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Occurrence of (+)- and (−)-Gossypol in Wild Species of Cotton and in *Gossypium hirsutum* Var. *marie-galante* (Watt) Hutchinson

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Gossypol occurs as a mixture of enantiomers in cottonseed. These enantiomers exhibit different biological activities. The (−)-enantiomer is toxic to animals, but it has potential medicinal uses. Therefore, cottonseed with >95% (−)-gossypol could have biopharmaceutical applications. The (+)-enantiomer shows little, if any, toxicity to nonruminant animals. Thus, cottonseed with >95% (+)-gossypol could be more readily utilized as a feed for nonruminants. The (+)- to (−)-gossypol ratio in commercial Upland (*Gossypium hirsutum*) cottonseed is usually about 3:2, whereas that in commercial Pima cottonseed (*Gossypium barbadense*) is approximately 2:3. Herein are reported the (+)- to (−)-gossypol ratios in the seed from 28 wild species of cotton (194 accessions), 94 accessions of *G. hirsutum* var. *marie-galante*, and 3 domesticated species (11 accessions). It was found that some or all of the accessions of *Gossypium darwinii*, *Gossypium sturtianum*, *Gossypium areysianum*, *Gossypium longicalyx*, *Gossypium harknessii*, and *Gossypium costulatum* produce an excess of (−)-gossypol but none >65%. At least one accession of *Gossypium anomalum*, *Gossypium mustelinum*, *Gossypium gossypioides*, and *Gossypium capitis-viridis* contained >94% (+)-gossypol. One of the 94 accessions of *G. hirsutum* var. *marie-galante* (i.e., no. 2469) contained 97% (+)-gossypol.

KEYWORDS: Cotton; (+)-gossypol; (−)-gossypol; *Gossypium*; wild cotton species; *G. hirsutum* var. *marie-galante*

INTRODUCTION

Gossypol is derived from hemigossypol (*1*) through a free radical coupling reaction (**Figure 1**). This bimolecular coupling occurs in the cotton plant and usually produces one enantiomer in preference to the other. Restricted rotation around the binaphthyl bond gives rise to an optically active molecule because of axial dissymmetry (atropisomerism) (*2*).

There is a considerable body of evidence showing that for higher animal systems most biological activities of gossypol reside with the (−)-enantiomer. For example, (−)-gossypol inhibited the cell growth of various cancer cells more effectively than the (+)-enantiomer (*3*−*6*). Furthermore, (−)-gossypol is a more effective inhibitor of various enzymes than (+)-gossypol (*7*, *8*). (−)-Gossypol, but not (+)-gossypol, shows anti-HIV-1 activity in humans (*9*). (−)-Gossypol is also a more effective antiamoebic agent (*10*). Several investigators showed that (−)-gossypol, but not (+)-gossypol, has male antifertility activity and is more toxic to animals (*11*−*14*). In a feeding study, Bailey et al. (*15*) showed that broiler chickens fed a diet containing 5% of a cottonseed with a (+)- to (−)-gossypol ratio of 83:17 gained weight at the same rate as the 100% soybean control diet. Regression analysis also showed that cumulative weight gains of the chickens decreased ∼126 g for each 100 mg increase in (−)-gossypol consumed, whereas the cumulative weight gains were not significantly altered with increased (+)-gossypol consumption.

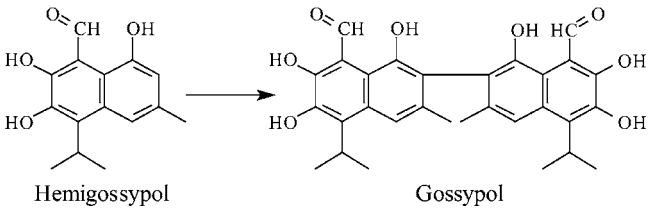

Figure 1. Biosynthesis of gossypol from hemigossypol.

