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## EXECUTIVE SUMMARY

This report is a summation of the background, objectives, and achievements of the research project funded by CRDC grant CSP47C. We would like to emphasize four particularly notable achievements.

- We assessed the risk of transgene escape from commercial cotton cultivars into wild Australian cotton species
- We synthesizing 18 new fertile intergenomic *Gossypium* polyploids
- We documented the production of unreduced gametes in hybrids between cultivated cottons and wild species indigenous to the Kimberley Plateau.
- We synthesized self-fertile cotton germplasm lines that incorporate genetic material from wild cotton species.

The significance of these achievements and their impact on sustainable cotton production in Australia are elaborated in later sections. Generally speaking, we have been successful in

- increasing our basic biological understanding of the wild Australian cotton species
- developing germplasm resources for exploiting the wild Australian cotton in the development of improved cotton cultivars, and
- assuring that the commercial release of transgenic cotton cultivars in Queensland and New South Wales will not endanger the wild cotton species or their ecosystems.

## GENERAL OBJECTIVES

Australia's 17 native wild cotton species do not have direct commercial potential because their lint lacks the mechanical properties necessary for spinning, however, they possess traits that would improve commercial cotton varieties (*G. barbadense* and *G. hirsutum*). In this project, we focused on the lack of 'gossypol' in the seeds of some of the wild Australian species. 'Gossypol' is a generic term for a suite of chemicals (terpenoid aldehydes) in cotton and its wild relatives that serve as effective natural insecticides and fungicides. Without 'gossypol' cotton plants would be virtually defenseless, unfortunately, this very toxicity that makes 'gossypol' effective against insects and fungi, also makes the seed oils and protein meals poisonous to non-ruminant animals. Removing 'gossypol' increases production costs and reduces the nutritional quality of the protein meal. The ideal cotton cultivar would have high concentrations of 'gossypol' in the leaves, stems, flowers, and fruit, but not in the seed. Among the 50+ *Gossypium* species distributed around the world, only a few Australian species were said to possess this trait. Unfortunately, much of the published literature about the 'gossypol' free seeds of the wild Australian cottons was contradictory or incomplete. Before we proceeded with a hybridization program to transfer this characteristic to cultivated cottons, we needed a better understanding of this trait and its distribution among the wild Australian cotton species.

The ability to transfer genes from wild species to cultivated relatives using traditional breeding rests on the ability to generate fertile hybrids. Hybrid fertility, in turn, depends a great deal on the ability of the chromosomes from the two parents to pair and segregate uniformly during meiosis. Because the wild cotton species have half (26) the number of chromosomes of the cultivated cottons (52), and these chromosomes differ greatly in structure, it was unlikely that they could make functional male or female gametes. We needed to overcome this barrier to develop useful germplasm lines. Literature reports suggested that for a few species, hybridization was possible, but the hybrid would be sterile. For the remaining species, there was no information. It was important then to determine which hybrid combinations were possible, and for those that were whether the sterility of the hybrid could be circumvented.

The process of trying to overcome the barriers to gene transfer between two species in the glasshouse would also allow us to estimate the probability that this could occur in nature. Ultimately this information would be particularly useful to government regulatory bodies (NRA and GMAC) charged with identifying and evaluating the potential environmental consequences of commercial release of transgenic cotton cultivars in New South Wales and Queensland. Wild cotton species that capture transgenes may have a selective advantage over other natural species. The result could be a new invasive weed with the potential to disrupt natural ecosystems. If this hypothetical weed spread to cotton fields, it could be difficult to control because cultivated cottons would be equally susceptible to effective herbicides. During the course of the grant, we expanded our objectives to include an assessment of the probability that transgenes could escape from transgenic cotton into wild species.

## BACKGROUND

Historically, the wild Australian *Gossypium* species have played a minor role in cotton breeding. Before 1980, this was mostly due to the rarity of material in germplasm collections, which contained only the commonest species (e.g., *G. australe* & *G. sturtianum*). A series of collecting expeditions in the 1980s addressed this situation (including CSP41C & CSP33C), and the CSIRO *Gossypium* germplasm collection now contains accessions of every known species. This presents the opportunity to use all the *Gossypium* species that evolved in Australia to develop better Australian cotton cultivars. This section summarises ongoing the strategies we used to overcome the extensive

crossing barriers that preclude exchange of genetic material between the wild Australian *Gossypium* species and the cultivated cottons in nature.

