Microscopy Facility. Material quality appeared to be improved if care was taken in critical point drying to avoid a rise in pressure to over 1250 psi. Ovules were generally better visualized if mounted on adhesive discs and heavily sputter coated without the use of conductive silver paint, although this allowed excessive charging of some large and hairy ovules. Most specimens were observed at an accelerating voltage of 10 kV and a working distance of 20 mm; images were stored as TIFF files.

Light microscopy

For measurements of fiber length, ovules were heated in deionized water to relax the fibers (Van’t Hof 1998). Fibers were combed out and measured under a dissecting microscope, using either an ocular micrometer for short fibers or a ruler in the case of longer fibers. The longest fibers on each ovule, typically found near the chalazal end, were measured. Fibers were measured at 2, 5, 7, 10, 15, 20, and 30 dpa; three to seven ovules were examined at each stage, and ovules from multiple capsules were used when possible. Gossypium arboreum AKA 8401 was harvested at 32 rather than 30 dpa, and no fertilized ovules of G. arboreum A2-47 were harvested at 7 dpa. Ovules could not be collected for several time points for G. anomalum, and hence growth curves are not reported for this species.

RESULTS

Observations of ovular surfaces during the initial stages of fiber development were made using scanning electron microscopy. Figure 3 shows whole-ovule and close-up examples of ovular surfaces in cultivated cotton species at anthesis, whereas similar views from selected wild species are shown in Figure 4. Figures 5 and 6, respectively, show ovular surfaces from wild and cultivated species at 2 dpa. A comparison of the panels in these figures demonstrates a principal finding of the SEM work, that is that the timing of fiber initiation is similar in these species, despite the taxonomic diversity encompassed in the study and the remarkable range in final fiber length and appearance (Fig. 2). This overall similarity is evidenced not only in both the shape and size of the fiber initials, but also in their apparent density (approximately one in four epidermal cells are fated to be trichomes), although the latter was not rigorously quantified.

Against this backdrop of relative evolutionary stability in the earliest stages of fiber initiation, we noted minor variations within and between species. For example, occasionally we encountered apparent delayed development, with little or no fiber elongation at 0 dpa and fibers remaining only slightly elongated by 2 dpa (Fig. 5C); however, this may be an artifact of environmental effects or lack of fertilization. With respect to the latter, we note that the taxa in which this pattern was most frequently observed (G. longicalyx and G. hirsutum TX2094) were also accessions that had the lowest reproductive success in our greenhouse. Thus, it may be that unfertilized ovules or ovules from plants in poor condition exhibit delayed developmental profiles at this stage. This suggestion is supported by the observation that although G. sturtianum initially appeared to display delayed development, examination of additional material (Figs. 4C and 4D, Figs. 6C and 6D) demonstrated typical development; it may be that the material initially studied came from unfertilized ovules.

A second type of variation evident from the SEM studies is that trichoblasts develop at varying rates on each ovule. Moreover, there may be taxonomic differences in the degree to which this type of variation exists. Ovules of G. raimondii, for example, were unique in initiating fiber development before anthesis on a small portion of the ovule, resulting in the presence of hairs of significant length at anthesis (Figs. 7A and 7B).

Growth curves generated from light microscopy observations are presented in Figures 8 and 9. These curves highlight interspecific rate similarities and differences for fibers from 2 dpa until maturity. Comparisons of data from 30 dpa to maturity (up to 60 dpa in some species), produced under similar growing conditions, indicates that virtually all length increase occurs by 30 dpa, as is the case in G. hirsutum (Schubert et al. 1973). The magnitudes of the standard deviations indicate considerable variation within species at many developmental stages, perhaps due in part to environmental variation.