High-(+)-gossypol cotton plants do not appear to be particularly susceptible to insect damage. Yang et al. (*16*) report that *Helicoverpa armigera* larvae raised on artificial diets con-

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taining (+)-gossypol matured more slowly, and the percent survival to the adult was lower as compared to those raised on (−)-gossypol. Thus, high levels of (+)-gossypol may even be desirable for resistance to some insects. In the case of pathogens, Puckhaber et al. (*17*) found that both (+)- and (−)-gossypol were equally effective at inhibiting the growth of the seedling pathogen *Rhizoctonia solani* and that neither enantiomer contributed significantly toward killing the pathogen. The most potent natural fungicides were hemigossypol and desoxyhemigossypol. Also, Yildirim-Aksoy et al. (*18*) found that (+)-gossypol was a better bacteriostat than racemic or (−)-gossypol.

The ratio of (+)- to (−)-gossypol in a particular cultivar appears to remain relatively constant across environments. For example, Meredith and collaborators (*19*) studied a large number of commercial *Gossypium hirsutum* and *Gossypium barbadense* cultivars growing at several locations across the United States. They found that for a specific cultivar, environment significantly affected the total amount of gossypol in the seed, but not the (+)- to (−)-gossypol ratios (*19*). Furthermore, Bell et al. (*20*) found that the ratio of (+)- to (−)-gossypol remained constant for a particular plant regardless of whether the seed came from bolls that were produced early or late in the season or whether the seed was from bolls at the bottom or top of the plant.

The ability of nonruminant animals to consume (+)-gossypol with no apparent ill effects indicates that a breeding program to incorporate a high-(+)-gossypol trait into seed of commercial cultivars would benefit the U.S. cotton industry through greater utilization of the seed meal. Alternatively, one can envision potential pharmaceutical applications for seed with high (−)-gossypol. Because cotton breeders have transferred both nuclear and cytoplasmic genes from many wild species into commercial cottons, it might be possible to breed cotton plants that express high levels of either (+)- or (−)-gossypol in seed, if the correct germplasm source could be identified.

Previous studies have focused primarily on the ratio of (+)- to (−)-gossypol in the seed from cotton species that are grown commercially. For example, the ratio has been reported to vary between 95:5 and 31:69 in *G. hirsutum* and *G. barbadense* (*20*−*23*). In addition, Jaroszewski et al. (*24*) reported the (+)- to (−)-gossypol ratios in *Gossypium arboreum* (77:23), *G. barbadense* (44:56), and *Gossypium herbaceum* (68:32). Cass et al. (*23*, *25*) reported the (+)- to (−)-gossypol ratios in seed from *Gossypium mustelinum* (57:43 and 65:35), *G. herbaceum* (63:37), and a *G. herbaceum* × *G. hirsutum* cross (48:52). No large-scale study has been conducted on the wild species of *Gossypium* to determine variations in the ratio of (+)- to (−)-gossypol. Our goal was to determine the range of (+)- and (−)-gossypol in wild cottonseed that could be utilized in a breeding program. Such a breeding program could have two goals: (1) to maximize (+)-gossypol in the seed so that it could be fed to nonruminant animals or (2) to maximize (−)-gossypol in the seed to provide a ready source of a pharmaceutically useful product. We report herein the results from our study on the total gossypol and the percent of (+)-gossypol in *Gossypium* seed from the wild species in the U.S. National Cotton Germplasm Collection housed at College Station, TX. For comparisons, we also examined the seed from several accessions from *G. barbadense* (Pima cotton), which is grown commercially in the United States, and from *G. herbaceum* and *G. arboreum*, which are grown commercially in other parts of the world. Within *G. hirsutum*, the highest percentage of (+)-gossypol has been reported in the variety *marie-galante* (*25*). To identify other *marie-galante* accessions with high ratios of (+)-gossypol, we analyzed seed from all *marie-galante* accessions in the U.S. collection.

MATERIALS AND METHODS

Equipment and Reagents. Analyses were performed on a Hewlett-Packard 1090 HPLC equipped with a diode array detector and operated under computer control. Solvents were all of HPLC grade. (*R*)-(−)-2-Amino-1-propanol (D-alaninol) was obtained from Aldrich Chemical Co., Milwaukee, WI.