**Evolutionary origin of the crossing barriers between cultivated cotton and the wild Australian *Gossypium* species:** The 44 diploid\* *Gossypium* species arose from a common ancestor approximately 24-33 million years ago and diverged into geographically distinct lineages that have subsequently evolved independently (Fryxell, 1979; Wendel and Albert, 1992). During the following millennia, as the foliage, flowers, and fruit differentiated to various degrees, the size and structure of the chromosomes in each lineage also diverged. Consequently, the diploid *Gossypium* species can be organised into groups, called genomes, based on similarities in chromosome size and structure (Table 1; Endrizzi *et al.*, 1985; Stewart, 1995). Each genome represents a group of morphologically similar species that, with few exceptions, are unable to form fertile hybrids with species from other genomes.

The Australian *Gossypium* species belong to one of three genomes, C, G, or K (Table 1; Fig. 1; Stewart, 1995). The C genome contains Sturt's Desert Rose (*G. sturtianum*) and its Western Australian relative, *G. robinsonii*. The G genome comprises the widespread *G. australe* (sometimes mistaken for Sturt's Desert Rose), the morphologically similar *G. nelsonii*, and *G. bickii*. The 12 K genome species are endemic to the monsoonal regions of northwestern Australia.

The cultivated cottons grown in Australia are tetraploids that contain both the African/Asian A genome and the New World D genome. The cultivated tetraploid cottons arose 1-2 million years ago when a diploid A genome species hybridised with a diploid D genome species (Wendel, 1989). Although this original hybrid no longer exists, it somehow gave rise to a plant with two full sets of A and D chromosomes. The resultant tetraploid survived and diverged into five species. Two of these species, *G. barbadense* and *G. hirsutum*, were domesticated by indigenous New World Indians. The modern cultivars of *G. barbadense* are known as Pima or Egyptian cotton. It is the modern *G. hirsutum* cultivars, however, that dominate world cotton commerce accounting for approximately 90% of the annual world cotton crop (Lee, 1984). The cottons cultivated in Australia belong mostly to *G. hirsutum* but some *G. barbadense* cultivars are grown.

The ancestor of the Australian C, G, and K genomes diverged from the ancestor of the A and D parents of the cultivated cottons very early in the evolution of *Gossypium* and thus the Australian C, G, and K genomes differ from the A and D subgenomes of the cultivated cottons by 24-33 million years of accumulated chromosomal differences as well as by the number of chromosomes. The Australian *Gossypium* species are diploids with 26 chromosomes while the Australian tetraploid cotton cultivars contain 26 A and 26 D genome chromosomes. When wild Australian *Gossypium* species are crossed directly to cultivated cottons, the triploid plants recovered cannot produce viable gametes and thus are sterile.

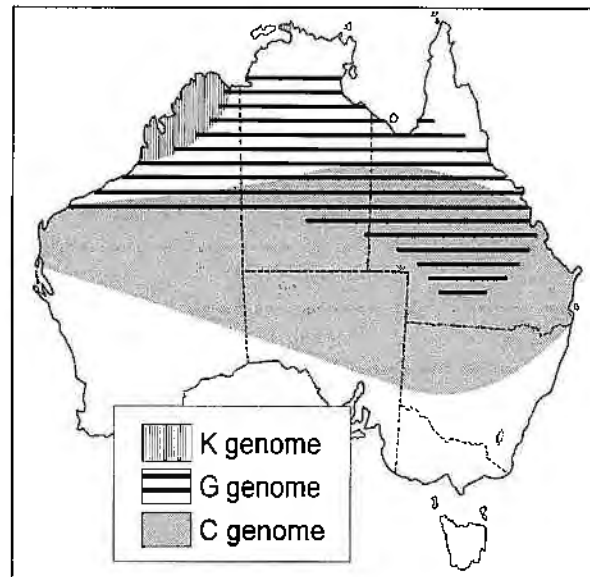


Fig. 1. Distribution of the three wild Australian *Gossypium* genomes (Adapted from Craven *et al.*, 1995)

Table 1. Geographic distribution of the diploid *Gossypium* genomes.

Genomes	No. of species	Location
A	2	Africa/Asia
B	4	Africa
C	2	Australia
D	13	New World
E	7	Arabia
F	1	Africa
G	3	Australia
K	12	Australia

\* Ploidy refers to the number of sets of chromosomes. In *Gossypium* each set contains 13 chromosomes. The prefix indicates the number of sets present. Diploids (the most typical condition) carry two sets of chromosomes, triploids carry three, tetraploids four, pentaploids five, and hexaploids six.