Schubert et al. (1973) found for G. hirsutum that rapid length increase occurred between 10 and 15 dpa, with growth
Fig. 2. Mature seeds from cultivated and wild *Gossypium hirsutum* (panels A and B, respectively), the wild tetraploid species *G. tomentosum* (panel C), cultivated *G. arboreum* (panels D), wild *G. herbaceum* (accession A1-JMS; panel E), and the wild diploid species *G. raimondii* (panel F), *G. davidsonii* (panel G), *G. longicalyx* (panel H), *G. anomalum* (panel I), and *G. sturtianum* (panel J). Variations in seed size and fiber characteristics are evident in the composite image of panels A through J (panel K).
this profile were the sole F-genome species. Among the wild diploids, the exceptions to these were the extremes of wild species (e.g., wild G. anomalum, not shown), C-genome (G. sturtianum), and D-genome (G. raimondii, G. davidsonii) appeared to achieve nearly full fiber expansion by 14 dpa. Among the wild diploids, the exceptions to this profile were the sole F-genome species G. longicalyx and the putatively wild A-genome species G. herbaceum, both of which exhibited fibers that continued elongation up until approximately 21 dpa. Fibers from one accession of cultivated G. arboream increased significantly in length between 20 and 30 dpa, a pattern that was also exhibited by the wild tetraploid and G. hirsutum accession TX2094 displayed little or no growth between 10 and 15 dpa, followed by a significantly increased rate between 15 and 20 dpa.

**DISCUSSION**

As summarized in Figure 1, *Gossypium* includes a diverse assemblage of species that share, among other characteristics, the unique epidermal seed trichomes that are the source of the world’s most important textile fiber. Trichome morphology varies greatly among wild species (Fryxell 1979), from pigmented trichomes only a few millimeters in length that are tightly crimped and closely appressed to the seed (many species) to smooth, curly hairs two centimeters long (e.g., wild G. herbaceum) to the stiff patent hairs of *G. australae* and *G. nelsonii*. Thus, considerable differentiation attributable to natural selection has occurred during the global radiation of the genus over approximately the past 20 Myr (Wendel and Albert 1992, Seelanan et al. 1997). Superimposed on this natural evolutionary pattern are the effects of human selection, which further altered the trichome morphology of four domesticated species, two at the diploid and two at the allopolyploid level. Thus, a comparative analysis of development offered the opportunity for insights into alterations resulting from both natural divergence and human selection. Various components of these alterations are discussed below.

**Fiber initiation**

In cultivated cottons, a distinction traditionally is made between “lint” and “fuzz” fibers, as the former—the source of commercial cotton—are longer and more easily detached than the latter. Previous studies of *G. hirsutum* (Lang 1938) have determined that lint develops from the first wave of fiber initials, which begins development within a day after anthesis, and fuzz develops from a second wave beginning several days later. Joshi et al. (1967) observed two distinct cohorts of putative lint fibers and a third of fuzz fibers; they also noted that the timing of fiber initiation is similar in A-genome species. Beasley (1975) and Stewart (1975) observed by SEM that lint fiber initials of *G. hirsutum* TM1 were generally consistent with this pattern. Most other species did not display significant length increase after 20 dpa, and wild B-genome (*G. anomalum*, not shown), C-genome (*G. sturtianum*), and D-genome (*G. raimondii*, *G. davidsonii*) appeared to achieve nearly full fiber expansion by 14 dpa. Among the wild diploids, the exceptions to this profile were the sole F-genome species *G. longicalyx* and the putatively wild A-genome species *G. herbaceum*, both of which exhibited fibers that continued elongation up until approximately 21 dpa. Fibers from one accession of cultivated *G. arboream* increased significantly in length between 20 and 30 dpa, a pattern that was also exhibited by the wild tetraploid and *G. hirsutum* accession TX2094 displayed little or no growth between 10 and 15 dpa, followed by a significantly increased rate between 15 and 20 dpa.

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**Table 1. Species of Gossypium studied, with genome designations (Endrizzi et al. 1985; Stewart 1995) and accession data.**

