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## Comparative genetic linkage maps of *Eucalyptus grandis*, *Eucalyptus globulus* and their F<sub>1</sub> hybrid based on a double pseudo-backcross mapping approach

Received: 25 February 2003 / Accepted: 12 May 2003 / Published online: 1 July 2003  
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**Abstract** Comparative genetic mapping in interspecific pedigrees presents a powerful approach to study genetic differentiation, genome evolution and reproductive isolation in diverging species. We used this approach for genetic analysis of an F<sub>1</sub> hybrid of two *Eucalyptus* tree species, *Eucalyptus grandis* (W. Hill ex Maiden.) and *Eucalyptus globulus* (Labill.). This wide interspecific cross is characterized by hybrid inviability and hybrid abnormality. Approximately 20% of loci in the genome of the F<sub>1</sub> hybrid are expected to be hemizygous due to a difference in genome size between *E. grandis* (640 Mbp) and *E. globulus* (530 Mbp). We investigated the extent of colinearity between the two genomes and the distribution of hemizygous loci in the F<sub>1</sub> hybrid using high-throughput, semi-automated AFLP marker analysis. Two pseudo-backcross families (backcrosses of an F<sub>1</sub> individual to non-parental individuals of the parental species) were each genotyped with more than 800 AFLP markers. This allowed construction of de novo comparative genetic linkage maps of the F<sub>1</sub> hybrid and the two backcross parents. All shared AFLP marker loci in the three single-tree parental maps were found to be colinear and little evidence was found for gross chromosomal rearrangements. Our results suggest that hemizygous AFLP loci are dispersed throughout the *E. grandis* chromosomes of the F<sub>1</sub> hybrid.

**Keywords** Comparative mapping · AFLP · *Eucalyptus* · Transmission ratio distortion · Genome synteny

### Introduction

*Eucalyptus* tree species constitute the most-widely planted exotic hardwood crop in tropical and subtropical regions of the world. Superior, fast-growing hybrids of *Eucalyptus* have been planted in many of these regions and this has resulted in large increases in productivity (Eldridge et al. 1993). Most successful hybrids of *Eucalyptus* have originated from crosses between species in three sections of the subgenus *Symphyomyrtus*, namely *Latoangulatae*, *Maidenaria* and *Exsertaria* (Brooker and Kleinig 1994; Steane et al. 2002). However, many of these superior hybrids represent rare genotypic combinations, especially in crosses between species in different sections of the subgenus. In these wide crosses, strong postzygotic reproductive barriers between the parental species frequently result in very low seed set and high proportions of abnormal hybrid plants (Griffin et al. 1988).

Very little information is available on genome evolution and genetic differentiation in *Eucalyptus*. This information is required to understand the nature and occurrence of reproductive barriers, and the genetic basis of hybrid superiority in this genus. Genetic linkage mapping in interspecific hybrids have been used to study the interactions of differentiated genomes in several crosses of plant species (reviewed by Rieseberg et al. 2000). These studies have revealed the polygenic nature of postzygotic reproductive barriers in plants and have suggested that the extreme phenotypes often observed in hybrid progeny may be caused by transgressive segregation. In *Eucalyptus*, genetic linkage maps have been produced in several interspecific crosses, all within the subgenus *Symphyomyrtus* (Grattapaglia and Sederoff 1994; Verhaegen and Plomion 1996; Marques et al. 1998; Brondani et al. 2002). These linkage maps provided excellent genetic frameworks for quantitative trait locus

Communicated by O. Savolainen

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(QTL) analysis of traits such as growth, vegetative propagation and wood quality in F<sub>1</sub> hybrid progeny (Grattapaglia et al. 1995; Verhaegen et al. 1997; Marques et al. 1999). However, despite the recent development of a genome-wide set of microsatellite markers for *Eucalyptus* (Brondani et al. 1998) and promise for a genus-wide reference linkage map (Brondani et al. 2002), no detailed comparative genome maps have been produced for any pair of eucalypt species.

The interspecific cross of *Eucalyptus grandis* (W. Hill ex Maiden.) and *Eucalyptus globulus* is an example of a wide cross between eucalypt tree species that results in high amounts of hybrid inviability and hybrid abnormality (Griffin et al. 2000). *E. grandis* (the Flooded Gum) is a subtropical species and a member of the section *Latoangulatae*, while *E. globulus* (the Tasmanian Blue Gum) is a temperate species and a member of the section *Maidenaria*. The two species have the same haploid chromosome number ( $n = 11$ ), but the estimated physical genome sizes of *E. grandis* (640 Mbp) and *E. globulus* (530 Mbp) differ significantly (Grattapaglia and Bradshaw 1994). Approximately 20% of the genetic material is expected to be hemizygous in F<sub>1</sub> hybrids of these two species. The distribution of hemizygous loci in the *E. grandis* chromosomes of F<sub>1</sub> hybrids relative to the *E. globulus* homologs, and possible effects of hemizygous loci on hybrid fitness is not known. The two species also have several contrasting wood properties, which make this interspecific cross an excellent framework for QTL analysis of commercially important traits such as wood density, lignin content and pulp yield.