**Breeding strategies using wild**

**Australian *Gossypium* species:** The first step to incorporating wild Australian germplasm into cotton cultivars is generating fertile breeding stocks that contain C, G, or K as well as A and D chromosomes (Stewart, 1995). The key to overcoming this barrier is to treat hybrid plants with colchicine, which doubles the number of chromosomes. This creates compatible pairs of chromosomes allowing the plant to produce viable gametes. This serves, however, to reduce rather than promote interaction between chromosomes of different genomes. Ultimate success depends on generating fertile intergenomic hybrids that promote interaction between the C, G, or K chromosomes and the A or D chromosomes of cultivated cottons so genes can be transferred. Without this transfer, the genes of interest will be lost in future backcross generations as the C, G, or K chromosomes are lost. Figure 2 illustrates the theoretical pathways for transferring wild Australian germplasm into cultivated cotton (Stewart, 1995).

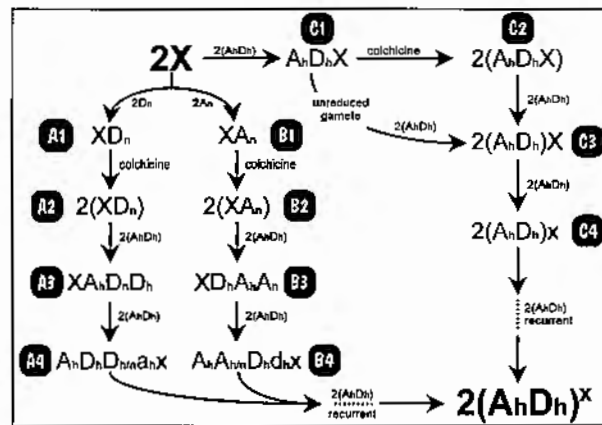


Fig. 2. Theoretical pathways for transferring wild Australian *Gossypium* genes to cultivated cotton. Upper case letters indicate complete chromosome sets; lower case letters indicate partial chromosome sets. Ah and Dh represent the A and D subgenomes of *G. hirsutum*; An, and Dn represent any of the A or D genome diploid species; X represents any of the C, G, or K wild Australian *Gossypium* genomes. Adapted from Stewart (1995).

The A1-4 and B1-4 pathways (Fig. 2) start with hybridizing a C, G, or K genome species with a diploid A or D genome species to produce a sterile diploid hybrid (A1 & B1). At this stage chromosomal interactions can be extensive (Phillips, 1966), but these genetic interchanges are lost because the hybrid is sterile. Using colchicine to double the chromosomes produces synthetic tetraploids (A2 & B2), but each chromosome set has a duplicate set with which to interact, reducing intergenomic interactions. To force the C, G, or K chromosomes to interact with either A or D chromosomes, the A3 and B3 synthetic tetraploids are crossed with *G. hirsutum* to create XADD or XDAA hybrids (A3 & B3; X represents any of the C, G, or K species). If these hybrids prove fertile, they can be backcrossed to *G. hirsutum*, hopefully leading to new *G. hirsutum* breeding stocks that carry genes from the wild Australian *Gossypium* species ( $2(A_hD_h)^*$ ). One significant limitation occurs at steps A4 and B4 (Fig. 2). Because of the incompatibility between C, G, or K chromosomes and A and D chromosomes, it is probable that many gametes produced by the A3 and B3 plants will not carry a full set of chromosomes and the A4 and B4 plants would not be full tetraploids. If this occurs it may be necessary to use colchicine to double the chromosomes again before continuing to backcross.

The third strategy (C1-4; Fig. 2) involves crossing a C, G, or K diploid directly with *G. hirsutum*. The triploid recovered is infertile but once colchicine doubled, the synthetic AADDXX hexaploid can be crossed with *G. hirsutum*. The result is a pentaploid with 5 sets of chromosomes: 2 sets of A and D chromosomes and one set of C, G, or K chromosomes. While the compatible interactions of the A and D chromosomes should be sufficiently stable to allow production of some fertile gametes, the wild Australian *Gossypium* chromosomes are free to interact with other chromosomes. With each succeeding generation these extra chromosomes will be shed until a true tetraploid AADD plant is recovered. Hopefully at some point in the preceding generations, a transfer of genetic material between the C, G, or K chromosomes and the A or D chromosomes would occur.

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## HIGHLIGHTS

### RISK ASSESSMENT OF TRANSGENIC COTTONS

Our research documented that the probability that transgenes could escape into the wild species (*G. australe* and *G. sturtianum*) native to the Queensland and New South Wales cotton growing regions is functionally zero. Hybrids between *G. australe* and cultivated cotton can only be synthesized using extreme human intervention (embryo rescue). Despite extensive effort, we could not synthesize any hybrids using moderate levels of intervention (hormone application). Of all the wild Australian cottons, *G. sturtianum* is the species that hybridizes most readily with cultivated cottons, and the hybrids are vigorous and floriferous. They are, however, completely sterile which represents an insurmountable barrier to gene leakage into wild populations.