<table>
<thead>
<tr>
<th>Genome</th>
<th>Species</th>
<th>Accession</th>
<th>Geographic origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_2</td>
<td>G. arboream L.</td>
<td>A_2-47</td>
<td>Sudan</td>
</tr>
<tr>
<td>A_2</td>
<td>G. arboream</td>
<td>A_2-73</td>
<td>India</td>
</tr>
<tr>
<td>A_1</td>
<td>G. herbaceum L. subsp. africanaum (Watt) Mauer</td>
<td>JMS (from J. McD. Stewart)</td>
<td>Southern Africa</td>
</tr>
<tr>
<td>A_1</td>
<td>G. herbaceum subsp. africanaum</td>
<td>B_1</td>
<td>Africa</td>
</tr>
<tr>
<td>B_1</td>
<td>G. anomalum Wawra &amp; Peyrisch</td>
<td>TM1 (Texas marker stock)</td>
<td>United States</td>
</tr>
<tr>
<td>AD_1</td>
<td>G. hirsutum L.</td>
<td>TX2094</td>
<td>Yucatan, Mexico</td>
</tr>
<tr>
<td>AD_1</td>
<td>G. hirsutum race yucatanense</td>
<td>WT936</td>
<td>Niihau, Hawaii</td>
</tr>
<tr>
<td>AD_1</td>
<td>G. tomentosum Nuttall ex Seemann</td>
<td>C_1</td>
<td>Australia</td>
</tr>
<tr>
<td>C_1</td>
<td>G. sturtianum J. H. Willis</td>
<td>D_3d-32</td>
<td>Baja California, Mexico</td>
</tr>
<tr>
<td>D_3d</td>
<td>G. davidsonii Kellogg</td>
<td>GG (from Glen Galau)</td>
<td>Peru</td>
</tr>
<tr>
<td>D_1</td>
<td>G. raimondii Ulbrich</td>
<td>F_1-3</td>
<td>Tanzania</td>
</tr>
<tr>
<td>F_1</td>
<td>G. longicalyx Hutchinson &amp; Lee</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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continuing up to 27 dpa; our observations of *G. hirsutum* accession TM1 were generally consistent with this pattern. More other species did not display significant length increase after 20 dpa, and wild B-genome (*G. anomalum*, not shown), C-genome (*G. sturtianum*), and D-genome (*G. raimondii*, *G. davidsonii*) appeared to achieve nearly full fiber expansion by 14 dpa. Among the wild diploids, the exceptions to this profile were the sole F-genome species *G. longicalyx* and the putatively wild A-genome species *G. herbaceum*, both of which exhibited fibers that continued elongation up until approximately 21 dpa. Fibers from one accession of cultivated *G. arboream* increased significantly in length between 20 and 30 dpa, a pattern that was also exhibited by the wild tetraploid and *G. hirsutum* accession TX2094 displayed little or no growth between 10 and 15 dpa, followed by a significantly increased rate between 15 and 20 dpa.
Fig. 3. Scanning electron micrographs of whole ovules (left) and fiber initials (right) from cultivated cotton species on the day of anthesis. Shown are *Gossypium hirsutum* cv. TM1 (panels A, B), *G. hirsutum* TX2094 (panels C, D), *G. herbaceum* subsp. *africanum* JMS (panels E, F), and *G. arboreum* A2-47 (panels G, H). Scale bars and magnification are indicated.
Fig. 4. Scanning electron micrographs of whole ovules (left) and fiber initials (right) from wild cotton species on the day of anthesis. Shown are *Gossypium tomentosum* (panels A, B), *G. sturtianum* (panels C, D), *G. davidsonii* (panels E, F), and *G. longicalyx* (panels G, H). Scale bars and magnification are indicated.
on their short length and usually tight adherence to the ovule, trichomes of these species seem to resemble fuzz more than lint. The use of the word *lint* to describe the longer hairs only of species in which two types exist may reflect an assumption that the seed hairs of other species are homologous to fuzz. However, since fuzz is qualitatively distinguished chiefly by a later initiation of development (Lang 1938; Joshi et al. 1967), this assumption would lead to the expectation that B-, D-, F-, and C-genome species would begin fiber growth several days after anthesis. By shortening the growth period compared to the cultivated cottons, this might contribute significantly to a lessened final fiber length. In the present study, however, all taxa were found to begin fiber development by two days post-anthesis, with few noteworthy differences among species. Most diploids observed displayed spherical fiber initials at the time the flower opens, a developmental stage comparable to that of *G. hirsutum* lint initials (Figs. 3 and 4). Species possessing the shortest trichomes measured in this study, *G. sturtianum* and *G. davidsonii*, similarly initiated fiber growth at 0 dpa (Figs. 4D and 4F). *Gossypium raimondii*, which has shorter mature fibers than A-genome species or *G. hirsutum*, began fiber elongation earlier than these species (Figs. 7A and 7B show an ovule at 0 dpa, on which hairs well into the elongation phase can be observed), resulting in increased fiber length at 2 dpa relative to other species examined (Figs. 7C and 7D). From these observations, we suggest that the lint of cultivated cottons may be homologous to the fibers of the wild species (assuming the latter to be homologous *inter se*), notwithstanding the qualitative differences between the hairs of short-fibered diploids and “true lint” fibers (e.g., Hutchinson et al. 1945). An alternative hypothesis is that fuzz fibers are homologous to fibers from the wild species, in which case lint fibers would represent a novel type of trichome whose development coincides with a delay in fuzz fiber initiation. In either case, it is clear that the time of fiber initiation does not appear to be a significant factor affecting interspecific differences in fiber length.