Seed. All seeds were obtained from the U.S. Cotton Germplasm Collection housed at College Station, TX. Because many of the wild species are photoperiodic, all seeds were obtained from plants grown in the greenhouse in College Station, TX. This should not affect the (+)- to (−)-gossypol ratios (*19*). The number of accessions available within the wild species varied from 1 to 28. The number of accessions varies depending on the number of contributors and the number of sites from which they were collected.

Chemical Analysis. We used a variation of the method developed by Kim et al. (*26*) to determine the total concentration of gossypol and the ratio of (+)- to (−)-gossypol in individual seed embryos. Seeds were dried under vacuum over anhydrous silica gel for 72 h and then stored in sealed containers until analysis. For analysis, a seed was dehulled, ground, and transferred to a tared 2 mL microfuge tube or 5 mL test tube and then weighed to the nearest 0.1 mg. A derivatizing reagent [88% UV-grade acetonitrile, 10% glacial acetic acid, and 2% (*R*)-(−)-2-amino-1-propanol] was prepared. Depending on the concentration of gossypol in the embryo, 0.25, 1.00, or 2.00 mL was pipetted onto the embryo tissue, and the tube was sealed. The sample was heated in a water bath at 70 °C for 30 min, vortexed, and then centrifuged. A portion of the clear sample was transferred to a vial for HPLC analysis. HPLC analysis used an injection volume of 2, 5, 10, or 50 μL depending on the concentration of the gossypol in the tissue. Analysis was conducted using a 150 × 3.0 mm i.d., 5 μm, Inertsil ODS-3 (GL Sciences, Tokyo, Japan) column maintained at 40 °C. The solvent system was an 80:20 isocratic mixture of acetonitrile and 10 mM KH_2PO_4, the latter adjusted to pH 3.0 with concentrated H_3PO_4, run at a flow rate of 0.60 mL/min. The run time was 8 min. The chromatogram was monitored at 254 nm (bandwidth of 20 nm) referenced to 550 nm (bandwidth of 100 nm). UV spectra were recorded over the 220−400 nm range. The gossypol−aminopropanol Schiff bases appeared at 3.0 and 4.5 min for the (+)- and (−)-enantiomers, respectively. The concentrations of (+)- and (−)-gossypol in the embryo were calculated using the Schiff base peak areas, standard peak area curves, the injection volume, the total sample volume, and the tissue weight. The total concentration of gossypol and the percentage of (+)-gossypol were calculated from the individual concentration values. For the wild and domesticated *Gossypium* accessions, three seeds were selected at random for analysis. In those cases where the concentration of total gossypol was low or variation in the results was high, additional seeds were analyzed. For *G. hirsutum* var. *marie-galante*, four seeds per accession were analyzed.

RESULTS AND DISCUSSION

Previous work has shown that environmental conditions can affect seed weight and total gossypol levels, but it does not affect the ratio of (+)- to (−)-gossypol (*19*). All seeds were obtained from plants grown under greenhouse conditions. Seed production occurred at different times of the year depending on the photoperiodic requirements of the individual species. Complete analytical results are given in the Supporting Information. **Table 1** summarizes the data on all genomes including the mean and range for embryo weights, total gossypol, and percentage of (+)-gossypol. **Table 2** provides mean and standard error (SE) on specific accessions within different genomes that are pertinent to the discussion.

Within the wild species, a relatively narrow variation for gossypol level and (+)- to (−)-gossypol ratios between seeds of any accession was observed as indicated by the small standard

Table 1. Mean and Range for the Weight, Total Gossypol, and Percent (+)-Gossypol in Embryos of the Species of *Gossypium*