This information was communicated to the cotton industry in a variety of ways. Dr. Brown testified at the December 1995 GMAC hearing and presented these data at The Commercialisation of Transgenic Crops Workshop (11-13 March 1997) held in Canberra. The workshop presentation was reiterated in a paper in the workshop proceedings (Brown et al., 1996). Dr. Brubaker subsequently provided Monsanto with a detailed report of our findings (Brubaker and Brown, 1997) and was later asked to attend the 25 September 1997 National Registration Authority hearing regarding the expansion of cotton production into central Queensland and western New South Wales.

Fortunately, the natural barriers between the wild cotton species of Queensland and New South Wales are sufficiently formidable to prevent the escape of transgenes. Our research suggests, however, that in the Ord, the probability of transgene escape may not be zero. Our data indicate that the wild cotton species of the Kimberley will hybridize with cultivated cottons with greater difficulty than for *G. sturtianum*, but without the extreme intervention necessary for *G. australe*. The reason for concern is that these hybrids can backcross directly to cultivated cotton. The result is a viable plant with low fertility. This is not to say that transgene escape in the Ord is a certainty, but that one of the primary barriers to gene exchange in the east (complete hybrid sterility) does not exist in the west. Our research highlights the need to insure that other natural barriers are sufficient to prevent transgene escape. We informally communicated these data to relevant cotton industry representatives and anticipate doing so formally in the near future. We will continue to provide the cotton industry with relevant data as it needs.

### SYNTHESIS OF 18 NEW FERTILE INTERGENOMIC ALLOPOLYPLOIDS

We synthesized 18 new fertile intergenomic allopolyploids between wild Australian *Gossypium* species and cultivated cotton (*G. hirsutum*). All of these new fertile hybrids are unique, and six represent new species combinations that have never before been synthesized.

Hybrids of this type are almost always sterile which prevents direct backcrossing to cotton (a few exceptions are described below). While those concerned with the risks of transgene escape find this reassuring, we needed overcome this barrier. The usual means of restoring fertility to intergenomic hybrids is to double the number of chromosomes with colchicine. One of the causes of infertility in

intergenomic hybrids is that the chromosomes from each parent are too dissimilar to pair uniformly at meiosis. The result is sterile gametes. Doubling the chromosomes provides every chromosome with a copy of itself with which to pair. In most cases, this restores sufficient fertility to allow backcrossing to cultivated cotton. We have been successful with 16 accessions and anticipate recovering more over the next year.

These new hybrids have a number of future uses. In keeping with one of the primary objectives of this grant, these hybrids can be backcrossed to cultivated cotton to provide avenues of gene introgression. Seven hybrids incorporate *G. sturtianum* as the male or female parent while the other parent is either a glanded or glandless cotton. These stocks will allow us to determine how the lack of terpenoid aldehyde production ('gossypol') in *G. sturtianum* seeds is expressed in hybrids with cultivated cottons. It is further anticipated that these hybrids will be used to study the parameters controlling chromosome structural evolution in nascent natural polyploids.

#### **CAPTURE OF UNREDUCED GAMETES FROM KIMBERLEY COTTON HYBRIDS**

Although, doubling chromosomes with colchicine is a reliable method of restoring fertility to intergenomic hybrids, it is time consuming. If intergenomic  $F_1$ s could be backcrossed directly, we could bypass several generations. This would shorten the time required to obtain the first backcross generation by one year. There was limited evidence that hybrids between cultivated cotton and two wild Australian species will produce unreduced gametes. This is a natural phenomenon that essentially doubles the number chromosomes in a small percentage of gametes. If these unreduced gametes are paired with fertile gametes from another source the result is a viable embryo:

In concert with efforts to double chromosome numbers, we backcrossed  $F_1$  hybrids to the cultivated parent for a variety of accessions. When [*G. hirsutum* x *G. sturtianum*] undoubled hybrids are backcrossed to *G. hirsutum* no progeny were obtained. This reinforces the conclusion that should any naturally occurring hybrids between cultivated cotton and *G. sturtianum* arise (none have ever been reported) their sterility safeguards against transgene escape. Hybrids between *G. hirsutum* and the wild Australian cotton species of the Kimberley Plateau, however, do form a small percentage of these unreduced gametes. This allowed us to proceed directly to backcrossing without the one year delay of chromosome doubling. We successfully generated 13 direct backcross progeny involving four Kimberley species. The fertility of these plants is low but not zero. Attempts at further backcrossing are underway and  $BC_2$  progeny have been generated.

