USING WILD AUSTRALIAN *GOSSYPIUM* GERMPLASM IN COTTON BREEDING

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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, 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 article summarises ongoing work to develop strategies 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

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. *Di*ploids (the most typical condition) carry two sets of chromosomes, *tri*ploids carry three, *tetra*ploids four, *penta*ploids five, and *hexa*ploids six.

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.

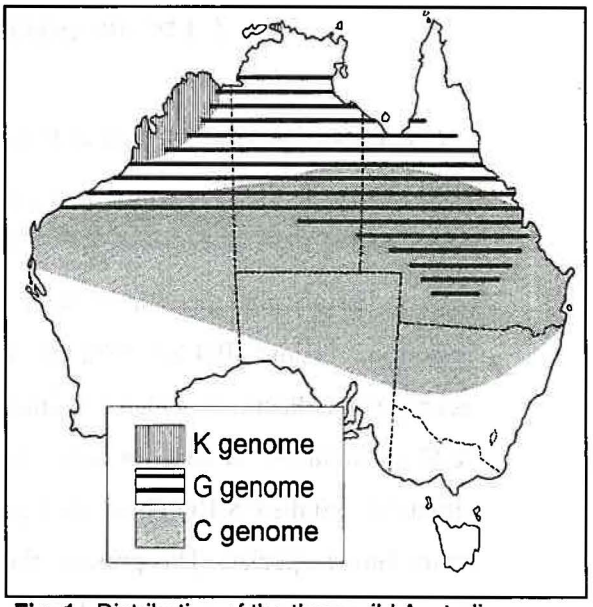

Fig. 1. Distribution of the three wild Australian *Gossypium* genomes (Adapted from Craven *et al.*, 1995)

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.

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).

The A1-4 and B1-4 pathways (Fig. 2) start with hybridising 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 ($2(AhDh)^x$). 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.

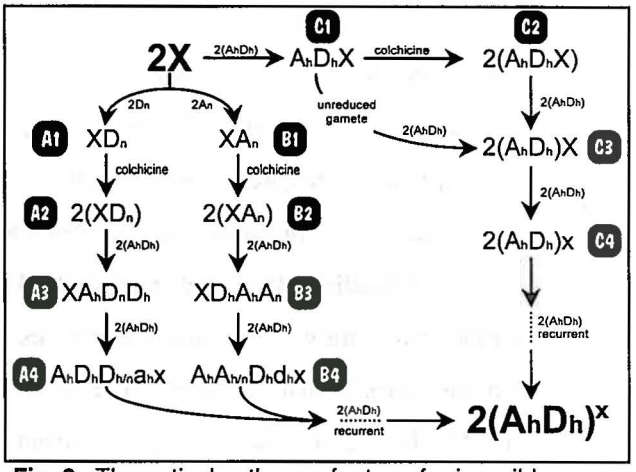

Fig. 2. Theoretical pathways for transferring wild Australian *Gossypium* genes to cultivated cotton. Upper case letters indicate complete chromosome sets; lower case letters indicate partial chromosome sets. Ah and Dh represent the A and D subgenomes of *G. hirsutum*; An, and Dn represent any of the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

The 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 Australian *Gossypium* chromosomes will be shed until a true tetraploid AADD plant is recovered. Hopefully at some point in the preceding generations, there has been a transfer of genetic material between the C, G, or K chromosomes and the A or D chromosomes.

Status of wild Australian *Gossypium* species cotton breeding: In the past two years, we have actively pursued all strategies diagrammed in Fig. 2.

Strategy A is based on a *G. thurberi* x *G. sturtianum* synthetic CCDD tetraploid (A2). This accession readily produces hybrids when crossed with *G. hirsutum*. The 10 CADD (A3) hybrids recovered to date, are relatively infertile. Maximum viable pollen (assayed using tetrazolium chloride; TTC) does not exceed 4%, but 170 crosses with *G. hirsutum* produced 51 seeds. Four ADDac (A4) plants have been recovered from the 20 seeds planted to date. Of these, one is reproductively mature and has produced a single seed from eight crosses to *G. hirsutum*.