genome	gossypium species	no. of accessions[a]	embryo weight (mg)		total gossypol (μg/mg)		% (+)-gossypol	
			mean	range	**mean**	range	**mean**	range
A1	*G. herbaceum*	6	**38.1**	22.9–61.3	**9.1**	3.5–18.1	**65.6**	59.3–71.0
A2	*G. arboreum*	6	**34.8**	21.2–46.5	**6.1**	2.3–12.1	**68.3**	54.8–78.8
(AD)1	*G. hirsutum* var. *marie-galante*	94	**55.8**	31.4–93.7	**4.7**	1.0–12.8	**82.8**	62.0–97.4
(AD)2	*G. barbadense*	4	**73.6**	62.9–81.2	**4.5**	2.3–7.0	**46.5**	41.6–55.3
(AD)3	*G. tomentosum*	1	**26.6**		**8.4**		**75.6**	
(AD)4	*G. mustelinum*	9	**28.6**	19.8–36.9	**9.0**	3.1–12.7	**69.6**	60.4–94.5
(AD)5	*G. darwinii*	28	**17.8**	7.2–47.6	**15.0**	2.7–28.5	**55.9**	38.1–70.0
B1	*G. anomalum*	7	**10.7**	8.6–12.7	**1.7**	1.0–2.7	**96.1**	90.2–98.2
B2	*G. triphyllum*	1	**13.1**		**0.43**		**82.8**	
B3	*G. capitis-viridis*	1	**11.3**		**0.98**		**96.2**	
C1	*G. sturtianum*	7	**5.1**	3.7–7.3	**0.04**	0.02–0.06	**60.4**	41.1–81.8
C1N	*G. nandewarense*	2	**4.0**	3.5–4.4	**0.13**	0.04–0.22	**64.6**	63.7–65.5
C2	*G. robinsonii*	1	**6.9**		**0.01**		**70.5**	
D1	*G. thurberi*	27	**13.6**	6.2–19.3	**9.2**	3.1–17.0	**70.1**	58.3–79.9
D21	*G. armourianum*	6	**40.3**	21.3–50.8	**4.8**	0.9–12.9	**60.7**	52.5–73.5
D22	*G. harknessii*	8	**39.4**	26.4–61.3	**11.8**	3.7–23.1	**62.1**	46.2–80.0
D3D	*G. davidsonii*	21	**17.6**	8.2–25.1	**36.4**	10.0–56.8	**67.9**	60.5–74.3
D3K	*G. klotzschianum*	1	**19.8**		**18.7**		**63.7**	
D4	*G. aridum*	5	**17.7**	9.1–21.4	**8.9**	3.3–14.8	**75.6**	72.5–79.2
D5	*G. raimondii*	22	**17.3**	10.4–25.1	**10.4**	6.0–12.7	**61.1**	58.7–63.3
D6	*G. gossypioides*	4	**20.0**	17.0–25.2	**1.3**	1.1–1.5	**93.9**	93.1–94.1
D7	*G. lobatum*	2	**17.0**	13.6–20.4	**3.4**	2.5–4.3	**78.7**	72.7–84.8
D8	*G. trilobum*	10	**8.8**	5.6–12.7	**5.3**	1.8–8.9	**67.2**	61.8–72.5
E1	*G. stocksii*	3	**11.9**	10.4–14.6	**0.10**	0.06–0.13	**57.2**	55.0–59.7
E2	*G. somalense*	2	**13.4**	13.0–13.8	**0.17**	0.14–0.20	**59.0**	58.4–59.6
E3	*G. areysianum*	1	**13.5**		**0.02**		**39.2**	
E4	*G. incanum*	1	**14.7**		**0.17**		**48.6**	
F1	*G. longicalyx*	3	**26.5**	24.4–28.0	**8.8**	6.1–13.4	**62.5**	37.9–77.9
G1	*G. bickii*	5	**6.0**	4.7–8.1	**0.03**	0.02–0.04	**71.3**	58.2–82.8
G2	*G. australe*	11	**4.9**	2.3–6.4	**0.05**	0.02–0.19	**64.1**	50.4–83.9
G3	*G. nelsonii*	4	**7.0**	6.0–7.9	**0.03**	0.02–0.04	**72.6**	69.4–75.8
K1	*G. costulatum*	1	**31.9**		**0.04**		**36.8**	

[a] Number of accessions investigated.

error. However, considerable variation was evident between species and between accessions of the same species in several of the groups surveyed. Most accessions of AD tetraploids and of D genome diploids contain considerable amounts of gossypol, ranging from 1 to 60 μg/mg. Accessions of D3 genome diploids (*Gossypium davidsonii*) (**Table 2**), and to a lesser extent D3K (*Gossypium klotzschianum*) (**Table 1**) and the tetraploids AD5 (*G. darwinii*) (**Table 2**), stand out with exceptionally high gossypol levels. In contrast, most or all accessions of B, C, E, G, and K genome diploids contain very low gossypol levels (1−3 μg/mg for B1 and B3 accessions and <0.2 μg/mg for other C, E, G, and K genome accessions).