#### **RESTORATION OF SELF-FERTILITY IN BACKCROSS PROGENY**

The restoration of fertility in backcross progeny between cultivated cotton and the wild Australian species although necessary is not sufficient to transfer traits between species. The fertility of the initial hybrids is too low to generate self-fertilized seed and deliberate crossing is required to generate further backcross progeny. Continued backcrossing, however, eventually produces progeny that are self-fertile.

In parallel with our efforts to develop new fertile intergenomic hybrids, we have been backcrossing two hexaploid stocks that incorporate *G. australe* and *G. sturtianum* germplasm. These two species are particularly interesting because they have seeds that are truly 'gossypol'-free (see below). Although the initial hybrids had very low fertilities, we have successfully backcrossed for enough generations to generate self-fertile derivatives. This means that within the next year, we will have sufficient seed stocks to begin screening lines for the 'gossypol'-free trait.

**SPECIFIC OBJECTIVES**

The primary grant objective was to develop new hybrid germplasm lines that facilitate the transfer of genes from indigenous Australian *Gossypium* species to cotton cultivars or provide useful genetic stocks for experimental genetics studies. To this end we made more than 10,000 crosses over the past three years. Not only will the hybrids generated be useful in a variety of research projects for the next decade, the data gathered while generating these crosses has been directly relevant to the risk assessment of transgenic cottons.

**Synthesis of intergenomic hybrids between *Gossypium arboreum* and wild Australian species of *Gossypium* (Table 2).** We generated 16 intergenomic hybrids between the Old World cultivated cotton, *G. arboreum*, and six *Gossypium* species indigenous to Australia. For *G. australe*, *G. bickii*, and *G. nelsonii* this was necessary because we were unable to generate hybrids between these species and *G. hirsutum*. The others were generated to determine if the hybrid was possible.

Table 2. Number of established hybrids between *G. arboreum* x Australian *Gossypium* species

Female	Male	Plants
<i>G. arboreum</i>	<i>G. australe</i>	3
"	<i>G. bickii</i>	3
"	<i>G. costulatum</i>	1
"	<i>G. nelsonii</i>	6
"	<i>G. nobile</i>	2
"	<i>G. pilosum</i>	1

**Synthesis of intergenomic hybrids between *Gossypium hirsutum* cultivars and wild Australian species of *Gossypium* (Table 3).** We generated 80 intergenomic hybrids between *G. hirsutum* and 12 species of Australian *Gossypium*. These plants were used in risk assessment studies and for generating fertile synthetic polyploids.

Table 3. Number of established hybrids between *G. hirsutum* and Australian *Gossypium* species..

Female	Male	plants	Female	Male	plants
Glandless cultivars	<i>G. enthyale</i>	3	Glanded cultivars	<i>G. costulatum</i>	5
(Gregg 25V,	<i>G. exiguum</i>	4	(Sicala V2, Siokra V15,	<i>G. exiguum</i>	3
Paymaster 784)	<i>G. nobile</i>	5	CS189+,	<i>G. londonderriense</i>	2
	<i>G. pilosum</i>	8	Tamcot CAMDE)	<i>G. nobile</i>	1
	<i>G. populifolium</i>	5		<i>G. pilosum</i>	8
	<i>G. pulchellum</i>	1		<i>G. robinsonii</i>	5
	<i>G. robinsonii</i>	5		<i>G. rotundifolium</i>	3
	<i>G. rotundifolium</i>	7		<i>G. anapoides</i>	3
	<i>G. anapoides</i>	3		<i>G. sturtianum</i>	4
	<i>G. sturtianum</i>	5			

Table 4. Summary of newly synthesized fertile intergenomic tetraploids and hexaploids incorporating wild Australian *Gossypium* germplasm.