Variation in fiber development within individual ovules

Lang (1938) noted that fibers develop first on the chalazal portion of the ovule, with fiber origin delayed at the micropylar end. In some cases, this progression of development is discontinuous and corresponds with the growth pattern of certain cultivars in which lint and fuzz are spatially separated. Stewart (1975) observed by SEM that lint fiber initials appear first on the crest of the funiculus, and that fiber-initial development spreads from that point laterally around the ovule toward the chalazal cap, and lastly down the ovule toward the micropyle; new fibers continue to arise for up to four days at the micropylar end. This pattern seemed, in this study, to generally be true for other species of *Gossypium*. Figures 5E and 6E show ovules at 2 dpa in which hair growth near the micropyte was noticeably retarded. In *G. raimondii*, precocious initiation of fiber development on a very small portion of the pre-anthesis ovule appeared to begin in the area of the funicular crest (Figs. 7A and 7B); SEM examination of ovules at least 2 dpa (not shown) revealed that a few fibers had already begun elongation in that region. It is reasonable that this pattern of fiber initiation would be consistent throughout the genus, if Stewart (1975) is correct in suggesting that fiber growth is influenced by the production and diffusion of a stimulus from the crest of the funiculus, or conversely, an inhibitor from the micropylar end. However, Tiwari and Wilkins (1995) found that fiber initiation may begin at either the chalazal end or the funicular crest on different ovules from the same plant.

**Fiber density**

Another aspect of growth pattern that has the potential to dramatically affect mature seed morphology is the density of fiber initials. If fibers of equal length are produced more densely, perceived hairiness and total fiber production will be greater. In some cases, the ratio of fiber cells to non-fiber cells can change during development due to later divisions of epidermal cells (Farr 1933; Stewart 1975), which may vary among species, or to later differentiation of epidermal cells into fuzz fibers (Lang 1938). Stewart (1975) observed that the ratio of fiber initials to total epidermal cells in *G. hirsutum* is about 1:3.7 at 0 dpa, a ratio generally consistent with our casual observations for most species.

We did not formally estimate trichome densities in this study because mucilaginous or waxy deposits on ovules reduced the quality of the material beyond the point where cellular limits could confidently be visualized. However, it appeared that interspecific differences were not dramatic in this respect. To be sure, some interspecific variation was apparent; for example, *G. hirsutum* (Figs. 3A and 3B) appears to have a greater initial trichome density than does *G. herbaceum* (Figs. 3E and 3F) or *G. longicalyx* (Figs. 4G and 4H). These differences do not, however, appear to be of sufficiently large magnitude to account for the variation evident in mature seeds (Fig. 2). Fiber density at anthesis may not directly reflect mature fiber density, as only a subset of the first fiber initials may persist and elongate to a mature length, whereas more fibers may develop after anthesis in some taxa (Joshi et al. 1967).