In general, the ratio of (+)- to (−)-gossypol varied widely among accessions. This is true for accessions from the subgenome groups that have high overall gossypol levels and accessions from subgenome groups that have low overall levels. There were a few accessions in which the ratios remained very tight. These include B1, C1N, D6, E1, and E2.

Previously, only *G. barbadense* cultivars were reported to produce an excess of (−)-gossypol. We now report that accessions of *G. darwinii* (AD5), *Gossypium sturtianum* (C1), *Gossypium areysianum* (E3), *Gossypium longicalyx* (F1), *Gossypium harknessii* (D22), and *Gossypium costulatum* (K1) also produce an excess of this enantiomer [shown in **Table** 1 as (+)-gossypol levels below 50%]. Of these, *G. longicalyx* accession F1-1 and *G. darwinii* accessions AD5-49 and AD5-50 produce the highest ratios of (−)-gossypol in conjunction with a respectable amount of total gossypol (**Table 2**).

Gossypium anomalum (B1), *Gossypium mustelinum* (AD4), *Gossypium gossypioides* (D6), and *Gossypium capitis-viridis* (B3) had at least one accession containing ≥94% (+)-gossypol (**Table 2**). Of this group, seven accessions had (+)-gossypol ratios in excess of 95%, including *G. anomalum* accessions B1-1, B1-2, B1-3, B1-4, B1-5, and B1-7 [96−98% (+)-gossypol, 0.10−0.25% total gossypol] and *G. capitis-viridis* accession B3-1 [96% (+)-gossypol, 0.10% total gossypol] **(Table 2**).

Among all known varieties of *G. hirsutum*, only *G. hirsutum* var. *marie-galante* has high levels of (+)-gossypol. Within the U.S. National Cotton Germplasm Collection, there are 94 accessions classified as *G. hirsutum* var. *marie-galante*. In view of the potential applications of high-(+)-gossypol cottonseed, we examined these accessions to identify those that might be most useful for breeding purposes. We found significantly more variation within the *marie-galante* accessions than was observed with the accessions from the wild collection (**Table 1**). However, the seed from some of these accessions showed consistently high (+)- to (−)-gossypol ratios. Three accessions had standard errors of <0.5 and (+)-gossypol of >95% [i.e., no. 2505 (95.7%), no. 2452 (96.7%), and no. 2469 (97.4%)], and four accessions had seed with standard errors of <0.75 and levels of (+)-gossypol of >94% (i.e., no. 2425, 2442, 2471, and 2481). Accession 2469 was especially noteworthy, giving a range of 96.4−98.3% with a standard error of 0.4. Many accessions of *marie-galante* did have lower ratios of (+)-gossypol, including no. 2488, which showed the lowest (+)- to (−)-gossypol ratio (i.e., 62:38).

Seeds were obtained from plants grown in a greenhouse at College Station, TX. Our results indicate that when the plants were grown under similar conditions, the capacity to produce relatively high levels of gossypol was developed or maintained to varying degrees in species belonging to several African and New World subgenome classes (A, D, AD, and F). However,

Table 2. Mean and Standard Error for the Weight, Total Gossypol, and Percent (+)-Gossypol in Embryos of Selected Accessions of Wild Species of *Gossypium*