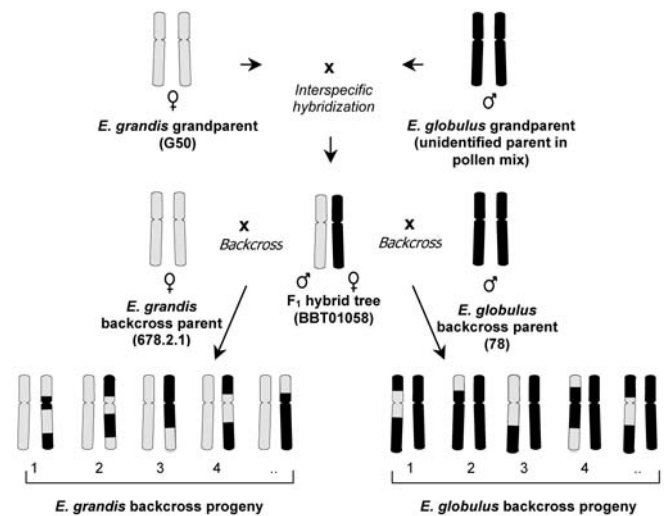
Here we present results of a survey of colinearity between the genomes of *E. grandis* and *E. globulus* based on the comparative mapping of shared AFLP markers in an interspecific pseudo-backcross pedigree of these two species. We report the first nearly complete genetic linkage map of a wide interspecific hybrid of *Eucalyptus*. Phase-known, paternal and maternal genetic maps of an F<sub>1</sub> hybrid of *E. grandis* and *E. globulus* are presented, together with genetic maps of the *E. grandis* and *E. globulus* individuals used as backcross parents. We also demonstrate the efficient use of the “double pseudo-backcross” mapping approach and high-throughput molecular marker technology to obtain comparative genetic linkage maps of the parental genomes involved in this three-generation, outbred pedigree.

## Materials and methods

### High-throughput AFLP marker analysis

#### Plant materials

All plant materials used in this study were generated by Shell Forestry and maintained by Shell Uruguay Renewables S.A. at a field site near Paysandu, Uruguay. A large interspecific, polymix F<sub>1</sub> hybrid progeny set was produced by controlled pollination of selected *E. grandis* mother trees with two 10-tree *E. globulus* pollen mixes. A single superior F<sub>1</sub> individual, tree BBT01058 (Forestal Oriental S.A., Uruguay), from the progeny of one of the *E. grandis*



**Fig. 1** The pseudo-backcross crossing scheme and genetic structure of the mapping populations. One homologous chromosome pair is shown for each tree. The sex of the parents used in the crosses is shown below each genotype. Note: the F<sub>1</sub> hybrid was used as a male (pollen) parent in the backcross to *E. grandis* and a female (seed) parent in the backcross to *E. globulus*. Although all *E. grandis* DNA is shown in white and *E. globulus* DNA in black, the parental trees represented here are all highly heterozygous

mother trees (G50, CSIR, South Africa), was selected for backcrossing to the parental species (Fig. 1). The backcrosses were made to unrelated individuals in *E. grandis* and *E. globulus* to avoid potential inbreeding depression in the backcross progeny. The hybrid tree (referred to here as the F<sub>1</sub> hybrid) was used as the female parent in the backcross to *E. globulus* tree 78, (Forestal y Agrícola Monte Aguila S.A., Chile). In the backcross to *E. grandis* tree 678.2.1 (Forestal Oriental S.A., Uruguay) the F<sub>1</sub> hybrid was used as a male parent. The backcrosses were made in this direction to avoid a unilateral crossing barrier (Gore et al. 1990) between the large-flowered *E. globulus* and small-flowered *E. grandis* parents.

Seedlings of the two backcross families were grown in trays in a seedling nursery. Leaf samples were obtained from the seedlings for DNA extraction before they were planted out in a field site near Paysandu, Uruguay, for phenotypic evaluation.

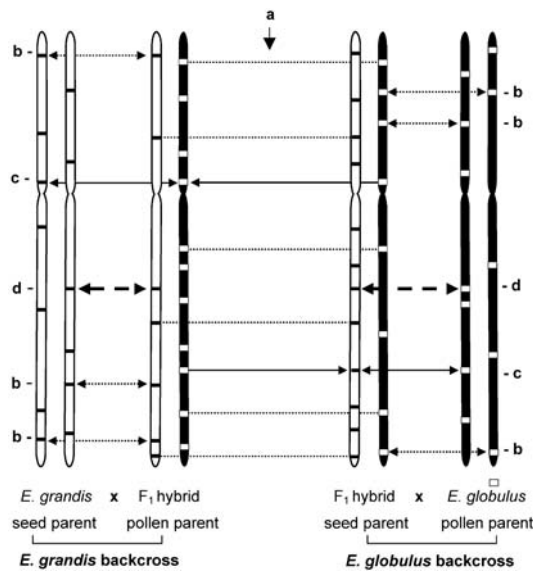
#### DNA isolation

DNA extraction was performed as described elsewhere (Myburg et al. 2001). After homogenization of fresh leaf material using a FastPrep FP120 Instrument (QBIogene, Carlsbad, Calif., USA), high quality genomic DNA samples were obtained using a 96-well DNA isolation method as implemented in the DNeasy 96 Plant kit (QIAGEN, Valencia, Calif., USA).

#### High-throughput AFLP analysis

We modified the original AFLP procedure (Vos et al. 1995) to allow high-throughput, multiplexed AFLP genotyping based on infrared detection of labeled AFLP fragments on LI-COR (Lincoln, Neb.) automated DNA sequencers (Remington et al. 1999; Myburg et al. 2001). This procedure was used to obtain AFLP banding patterns for 24 *EcoRI/MseI* (+3/+3) selective primer combinations in all the backcross progeny. The 24 AFLP primer combinations were previously selected by primer screening in *Eucalyptus* (Marques et al. 1998).