Strategy B is based on a *G. arboreum* x *G. australe* synthetic AAGG tetraploid (B2). Twenty-seven crosses to *G. hirsutum* produced 17 seeds from which 15 plants have been recovered. Four plants died soon after reaching reproductive maturity; the remaining 11 GDAA (B3) plants have produced 6 seed from 89 crosses to *G. hirsutum*. Of the five plants assayed for pollen fertility, maximum percent viable pollen ranges from 3 to 8 percent.

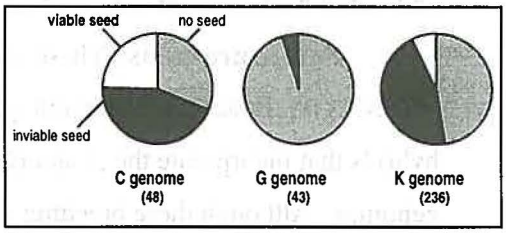

Fig. 3. Proportion of *G. hirsutum* x wild Australian *Gossypium* crosses producing no seed, inviable seed, and viable seed. Parenthetic numbers indicate number of crosses attempted.

Strategy C has been the primary focus. The initial step was to generate as many ADC, ADG, and ADK triploids as possible (Fig. 3). ADC triploids are relatively easy to generate: 39 of 48 crosses produced 283 seed, and 19 plants have been recovered from the 73 seeds planted. ADG triploids are difficult to generate, 43 *G. hirsutum* x G genome species crosses produced no viable seed. Importing a *G. hirsutum* x *G. australe* synthetic hexaploid from Belgium (see below) obviated the need for continued crossing. ADK triploids can be obtained but at lower frequencies than ADC triploids. Two hundred and thirty-six crosses produced 552 seed; 64 plants were recovered from 464 planted seeds. Although 15 plants have subsequently died, many that remain are reasonably vigorous.

There is some evidence that ADK triploids will produce unreduced gametes (Stewart, 1995). This eliminates the need to create colchicine-induced hybrids and bypasses the C2 generation (Fig. 2). In initial crossing experiments, involving the two C genome species and three K genome species, of 131 crosses to *G. hirsutum* only the *G. hirsutum* x *G. populifolium* triploid produced seeds (6) from which three healthy presumably pentaploid AADDK C3 seedlings were recovered. While most of the ADK triploids generated will be treated with colchicine, a fraction will be retained and crossed to *G. hirsutum* immediately.

Two synthetic hexaploids, 2(*G. hirsutum* x *G. australe*) and 2(*G. hirsutum* x *G. sturtianum*) were imported from Belgium and crossed with *G. hirsutum* (Maréchal, 1983). When these synthetic hexaploids are crossed to *G. hirsutum*, the AADDC and AADDG pentaploids (C3) recovered are nearly male sterile with no greater than 5

percent viable pollen, but they do have sufficient female fertility to generate C4 generation 4N+ derivatives. To date 25 C4 plants have been recovered, most of which are reproductively mature. The morphological characteristics and fertility of these hybrids varies considerably with the number of chromosomes retained and the genetic information they contain. Initial estimates of pollen fertility range from 0-72 percent and initial backcrossing indicate that most of these C4 plants are also female fertile.

Future prospects: These results indicate that it is possible, using the three strategies (A, B, & C) detailed in Fig. 2, to generate functionally fertile intergenomic hybrids that incorporate the A and D subgenomes of *G. hirsutum* with the C, G, or K genomes. Although these breeding strategies should optimise intergenomic interactions, transfer of genes of agronomic utility will be infrequent. As this work progresses, increasing attention will be devoted to developing methodologies to promote gene transfers as well as the screening protocols necessary to identify progeny with new agronomically useful traits.

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