accession no.	species/genome	no. of replicates	embryo weight (mg)		total gossypol (μg/mg)		% (+)-gossypol	
			mean	SE	**mean**	SE	**mean**	SE
(AD)4-17	*G. mustelinum*/AD4	3	**28.60**	6.96	**3.10**	0.69	**94.46**	1.05
(AD)5-32	*G. darwinii*/AD5	3	**11.67**	1.00	**26.17**	2.00	**56.27**	1.21
(AD)5-35		3	**8.43**	1.18	**28.48**	5.13	**59.57**	1.69
(AD)5-49		3	**22.33**	2.43	**17.20**	4.24	**38.09**	3.55
(AD)5-50		4	**23.75**	1.94	**23.44**	2.45	**40.95**	1.58
(AD)5-51		5	**16.34**	1.15	**26.90**	3.16	**48.65**	0.68
(AD)5-52		4	**11.85**	2.06	**24.12**	2.50	**48.94**	2.14
(AD)5-53		3	**13.10**	1.21	**19.19**	4.03	**48.48**	1.54
(AD)5-55		3	**19.21**	1.41	**27.19**	7.38	**49.12**	2.25
B1-1	*G. anomalum*/B1	3	**8.57**	0.96	**1.15**	0.03	**96.72**	0.21
B1-2		3	**11.20**	0.15	**0.97**	0.09	**96.82**	0.16
B1-3		3	**12.67**	1.38	**1.15**	0.23	**98.18**	1.26
B1-4		3	**12.53**	1.79	**2.47**	0.35	**97.54**	0.18
B1-5		4	**10.19**	0.80	**1.17**	0.39	**95.91**	1.65
B1-7		3	**11.23**	1.43	**2.09**	0.33	**97.12**	0.33
B3-1	*G. capitis-viridis*/B3	3	**11.27**	0.73	**0.98**	0.10	**96.24**	0.45
C1-4	*G. sturtianum*/C1	3	**5.60**	0.78	**0.05**	0.03	**41.06**	2.54
C1-6		3	**4.47**	0.43	**0.06**	0.03	**44.75**	5.61
D22-17	*G. harknessii*/D22	3	**28.47**	1.37	**8.50**	0.57	**46.22**	2.05
D3D-1	*G. davidsonii*/D3D	3	**25.13**	0.82	**56.83**	4.80	**69.53**	0.91
D3D-2		3	**24.03**	1.49	**43.14**	3.94	**63.22**	0.55
D3D-3		3	**14.93**	3.75	**38.89**	6.78	**73.30**	1.12
D3D-4		3	**24.87**	2.79	**50.56**	4.15	**71.63**	1.51
D3D-6		5	**19.18**	1.84	**30.62**	5.41	**74.30**	1.35
D3D-8		3	**15.60**	0.97	**32.87**	2.15	**71.27**	0.69
D3D-10		4	**24.35**	2.12	**44.31**	6.30	**66.49**	2.46
D3D-11		4	**25.00**	1.77	**49.62**	7.72	**65.87**	1.74
D3D-12		3	**18.40**	3.08	**36.57**	5.92	**69.87**	0.51
D3D-15		4	**21.98**	1.46	**39.42**	7.72	**69.70**	0.81
D3D-16		3	**18.60**	0.91	**37.15**	4.53	**66.14**	0.38
D3D-17		4	**19.00**	0.44	**52.89**	6.18	**71.86**	0.64
D3D-18		3	**13.47**	3.52	**43.34**	10.29	**63.16**	2.33
D3D-22		3	**16.13**	1.74	**32.79**	6.59	**63.86**	0.94
D3D-25		3	**16.47**	4.65	**37.42**	2.77	**65.94**	1.02
D3D-26		3	**14.13**	0.93	**25.50**	3.88	**66.34**	1.32
D3D-28		3	**10.67**	3.10	**30.20**	7.61	**60.54**	0.45
D3D-30		3	**12.63**	0.87	**29.07**	3.14	**68.36**	0.44
D6-1	*G. gossypioides*/D6	3	**17.80**	2.16	**1.24**	0.29	**93.71**	0.81
D6-2		4	**17.00**	3.08	**1.25**	0.35	**94.08**	0.41
D6-3		4	**25.20**	1.29	**1.52**	0.11	**94.09**	1.04
D6-4		3	**20.07**	3.10	**1.13**	0.16	**93.76**	1.11
E3-1	*G. areysianum*/E3	5	**13.50**	1.46	**0.02**	0.00	**39.23**	1.45
E4-4	*G. incanum*/E4	5	**14.68**	2.75	**0.17**	0.06	**48.62**	1.87
F1-1	*G. longicalyx*/F1	3	**27.27**	2.82	**13.38**	1.26	**37.89**	1.31
K1-3	*G. costulatum*/K1	4	**31.95**	1.17	**0.04**	0.01	**36.83**	3.94

this capacity to produce high levels of gossypol was not developed or it was lost in all species from the African and Australian subgenome groups B, C, E, G, and K.