The LI-COR TIFF images were scored using the AFLP-Quantar software program (version 1.05, Keygene products B.V., Wageningen-



**Fig. 2** Schematic of the “double pseudo-backcross” approach used for comparative mapping in this interspecific pedigree. Only one homologous chromosome pair of each parent is shown, with the physical positions of polymorphic marker loci along each homolog. Marker loci on each homolog represent one linkage phase of the corresponding genetic linkage group. Different types of shared markers are indicated: *a* shared testcross (1:1) markers were used to align the paternal and maternal maps of the  $F_1$  hybrid. *b* Intercross (3:1) markers were used to align the maps of each backcross parent with those of the  $F_1$  hybrid. *c* Markers in testcross configuration in one backcross, but intercross configuration in the other backcross, and *d* shared intercross fragments provided additional information for comparative mapping. Note that in each direction of backcrossing, more marker loci were polymorphic in the donor homolog (or linkage phase) of the  $F_1$  hybrid than in the recurrent homolog

gen, The Netherlands) with band scoring parameters set as described in Myburg et al. (2001). Data points flagged as unreliable by AFLP-Quantar were manually re-evaluated and treated as missing data if a reliable marker score was not possible. Fragments that could not be scored reliably, or contained an excess of unreliable scores (>5%) were excluded from the study.

#### De novo framework map construction

##### Mapping approach and data quality

Each pseudo-backcross family was analyzed using the “two-way pseudo-testcross” mapping approach (Grattapaglia and Sederoff 1994). The AFLP marker data were divided into three parts based on the parental genotype of each AFLP fragment: (1) 1:1 segregating (testcross) fragments present only in the  $F_1$  hybrid, (2) 1:1 segregating (testcross) fragments present only in the backcross parent, and (3) 3:1 segregating (intercross) markers present in both parents of the backcross family. Four testcross marker sets were therefore obtained from the two backcrosses (Fig. 2) and were used for construction of four separate single-tree, framework linkage maps; i.e. a maternal map of the *E. grandis* backcross parent, a paternal map of the *E. globulus* backcross parent, and separate paternal and maternal maps of the  $F_1$  hybrid. The intercross fragments segregating in each backcross family were not used for framework map construction, but were used to align the maps of the two parents of each backcross family.

Backcross individuals with more than 10% missing marker data were dropped from the input data sets before framework map construction in MAPMAKER version 2.0 for Macintosh (Lander et

al. 1987). A chi-square analysis was performed on all AFLP fragments to test for departure from expected Mendelian genotypic ratios. As expected for this wide interspecific cross, a large proportion of AFLP fragments with distorted genotypic ratios was observed. Because removal of these fragments would have drastically lowered map coverage, the distorted markers were included in all phases of linkage map construction.

##### Marker grouping and framework mapping

The  $F_2$  backcross model of MAPMAKER was used for marker grouping and ordering. Marker grouping was evaluated for each marker set at LOD linkage thresholds of 7.0 to 12.0 in steps of 1.0. All of the mapping sets were separated into at least 11 major linkage groups at LOD thresholds ranging from 10.0 to 12.0. Marker ordering and framework marker selection were performed on the groups obtained at these thresholds.

The criteria used for framework marker selection in each linkage group were as follows: after initial ordering of all the testcross fragments using the “First Order” command of MAPMAKER, internal markers that expanded the map length by more than 7.0 cM (based on output from the “Drop Marker” command) were removed. The “First Order” step was repeated after removing each marker. Support for the order of remaining internal markers was subsequently evaluated using the “Ripple” function of MAPMAKER. Markers that were too close to other markers to obtain a LOD interval support of at least 3.0 for all three-point orders involved, were removed from the map. The “First Order” step was repeated again after removing each marker. Terminal markers were evaluated using the “TwoPoint/LOD Table” command of MAPMAKER. End markers that showed stronger pairwise linkage to internal markers other than their immediate neighbors were removed. This process was repeated iteratively until all internal and terminal markers conformed to the framework marker criteria described above.

The classical estimate of recombination is often biased when distorted genotypic ratios occur in neighboring markers. This bias is especially pronounced when distortion is in opposite directions, or when the level of distortion differs greatly (Lorieux et al. 1995). We used Bailey’s estimate of recombination frequency (Bailey 1949) to re-calculate all framework map distances to evaluate the effect of transmission ratio distortion on each framework marker interval.

##### Species identity of linkage phases in the $F_1$ hybrid

The two linkage phases of the maps of the  $F_1$  hybrid represent haplotype maps of the *E. grandis* and *E. globulus* gametes that the  $F_1$  hybrid received from its parents. The parental origin of AFLP fragments in the  $F_1$  hybrid was determined by genotyping the seed parent of the  $F_1$  hybrid, i.e. *E. grandis* tree G50 (Fig. 1). AFLP fragments that were absent from *E. grandis* tree G50, but present in the  $F_1$  hybrid, were identified as markers inherited from the (unknown) *E. globulus* pollen parent of the hybrid tree. Based on the linkage-phase assignment of these markers, it was possible to identify the *E. globulus* linkage phase (and *E. grandis* linkage phase) of each linkage group in the  $F_1$  hybrid.

##### Estimated genome length and coverage

The total genome length of each parental framework map was estimated with the commonly used Hulbert estimate (Hulbert et al. 1988), as implemented in method 3 of Chakravarti et al. (1991). We also used a modified estimator described by Remington et al. (1999). This estimate corrects for an upward bias related to chromosome ends. A pairwise linkage threshold of LOD 11.0 was used for the estimation of genome length, because all of the parental testcross sets were separated into at least 11 major linkage groups at LOD 11.0, and the set of pairwise linkages observed at

this empirically determined threshold should include very few false-linkages.

Genome coverage was calculated for each framework map using  $c = 1 - e^{-2dn/L}$ , where  $c$  is the proportion of the genome within  $d$  cM of a framework marker,  $L$  is the estimated genome length and  $n$  is the number of framework markers in the map (Lange and Boehnke 1982).

#### Bin mapping of accessory markers

AFLP fragments that were not selected as framework markers were mapped to framework marker intervals using the bin-mapping function of the software program MapPop version 1.0 (Vision et al. 2000). Based on a previous estimate of scoring error in the same marker data (Myburg et al. 2001), an expected scoring error of 2% was used for the bin-mapping procedure. The bin-mapping procedure of MapPop did not support the placement of accessory markers outside the terminal framework markers of each linkage group. Therefore, AFLP fragments that were not successfully placed during the bin-mapping procedure were evaluated for linkage to terminal framework markers using the "TwoPoint/LODs" command of MAPMAKER.