**Variation in fiber elongation phase**

Growth curve data (Figs. 8 and 9) showed that there was considerable interspecific variability in the duration of the cell elongation phase, in the timing of the period of the maximum
Fig. 5. Scanning electron micrographs of whole ovules (left) and fiber initials (right) from cultivated cotton species 2 dpa. Shown are *Gossypium hirsutum* cv. TM1 (panels A, B), *G. hirsutum* TX2094 (panels C, D), *G. herbaceum* subsp. *africanum* JMS (panels E, F), and *G. arboreum* A2-47 (panels G, H). Scale bars and magnification are indicated.
Fig. 6. Scanning electron micrographs of whole ovules (left) and fiber initials (right) from wild cotton species 2 dpa. Shown are *Gossypium longicalyx* (panels A, B), *G. sturtianum* (panels C, D), *G. tomentosum* (panels E, F), and *G. davidsonii* (panels G, H). Scale bars and magnification are indicated.
rate of elongation, and in the absolute growth rate during different stages of development. Given the foregoing demonstration that fiber initiation occurs approximately at the same time in all taxa, variation in the duration of fiber elongation will be dependent upon the date at which elongation ceases. Fiber elongation in *G. hirsutum* may continue until 24–34 dpa (Schubert et al. 1973; Quisenberry and Kohel 1975). Quisenberry and Kohel (1975) observed that differences in the duration and rate of elongation among *G. hirsutum* cultivars are significantly influenced by environmental as well as genetic factors: fibers of plants raised at higher temperatures have shorter elongation periods but increased rates of growth. Thus, growth curves presented in this article reflect only the performance of the included taxa under a single set of conditions. It is possible that further study might unmask additional intrataxon variation.

Growth curves for diploid species are presented in Figure 8; all of the species in panels A and B are wild, with the exception of *G. arboreum*, known only from cultivation (Stanton et al. 1994; Wendel et al. 1989). Most wild diploids had similar growth curves, in which fibers largely attained their final length by approximately two weeks post-anthesis; this
was true of representatives of the B-, C-, and D-genome groups. A-genome species (*G. herbaceum* and *G. arboreum*) differed from this pattern in that fibers continued to elongate for about an additional week. This prolongation of the elongation phase also occurred in the sole representative of the F-genome, *G. longicalyx*, which is cladistically resolved as the sister taxon to the A-genome in molecular phylogenetic analyses of both plastid and nuclear gene sequences (Fig. 1; see introduction). If a longer growth period were found only in the A-genome species, it might be argued that this effect was a product of domestication and that the supposedly wild accession of *G. herbaceum* in fact had a history of prior domestication. The fact that the short-fibered, undomesticated sister-species shared this feature constitutes evidence that the nearly 50% increase in the elongation phase represents a developmental synapomorphy for the A- + F-genome clade.

**Effects of domestication on fiber elongation phase**

Figures 8C and 8D show growth curve data for fibers from all A-genome accessions examined; of these, both *G. herbaceum* subsp. *africanum* accessions are wild and both *G. arboreum* accessions are cultivated. These two species, which have longer fibers than other diploid species, showed continued elongation after 15 dpa. For three of these accessions, the rate of fiber elongation declines sharply or elongation ceases by 20 dpa. The exception was *G. arboreum* AKA 8401, which continued to elongate until 30 dpa, resulting in a greater mature fiber length. Since this accession represents an elite diploid cultivar, artificial (agronomic) selection is presumed to be responsible for the additional increase in the elongation period beyond three weeks post-anthesis.

This effect of artificial selection may be paralleled at the tetraploid level. As shown in Figure 9, lint fibers of wild polyploids (*G. tomentosum* and *G. hirsutum* TX2094) con-
continued to elongate until about three weeks post-anthesis, as do the seed fibers from their A-genome ancestor. Hence, it is likely that this extended elongation phase was inherited directly from the A-genome parent at the time of allopolyploid formation. There was also an additional prolongation of fiber elongation in the most agronomically advanced tetraploid studied, *G. hirsutum* TM1 (Fig. 9), a pattern similar to that observed with diploid *G. arboreum* AKA 8401 (Fig. 8). As evidenced in the comparative chart shown in Figures 9C and 9D, the elongation periods for these two cultivated accessions, one diploid and one allopolyploid, are markedly similar, suggesting parallel artificial selection for increased duration of fiber growth.