Female	Ploidy	Genomic Designation
<i>G. arboreum</i> x <i>G. bickii</i>	4N	AAGG
<i>G. arboreum</i> x <i>G. bickii</i>	4N	AAGG
<i>G. hirsutum</i> x <i>G. anapoides</i>	6N	AADDKK
<i>G. hirsutum</i> x <i>G. enthyale</i> x <i>G. australe</i>	6N	AADDKG
<i>G. hirsutum</i> x <i>G. exiguum</i>	6N	AADDKK
<i>G. hirsutum</i> x <i>G. populifolium</i> x <i>G. australe</i>	6N	AADDKG
<i>G. hirsutum</i> x <i>G. robinsonii</i>	6N	AADDCC
<i>G. hirsutum</i> x <i>G. sturtianum</i> -1	6N	AADDCC
<i>G. hirsutum</i> x <i>G. sturtianum</i> -2	6N	AADDCC
<i>G. hirsutum</i> x <i>G. sturtianum</i> -3	6N	AADDCC
<i>G. hirsutum</i> x <i>G. sturtianum</i> -4	6N	AADDCC
<i>G. hirsutum</i> x <i>G. sturtianum</i> -5	6N	AADDCC
<i>G. hirsutum</i> x <i>G. sturtianum</i> -6	6N	AADDCC
<i>G. hirsutum</i> x <i>G. sturtianum</i> -7	6N	AADDCC
<i>G. hirsutum</i> x <i>G. sturtianum</i> -8	6N	AADDCC
<i>G. hirsutum</i> x <i>G. sturtianum</i> x <i>G. australe</i>	6N	AADDCCG
<i>G. sturtianum</i> x <i>G. hirsutum</i>	6N	CCAADD
<i>G. sturtianum</i> x <i>G. hirsutum</i> x <i>G. exiguum</i>	6N	CADADK

**Generation of Synthetic Polyploids:**

The original purpose of synthesizing intergenomic hybrids between cultivated cottons and the wild Australian *Gossypium* species was to generate new breeding stocks. To do this, the sterile triploids described above were treated with colchicine. This chemical doubles the number of chromosomes, restoring at least partial fertility to intergenomic hybrids. We have so far been successful with 18 hybrids (Table 4). We anticipate more in the future. All of these newly fertile hybrids are unique and many combine species from which fertile hybrids have never been successfully synthesized.

**Backcrossing synthetic hybrids to**

**cultivated cotton.** To speed the development of new germplasm, we imported a number of existing synthetic polyploids. We focused on the four listed in Table 5. These four hybrids

Table 5. Synthetic polyploids imported from Belgium.

GOS-5267	2X[ <i>G. arboreum</i> $\boxtimes$ <i>G. australe</i> ] A <sub>2</sub> A <sub>2</sub> C <sub>3</sub> C <sub>3</sub>
GOS-5268	2X[ <i>G. thurberi</i> $\boxtimes$ <i>G. sturtianum</i> ] D <sub>1</sub> D <sub>1</sub> C <sub>1</sub> C <sub>1</sub>
GOS-5271	2X[ <i>G. hirsutum</i> $\boxtimes$ <i>G. sturtianum</i> ] A <sub>3</sub> A <sub>3</sub> D <sub>3</sub> D <sub>3</sub> C <sub>1</sub> C <sub>1</sub>
GOS-5269	2X[ <i>G. hirsutum</i> $\boxtimes$ <i>G. australe</i> ] A <sub>3</sub> A <sub>3</sub> D <sub>3</sub> D <sub>3</sub> C <sub>3</sub> C <sub>3</sub>

incorporate wild Australian species that have 'gossypol'-free seeds. It proved difficult to generate backcross progeny with the synthetic tetraploids (GOS-5267 & GOS-5268) and these lines have been abandoned. The hexaploids lines have been amenable to backcrossing, however. An initial pentaploid hybrid between GOS-5271 and *G. hirsutum* led to the development of 10 BC<sub>1</sub> lines. From these, we have obtained eight BC<sub>2</sub> lines. Three of BC<sub>1</sub> and three BC<sub>2</sub> lines are sufficiently self-fertile that BC<sub>1</sub>F<sub>2</sub> and BC<sub>2</sub>F<sub>2</sub> seed are being obtained. We have had greater success with GOS-5269. From the initial F<sub>1</sub> hybrid, we generated 19 BC<sub>1</sub> lines. Twelve of these were sufficiently self-fertile to generate BC<sub>1</sub>F<sub>2</sub> seed. We have also derived 14 BC<sub>2</sub> lines from which were are beginning to get BC<sub>2</sub>F<sub>2</sub> and BC<sub>3</sub> seed. The earliest stages of this process are arduous, but with the each generation the level of self-fertility increases. This will allow us to generate enough seed for small scale trials in the near future.