#### Comparative mapping

Linkage group synteny was first established for the paternal and maternal maps of the  $F_1$  hybrid by identifying shared framework markers in these two maps. These markers were all testcross AFLP fragments that were inherited from one of the parents of the  $F_1$  hybrid, but were absent in both of the backcross parents. Linkage groups of the paternal and maternal maps of the hybrid were manually aligned using the shared framework markers.

Intercross fragments (heterozygous in both parents of a backcross family) were used to establish synteny of the backcross parent maps to that of the  $F_1$  hybrid. In order to use the  $F_2$  backcross model of MAPMAKER for the placement of intercross markers in a fixed testcross marker map, we re-coded the band-present data of intercross markers as missing data. This resulted in a maximum of only 25% of individuals being informative for linkage evaluation between the dominantly scored intercross markers and testcross markers in the framework map. We therefore had to use a lower LOD threshold for linkage than for pairs of testcross markers. The distance between each intercross marker and the nearest framework marker was first determined with the "TwoPoint/Near" command of MAPMAKER using a LOD threshold of 5.0 and  $\theta = 0.2$ . The most-likely interval placement of the intercross marker was then determined using the "Multipoint/Try" command of MAPMAKER. The approximate positions of intercross markers that could be placed in both parental maps of the particular backcross were used to manually align the maps of the backcross parent with the maps of the  $F_1$  hybrid.

## Results

### AFLP marker analysis

#### Data quality

A total of 186 individuals of the *E. grandis* backcross family and 188 of the *E. globulus* backcross family were genotyped for linkage mapping using 24 AFLP primer combinations. Approximately 50% of scored AFLP fragments were classified as framework quality (f) fragments based on the ease and accuracy of band-calling during the semi-automated scoring procedure in AFLP-Quantar. The remaining fragments that were close to other polymorphic or monomorphic fragments, or required manual editing after semi-automated scoring, were labeled as putative accessory (a) markers. This approach allowed us to enrich the framework maps with high quality AFLP fragments. Only individuals with more than 90% of scored marker data were included in the final testcross data sets (156 in the *E. grandis* backcross and 177 in the *E. globulus* backcross). This criterion resulted in overall rates of missing data of approximately 3%, of which approximately half could be accounted for by data points labeled as unreliable by AFLP-Quantar.

#### Marker polymorphism

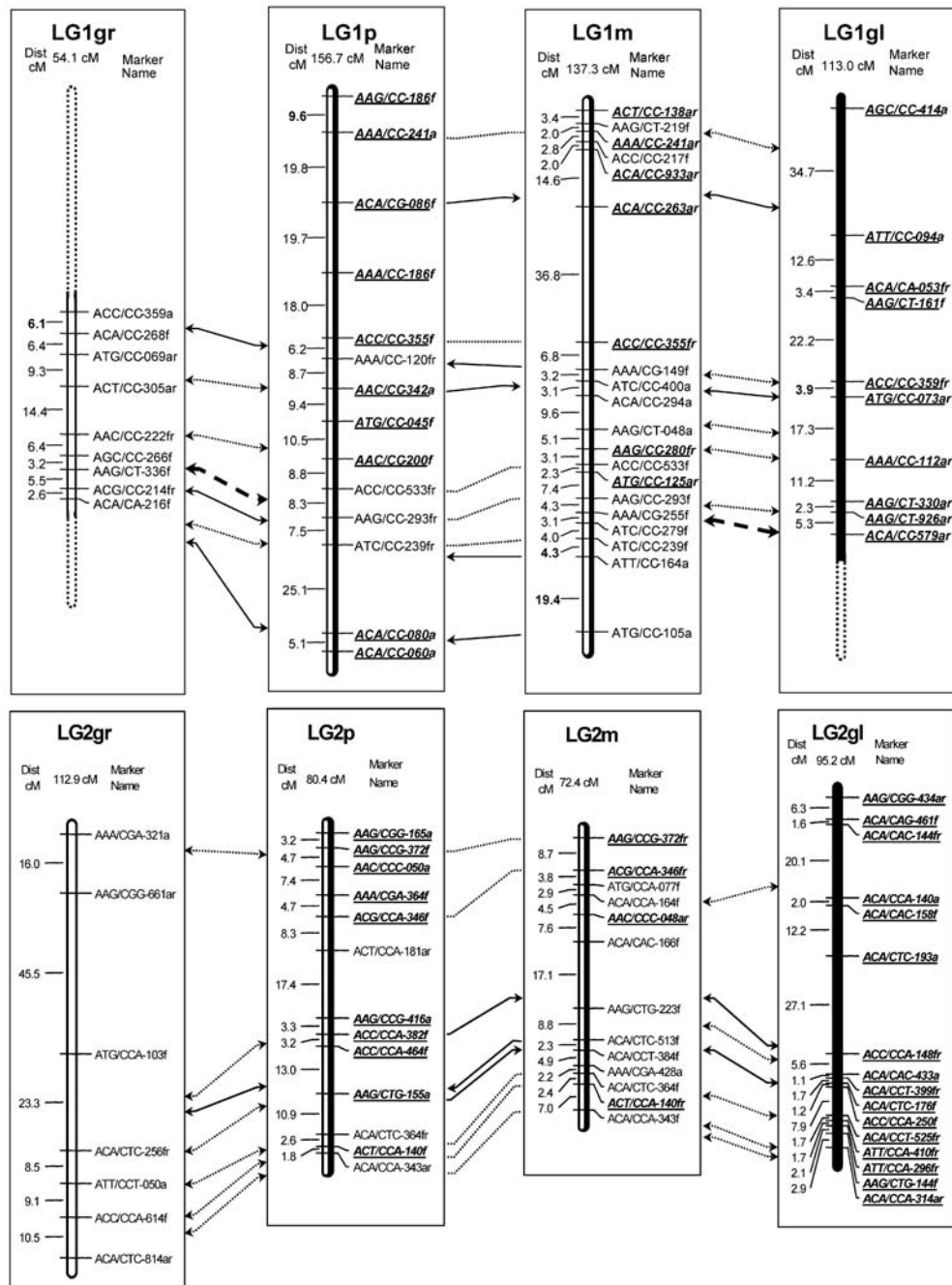
An average of 35 (50%) of the AFLP fragments produced by each primer combination were polymorphic and scorable in the two backcross families. The majority of testcross fragments in each backcross family segregated out of the  $F_1$  hybrid (Table 1), consistent with the higher expected heterozygosity of the  $F_1$  hybrid relative to that of the two backcross parents. In addition, approximately 25% more testcross loci segregated in the maternal map of the  $F_1$  hybrid (*E. globulus* backcross), than in the paternal map (*E. grandis* backcross, Table 1).