**Timing of fiber elongation**

The timing of the period of maximum fiber elongation is also of interest. In this study, species that terminated growth by 15 dpa or shortly thereafter generally showed a nearly linear rate of elongation over most of the growing period, suddenly tapering off between 10 and 15 dpa. Long-linted species exhibited more complex growth curves. Schubert et al. (1973) presented a roughly sigmoidal growth curve for *G. hirsutum*, in which growth rates increase over time, reach a maximum between 10 and 15 dpa, and decline thereafter. Van’t Hof (1998) also observed an increasing rate of elongation in fertilized ovules over the first 10 dpa. Quisenberry and Kohel’s (1975) results indicate that the period of maximum growth is later under cool conditions.

In the present study, the agronomically advanced cultivar TM1 of *G. hirsutum* showed an increased rate of elongation between 10 and 15 dpa, similar to that reported in previous studies for *G. hirsutum*. A similar growth spurt was apparent in one of the two accessions (A-1) of *G. arboreum* (Figs. 8 and 9). However, in *G. herbaceum*, *G. tomentosum*, and the
putatively wild (Brubaker and Wendel 1994) accession of *G. hirsutum* (TX2094), the comparable period of maximum growth was delayed until between 15 and 20 dpa, followed by a quicker cessation of growth than that observed by Schubert et al. (1973). Some of the long-linted taxa (e.g., *G. arboreum* AKA8401) had almost linear growth rates without conspicuous variation.

Whereas it is likely that the above differences reflect actual variation in developmental programs, it is also probable that there were environmental influences and sampling error associated with measurements on relatively small numbers of ovules/taxon. Nevertheless, the data suggest a precocious period of maximum growth that was separately developed in cultivated *G. hirsutum* and *G. arboreum*. Independent origin of this feature is implicated, as opposed to a single origin at the diploid level followed by inheritance at the time of polyploid formation, by the fact that no wild diploid cotton exhibits this feature and by the observation that it is evidenced only by the advanced cultivated tetraploid, being absent from both agronomically primitive *G. hirsutum* and the wild Hawaiian species *G. tomentosum*. Whereas the agronomic significance of the precocious elongation spurt remains unknown, its repeated origin within cultivated species suggests that it may be related to important fiber properties. Ascertain-ting the genetic basis of this developmental change is of special interest, particularly inasmuch as this would permit an evaluation of whether the developmental parallelism itself is mirrored in the underlying genetic mechanisms.

**Fiber elongation rate**

Comparison of growth curves across species, expressed as percentage of mature length, (Figs. 8B, 8D, 9B, and 9D) suggest that absolute fiber elongation rate was probably the most important factor in determining final fiber length. This is clearly evidenced by species-pairs with similar growth curves (e.g., *G. longicalyx* and *G. herbaceum*; *G. sturtianum* and *G. raimondii*) where the two members had different ultimate fiber lengths. To the extent that the growth curves reflect comparable developmental stages, this implies that during the entire elongation period, *G. sturtianum* and *G. raimondii*, for example, were in homologous stages of growth but that the absolute growth rate of *G. raimondii* fibers was always greater. Similarly, whereas fibers from *G. tomentosum* showed a growth pattern similar to that of *G. herbaceum* and *G. hirsutum* TX2094, its final fiber length was only about 60% of that of the latter two species, clearly because fiber growth rate during elongation was comparatively low.

It did not appear that differences in mature fiber length reflected a mere compounding of early-established differences. Although short-fibered species (*G. sturtianum*, *G. davidsonii*) did tend to have shorter hairs at 2 and 5 dpa, quite the opposite was true for *G. raimondii*. Similarly, fiber length at 10 dpa did not correlate well with final length among the A- and AD-genome accessions. Periods of rapid elongation could occur at almost any time before 30 dpa, and absolute length gained during those periods appeared to be primarily responsible for mature fiber length. It is possible that slight variations in the length of the growth spurt, undetected in this study, would have a significant influence on fiber length; absolute rate, however, appears to be of primary importance.

A final comment with respect to growth rate concerns the variability of fibers within individual ovules. As discussed above, we often observed variation in the fiber development with respect to position relative to the chalazal or micropylar end. Superimposed on this may be other, later developmental inequalities. A case in point is *G. sturtianum*, which has sparse hair on the sides of mature seeds, except in limited areas; at 0 dpa, however, developing initials were present in similarly high density across the entire epidermal surface (Figs. 4C and 4D). Perhaps fiber elongation is prematurely terminated on portions of the developing ovule, or alternatively, elongation is prolonged in the complementary portions.