**Developing interspecific hybrids among the indigenous Australian *Gossypium* species.** Diploid hybrids among the wild Australian *Gossypium* species represent important genetic stocks in research programs designed to unravel the genetic basis of traits of possible agronomic utility (Table 6). Three of these hybrids are particularly important. HYB-601 is a cross between *G. nelsonii* and *G. australe*. This hybrid is self-fertile and has been used to generate an F<sub>2</sub> mapping population from which we will develop the first molecular genetic map of a Australian *Gossypium* genome. Equally important are the *G. sturtianum* x *G. nobile* hybrids. Contrary to expectations these hybrids can be backcrossed to *G. sturtianum* yielding BC<sub>1</sub> seed. Because these hybrids combine a species with 'gossypol'-free seeds (*G. sturtianum*) with a species that has 'gossypol' in its seeds (*G. nobile*), this

Table 6. Amount of F<sub>1</sub> seed generated from inter-specific hybrids among the wild species of Australian *Gossypium*.

Female	Male	Seed	Female	Male	Seed
<i>G. australe</i>	<i>G. bickii</i>	8	<i>G. populifolium</i>	<i>G. australe</i>	15
"	<i>G. nelsonii</i>	22	"	<i>G. bickii</i>	4
"	<i>G. sturtianum</i>	2	"	<i>G. enthyale</i>	15
<i>G. bickii</i>	<i>G. australe</i>	153	"	<i>G. exiguum</i>	11
<i>G. cunninghamii</i>	<i>G. costulatum</i>	12	"	<i>G. pilosum</i>	2
<i>G. enthyale</i>	<i>G. exiguum</i>	2	<i>G. pulchellum</i>	<i>G. londonderriense</i>	4
"	<i>G. londonderriense</i>	1	"	<i>G. marchantii</i>	4
"	<i>G. marchantii</i>	5	"	<i>G. nobile</i>	3
"	<i>G. nobile</i>	3	"	<i>G. populifolium</i>	4
"	<i>G. pilosum</i>	1	"	<i>G. rotundifolium</i>	11
"	<i>G. populifolium</i>	5	<i>G. robinsonii</i>	<i>G. sturtianum</i>	26
"	<i>G. pulchellum</i>	2	<i>G. rotundifolium</i>	<i>G. exiguum</i>	1
"	<i>G. rotundifolium</i>	1	"	<i>G. marchantii</i>	14
<i>G. exiguum</i>	<i>G. costulatum</i>	2	"	<i>G. nobile</i>	10
"	<i>G. londonderriense</i>	13	"	<i>G. pilosum</i>	4
"	<i>G. marchantii</i>	12	"	<i>G. populifolium</i>	1
"	<i>G. nobile</i>	4	<i>G. sturtianum</i>	<i>G. australe</i>	20
"	<i>G. pilosum</i>	1	"	<i>G. bickii</i>	1
"	<i>G. populifolium</i>	11	"	<i>G. enthyale</i>	53
<i>G. nelsonii</i>	<i>G. australe</i>	14	"	<i>G. exiguum</i>	26
<i>G. nobile</i>	<i>G. enthyale</i>	4	"	<i>G. nelsonii</i>	27
"	<i>G. marchantii</i>	3	"	<i>G. nobile</i>	11
"	<i>G. populifolium</i>	3	"	<i>G. populifolium</i>	24
"	<i>G. rotundifolium</i>	5	"	<i>G. pulchellum</i>	41
<i>G. pilosum</i>	<i>G. londonderriense</i>	1	"	<i>G. robinsonii</i>	39
"	<i>G. populifolium</i>	8	"	<i>G. rotundifolium</i>	72
			"	<i>G. species nova</i>	53

presents an unprecedented opportunity to characterize the genetics of this trait.

**Analysis of the 'gossypol'-free seed trait.** As stated earlier, the literature presents a contradictory picture of the distribution and anatomic basis for the 'gossypol'-free seed trait among the wild Australian cottons. We clarified this confusion by surveying, anatomically and chemically, seeds of all the known Australian *Gossypium* species. These data are reported in manuscript published in the *Australian Journal of Botany* (Brubaker et al, 1996). The abstract of this manuscript appears below. This manuscript represents the outcome of a productive and ongoing collaboration with DN Leach and CG Benson of the University of Western Sydney--Hawkesbury.