The two backcross families shared a significant number of polymorphic AFLP fragments. The total number of AFLP fragments scored in the two backcross families was 1,627. A total of 368 AFLP fragments (45% in each family) segregated as testcross and/or intercross markers in both families. Of these, 183 (22.5%) were shared testcross fragments in the maternal and paternal

**Table 1** Summary of AFLP fragments scored in each backcross family and levels of transmission ratio distortion observed for each parental marker set

Segregation type	<i>E. grandis</i> BC family	<i>E. globulus</i> BC family
$F_1$ hybrid – # testcross (1:1) fragments	365 (45.2%) Paternal	457 (55.0%) Maternal
% Distorted <sup>a</sup>	28.5%	30.6%
Backcross parent – # testcross (1:1) fragments	285 (35.7%) Maternal	247 (30.6%) Paternal
% Distorted <sup>a</sup>	30.9%	20.6%
Both parents – # intercross (3:1) fragments	153 (19.1%)	120 (14.4%)
% Distorted <sup>a</sup>	32.0%	25.8%
Total	803	824

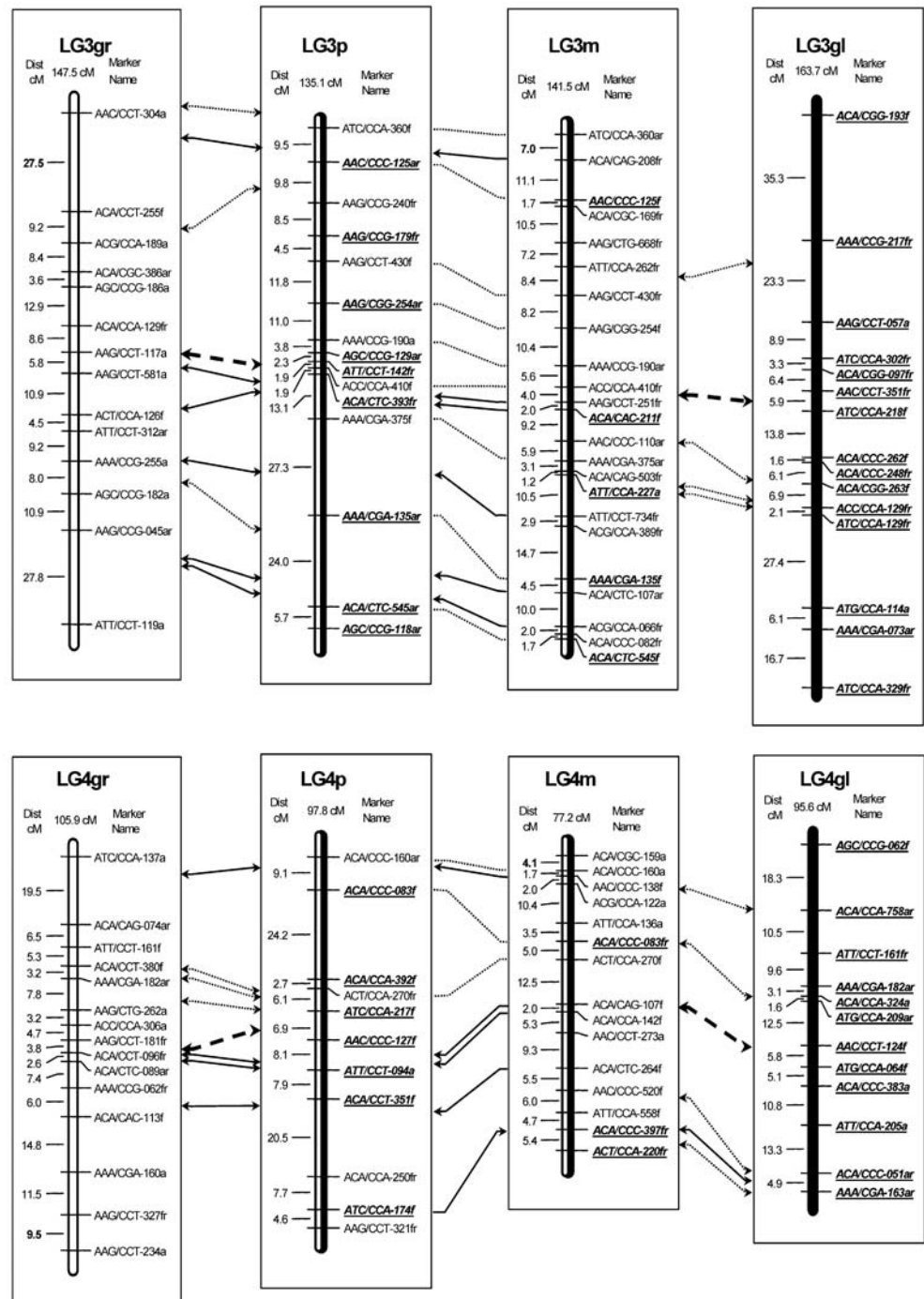
<sup>a</sup> Percentage of fragments in each testcross or intercross set with genotypic ratios that deviated significantly from the expected ratio of 1:1 (for testcross) or 3:1 (for intercross) at the 0.05 level of significance