The results from the variations observed in the ratios of (+)- and (−)-gossypol (**Table 1**) suggest that either a full complement of alleles specifying gossypol ratios went through speciation bottlenecks or that allelic variation arose de novo after speciation events. In the latter case, the nature of allelic variation that determines specific gossypol compositions is likely to be different between species or accessions. The high level of (+)-gossypol in *G. hirsutum* var. *marie-galante* may represent retention of originally broader allelic variation in only this one lineage, or novel allelic variation may have arisen at the foundation of this lineage. Alternatively, tetraploid interaction between the A and D genomes could account for its high (+)-gossypol ratio if this character was observed in the A1 or A2 species. The number of accessions within the Germplasm Collection for the A1 (*G. herbaceum*) and A2 (*G. arboreum*) genomes is extremely large, and reports on the (+)-gossypol ratios in these genomes are scattered through the literature. We selected six accessions at random from each of the A1 and A2 genomes. Only one was significantly outside the normal (+)- to (−)-gossypol range; this was A2-236, with 78% (+)-gossypol. Thus, neither our data nor those reported by others (*24*, *25*) support the hypothesis that tetraploid interaction accounts for the high (+)-gossypol ratio in *marie-galante* embryos. Selection could also account for the high (+)-gossypol ratio in *marie galante*. These accessions were collected in Central and South America and the Caribbean Islands. Folklore indicates that these seeds were used for medicinal purposes, animal feed, and human food. The latter uses could have favored the selection of the less toxic high-(+)-gossypol seed.

Among the wild species accessions surveyed, there was little correlation between embryo weight, total gossypol concentration, and percentage of (+)-gossypol. In the AD and D1 genome accessions, the total gossypol concentration tends to decrease

with increasing embryo weight and the percent (+)-gossypol tends to decrease with increasing total gossypol concentration. In the D3 and D5 genome accessions, the reverse was observed with total gossypol concentration tending to increase with embryo weight. However, all of these correlations were weak. In other groups of accessions, no correlations were observed. These results suggest that seed weight, gossypol concentration, and gossypol composition are under independent genetic control, and any type of coregulation is not maintained between accessions or species.

We did not find any accessions that contained >65% (−)-gossypol. Thus, it is unlikely that traditional breeding techniques could be used to attain a goal of >95% (−)-gossypol in cotton seed (**Table 2**). Thus, producing large amounts of "pure" (−)-gossypol for pharmaceutical applications directly from a domestic or wild source remains elusive.

We did identify several new sources of wild cottons that produce high levels of (+)-gossypol. However, introducing the high-(+)-gossypol trait into domestic cottons using these wild species would be at best problematic. Fortunately, within Upland cotton, accessions of *G. hirsutum* var. *marie-galante* have been identified that produce >95% (+)-gossypol in the seed (*25*). Among the *marie-galante* accessions, we found significant variations in percent (+)-gossypol within some accessions (Supporting Information). This variation is probably due to the lack of parental uniformity. Many of the *marie-galante* accessions were obtained from "backyard" collections, which were themselves of mixed lineage. Nevertheless, we have identified several new accessions including no. 2469 that are probably the best sources for the high-(+)-gossypol trait (Supporting Information). We are currently pursuing this goal using traditional breeding techniques (*20*).

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Supporting Information Available: Embryo weight, gossypol concentration, and percent (+)-gossypol for the seed embryos of the wild species and selected accessions from *G. herbaceum*, *G. arboreum*, and *G. barbadense*, as well as 94 accessions of *G. hirsutum* var. *marie-galante* available from the U.S. National Cotton Germplasm Collection. This information is available free of charge via the Internet at http://pubs.acs.org.

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