**"Occurrence of Terpenoid Aldehydes and Lysigenous Cavities in the 'Glandless' Seeds of Wild Australian *Gossypium*"**

C. L. Brubaker, C. G. Benson, C. Miller, and D. N. Leach

**Abstract:** The presence of lysigenous cavities filled with terpenoid aldehydes (generically termed 'gossypol') in most tissues of cultivated cottons and their relatives imparts natural resistance to a variety of insect, fungal, and bacterial pests. Deposition of terpenoid aldehydes in cultivated cotton seed, however, renders cottonseed oils and protein meals toxic to non-ruminant animals, including humans. Seeds of the so-called 'glandless-seeded' Australian *Gossypium* species (*Gossypium* subgenus *Sturtia* (R. Brown) Todaro) reportedly lack terpenoid aldehydes, and thus may represent an important genetic resource in the development of cottonseed free of these toxins. Information supporting this assertion, however, is fragmentary and contradictory. To resolve this, seeds of all known Australian *Gossypium* species were surveyed chemically and anatomically. Immature lysigenous cavities were present in seeds of all 18 species. Lysigenous cavities of sect. *Sturtia* and sect. *Hibiscoidea* Todaro seeds were unpigmented and invisible to the naked eye, while pigmented, macroscopically visible lysigenous cavities occurred in all the sect. *Grandicalyx* seeds. HPLC analysis revealed that sect. *Sturtia* and sect. *Hibiscoidea* seeds did not contain detectable levels of terpenoid aldehydes (> 0.035 ppm), but that sect. *Grandicalyx* (Fryxell) Fryxell seeds contained gossypol.

**Assaying variation in fatty acid profiles:** The proportional representation of various fatty acids in oils is the primary determinant of their culinary and industrial properties. Fatty acid profiles of representative accessions of each native Australian *Gossypium* species were obtained [in collaboration with Ross Downes (CSIRO Plant Industry)]. Preliminary evaluation of the data suggest that the variation observed is mostly distributed among three groups of species corresponding to the three taxonomic subdivisions of the native Australian *Gossypium* species.

**Capture of unreduced gametes from intergenomic hybrids:** The process of restoring fertility to intergenomic hybrids takes nearly a year. This delay can be avoided in those hybrids that produce some unreduced gametes. This is a natural chromosome doubling mechanism. When this occurs, however, only a small proportion of gametes are unreduced. To increase the probability of capturing unreduced gametes hybrids can be pollinated with pollen of a compatible and fully fertile species. There was limited evidence that intergenomic hybrids between *G. hirsutum* and the wild cotton species from the Kimberley Plateau (K genome) do produce some unreduced gametes. We re-investigated this phenomenon by pollination intergenomic triploids between *G. hirsutum* and the wild Australian *Gossypium* species. None of the hybrids between the C genome species and *G. hirsutum* produced viable gametes when crossed directly to *G. hirsutum*, but intergenomic hybrids between *G. hirsutum* and three of the Kimberley species (*G. anapoides*, *G. nobile*, and *G. populifolium*) produced nine pentaploid derivatives. We have already successfully backcrossed five of these pentaploids to *G. hirsutum* to produce BC<sub>2</sub> seed.

## **PUBLICATIONS**

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- Brubaker, C. L., A. H. D. Brown, J. P. Grace, and M. Kilby. 1996. Using wild Australian *Gossypium* germplasm in cotton breeding. Proceedings of the 8th Australian Cotton Conference, 14-16 August, 1996, Broadbeach, QLD, Australia. Australian Cotton Growers' Research Association.

## **COMMERCIAL POTENTIAL**

This research has two commercially important components. The process of hybridizing wild Australian *Gossypium* species to *G. hirsutum* has provided critical data for evaluating the risk of commercial cultivation of transgenic cottons in Queensland and New South Wales. Additional data will be necessary if transgenic cottons are to be approved for cultivation in Ord region of northern Western Australia. The 'gossypol'-free seed trait of some of the wild Australian *Gossypium* species would significantly improve the profitability of cotton.

## **IMPLEMENTATION OF THE RESEARCH**

This research project will continue along several avenues. We will continue to cooperate with the cotton industry in providing the data necessary for risk assessments of transgenic cottons. The plant germplasm generated provide the necessary basis for new research projects. Two of these have already begun. The *G. nelsonii* x *G. australe* hybrid was selected for the basis of molecular marker mapping. An F<sub>2</sub> population has been generated and is growing. This population will be used to develop the first molecular genetic map of an Australian *Gossypium* genome. At the same time *G. sturtianum* x *G. nobile* hybrids are being backcrossed to *G. sturtianum* to generate a population in which the gene(s) for the 'gossypol'-free seed trait will segregate, allowing us to determine the genetic control of this trait. At the same time we will continue develop our backcross lines toward field evaluation. This work is funded under a new CRDC grant (CSP85C). We are also organizing a collaboration with Dr. J. F. Wendel (Iowa State University) and Dr. J. M. Stewart (University of Arkansas) that would use our newly synthesized polyploids to characterize the genomic processes associated with the evolution of allopolyploids.