**Fig. 3** Comparative synteny maps of the  $F_1$  hybrid of *E. grandis* and *E. globulus* and of the two backcross parents. Linkage group numbers are followed by parental codes for the maternal (*gr*), paternal (*p*) and maternal (*m*) maps of the  $F_1$  hybrid tree, and paternal map of the *E. globulus* backcross parent (*gl*). The *E. grandis* linkage groups and the *E. grandis* linkage phase for the  $F_1$  hybrid maps are shown as white bars, while *E. globulus* linkage groups and linkage phases are shown as black bars. AFLP markers that originated in the *E. globulus* backcross parent or grandparent are typed in bold, italics and are underlined. *E. grandis* markers are in normal font. Shared marker types are the same as in Fig. 2. Horizontal dotted lines (type a) indicate testcross markers shared by the two maps of the  $F_1$  hybrid. Horizontal dotted arrows (type b) indicate the approximate

positions of intercross markers in the two parental maps of each backcross family. Solid arrows (type c) indicate AFLPs that segregated as intercross markers in one backcross and as testcross markers in the other backcross. Heavy dashed arrows (type d) indicate AFLPs that were heterozygous in all three parents and therefore segregated as intercross markers in both backcross families. Regions with low map coverage in the backcross parents are indicated with dotted lines. Distances in centiMorgan (cM) Kosambi are indicated on the left of each linkage group and the total map length of each group is given at the top. Intervals in bold were modified using the recombination estimate of Bailey (1949). Marker names consist of the selective nucleotides of the AFLP primer combination and the molecular size (bp) of each marker, followed by a quality (f or a) and phase (r) indicator

Fig. 3 (continued)

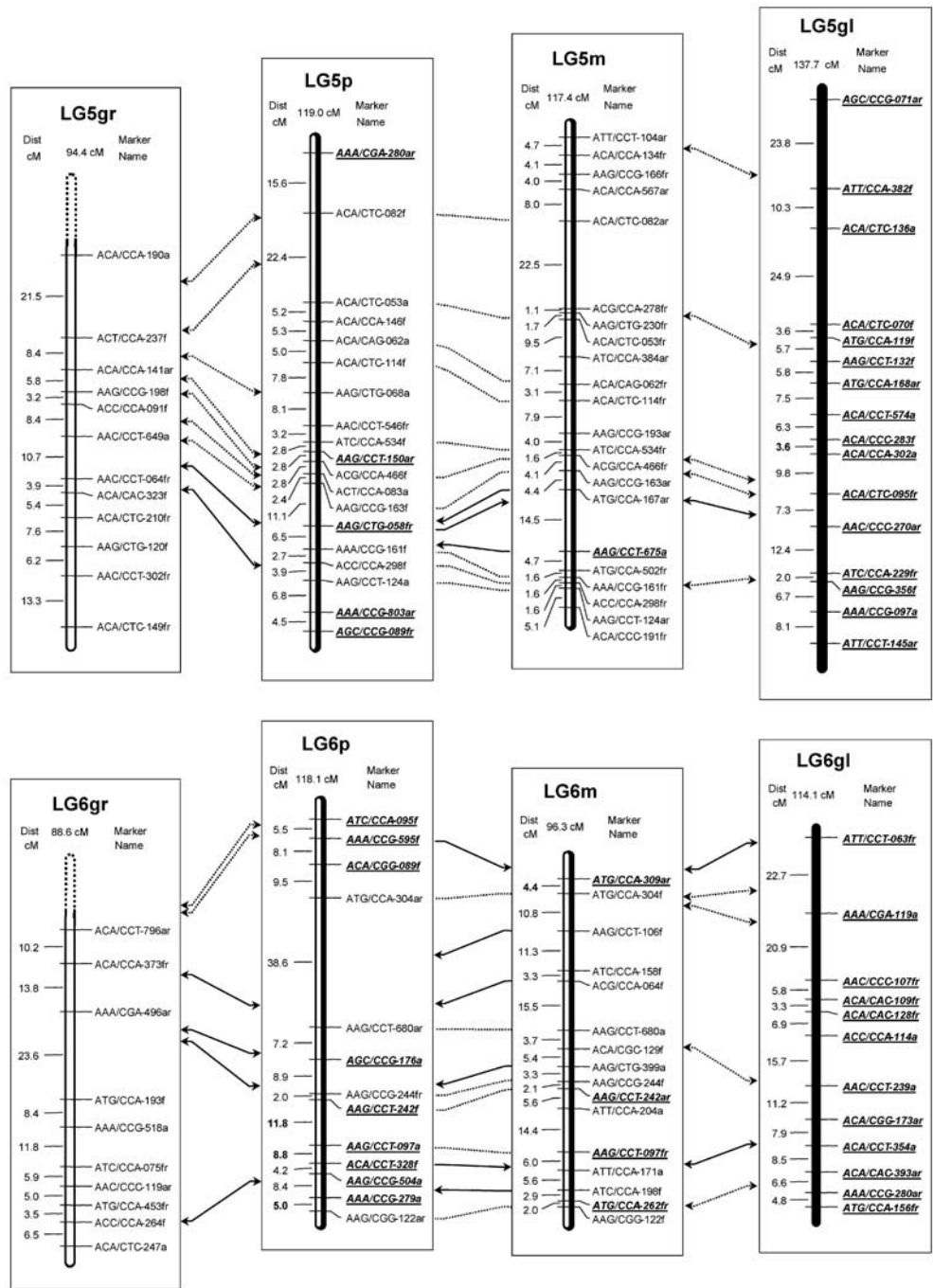


maps of the  $F_1$  hybrid. As expected from this wide cross, a much lower proportion (4.8%) of polymorphisms were shared between the pure-species (backcross) parents and only 2% of AFLP fragments were heterozygous in all three parents. The total number of unique AFLP fragments scored in the two backcross families was therefore 1,259. However, 42 pairs of putative allelic AFLP fragments were observed, which reduces the total number of AFLP loci genotyped in this study to 1,217.