**Fiber development and polyploidization**

Two cultivated allopolyploid cotton species (*G. hirsutum* and *G. barbadense*) presently dominate world cotton commerce, having supplanted the majority of Old World diploid cotton cultivation. One reasonable hypothesis for the longer lint of cultivated allopolyploid relative to cultivated diploid cotton (Fig. 2) is that it arose as a direct consequence of genome doubling and the concomitant doubling of fiber-related genes. This appears unsupported by our data, as the wild tetraploids examined did not surpass A-genome diploids in fiber length (e.g., *G. tomentosum*, Fig. 9). In fact, fiber growth curves for wild tetraploids appeared similar to those of the wild A-genome species (compare *G. hirsutum* TX2094 to *G. herbaceum* A-73 in Fig. 9, panels C and D). Moreover, these growth curves differ from those of D-genome species, the paternal donor to the AD-genome polyploids.

These observations permit a speculation that in wild *G. hirsutum* and perhaps other wild tetraploids, the developmental profiles of fibers are largely controlled by genes located in the A-subgenome. This need not imply that D-subgenome genes were preferentially silenced at the time of allopolyploid formation, but that the developmental program of the A-genome ancestor, when combined with a typical D-genome profile, appears morphogenetically “dominant.” In principle, this speculation leads to a testable hypothesis, one that perhaps is readily addressed by comparative microarray analysis. These experiments presently are being planned. As an extension of this reasoning, we note that there is considerable diversity in the fibers of tetraploid cotton species. By comparison to wild *G. hirsutum* or to A-genome diploids, for example, *G. tomentosum* has fiber that is short and of poor quality. The observation that its growth curve
still follows the A-genome ancestral pattern (Fig. 9B) is consistent with the foregoing hypothesis of dominance in superimposed developmental profiles. The short fiber, however, requires explanation. Perhaps there has been silencing of or a reduction in expression of one or more A-subgenome “fiber-length” genes as a result of selective or stochastic forces. Again, this poses a testable hypothesis for future work.

As discussed above, domestication of the AD-genome tetraploid cottons increased fiber length beyond that attained by high-quality A-genome diploid cultivars (as shown in Figs. 2 and 9C). This observation suggests that the genome-wide gene duplication caused by allopolyploidization provided the raw material necessary for the evolution of novel gene expression patterns. In this respect, the study of Jiang et al. (1998) is noteworthy. Using quantitative genetic approaches (QTL analysis), they reported that a majority of loci affecting fiber yield and quality in G. hirsutum are found in the D-subgenome, possibly explaining the superiority of the tetraploids. In the present study, domestication at the tetraploid level is shown not only to have prolonged the elongation period beyond three weeks, but also to have increased growth rate at earlier stages (Fig. 9C). Perhaps these shifts in developmental profiles were mediated by recruitment of novel expression patterns for D-subgenome genes, or perhaps novel expression of A-subgenome genes was permitted by virtue of gene duplication (Wendel 2000).

CONCLUSION

In summary, we have taken a phylogenetic approach to study the comparative developmental morphology of a trait that exhibits great diversity on both evolutionary and human time-scales. Our analysis has revealed effects of selection at both of these levels. The two most important factors governing interspecific differences in fiber length are (1) growth rate during rapid elongation, particularly during the period of maximum growth, and (2) total duration of the elongation phase. Domestication is associated with a prolonged elongation phase, both at the diploid and allopolyploid levels, suggesting parallel artificial selection for increased fiber length. The underlying genetic basis governing these shifts in developmental profiles and hence ultimate morphologies may include qualitative as well as quantitative components of gene expression.

Acknowledgments

We gratefully acknowledge Drs. Curt Brubaker, Harry Horner, James McD. Stewart, and Jack Van’t Hof for discussion; Dr. Paul A. Fryxell and an anonymous reviewer for helpful comments; Christine Notis for greenhouse and lab work; Tracey Pepper for SEM advice; and Anna Gardner and Greg Courtney for help with the plates. We also thank the National Science Foundation for support (to J. F. W.).

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