#### Transmission ratio distortion

The genotypic ratios of a relatively large proportion of testcross and intercross fragments deviated significantly from expectation in the two backcross families at the 0.05 level of significance (Table 1). In less than 0.5% of cases, distorted genotypic ratios of AFLP fragments could be explained by their misclassification as testcross or intercross markers due to incorrect parental genotypes (data not shown). The majority of the distorted AFLP fragments were successfully mapped onto the appropriate

Fig. 3 (continued)



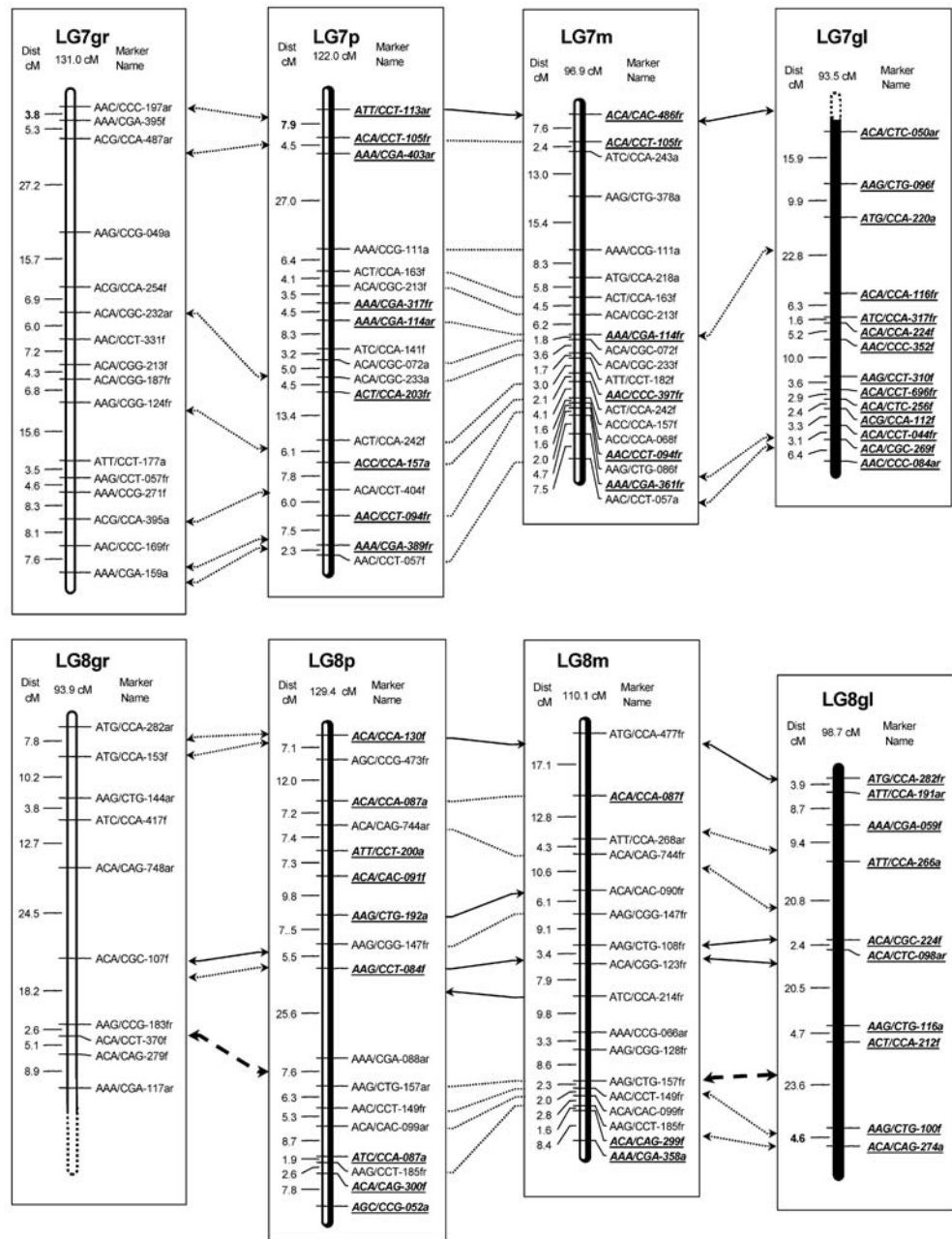
parental maps and were most likely distorted due to linked genetic factors that reduced the fertilization ability of F<sub>1</sub> hybrid gametes or the viability of backcross individuals. Interestingly, approximately the same proportion of testcross AFLPs were distorted in the two backcross parents as in the F<sub>1</sub> hybrid (Table 1), which suggests that genetic factors that affect hybrid fitness may also be segregating in these two parents. The results of a genome-wide analysis of transmission ratio distortion in the three parental trees are presented elsewhere (Myburg et al. 2003).

Framework map construction

Marker grouping

For each parental testcross data set, a threshold *P*-value for two-point linkage declaration was calculated to reduce the experiment-wide likelihood of obtaining false linkages to a target value of less than 0.05 (Remington et al. 1999). These *P*-values corresponded to LOD linkage thresholds of approximately 6.3 to 6.8 (data not shown). However, we found that LOD thresholds of 10.0 to 12.0

Fig. 3 (continued)



were required to separate the fragments in each parental set into at least 11 major groups. This requirement may be the result of false linkages introduced by the inclusion of a large proportion of fragments with severely distorted genotypic ratios.

In addition to the major linkage groups, several minor groups and single unlinked markers were obtained at the conservative LOD thresholds of 10.0 to 12.0. We were able to join the majority of these smaller linkage groups to major linkage groups by lowering the linkage threshold to LOD 7.0. Only three minor groups in the backcross parent-maps remained unlinked. Two of these were joined to the ends of major linkage groups based on the location of intercross markers shared with the F<sub>1</sub> hybrid (LG10gl

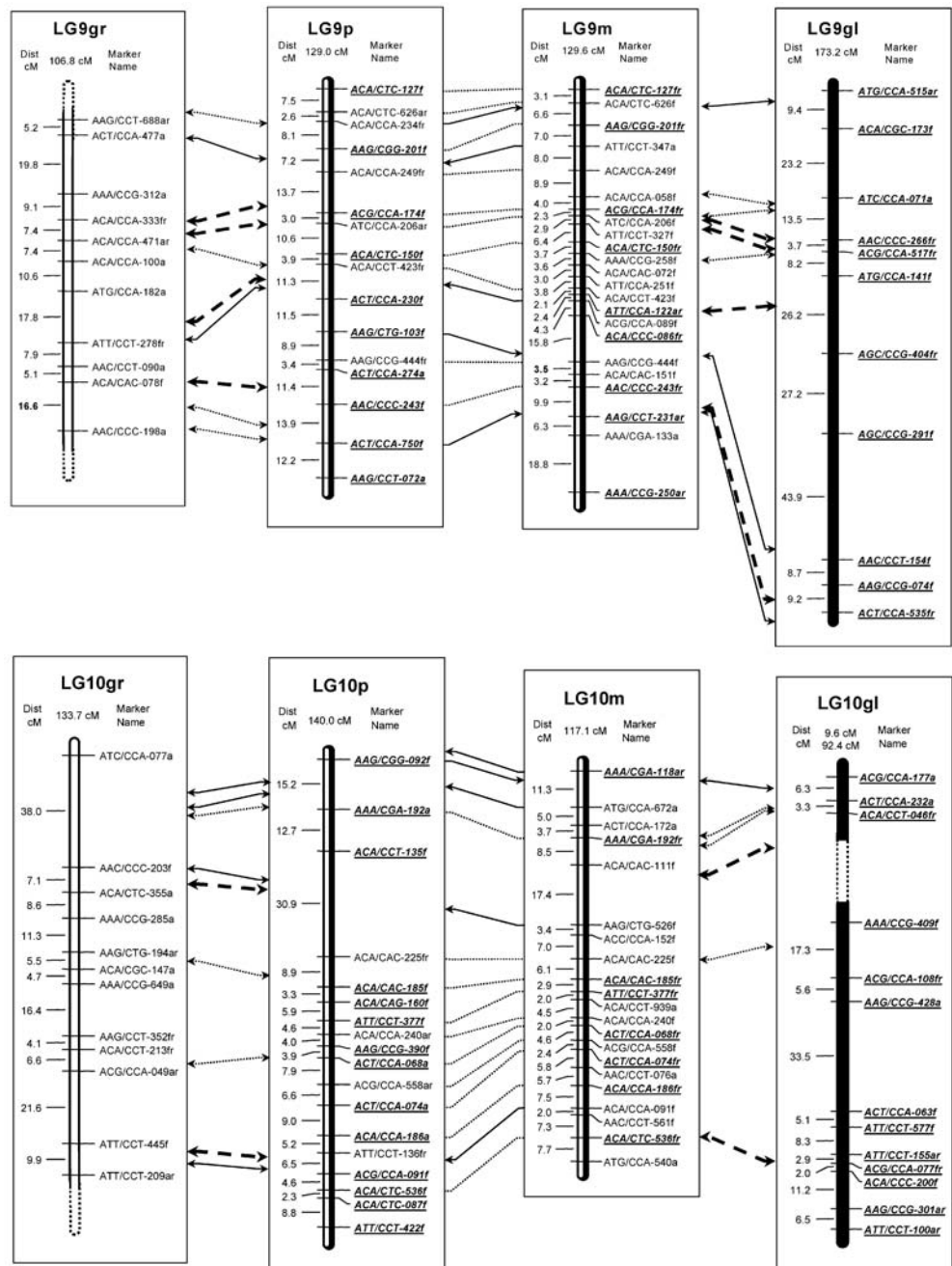
and LG11gl, Fig. 3). One small linkage group with four markers remained unlinked to any other linkage group in the *E. grandis* backcross parent after comparative mapping (data not shown).

#### Linkage phase identity

The AFLP marker profile of tree G50, the *E. grandis* seed parent of the F<sub>1</sub> hybrid, allowed us to determine the species origin of all testcross markers in the F<sub>1</sub> hybrid. Testcross markers that were absent in tree G50, and therefore inherited from the *E. globulus* pollen parent, were all found to be linked in coupling in the maps of the



Fig. 3 (continued)



F<sub>1</sub> hybrid. The opposite linkage phase of each linkage group always contained AFLP fragments that were shared between *E. grandis* tree G50 and the F<sub>1</sub> hybrid. This was consistent with the interpretation that the two linkage phases of each linkage group in the F<sub>1</sub> hybrid represent haplotype maps of the *E. grandis* and *E. globulus* homologs of a particular chromosome pair.

#### The framework maps

Four genetic linkage maps were constructed, one for each of the pure species parents and two for the F<sub>1</sub> hybrid

(Fig. 3). Each contained 11 major linkage groups, which equals the haploid chromosome number of *E. grandis* and *E. globulus*. The number of framework markers in these maps ranged from 138 to 209 and the average spacing between framework markers ranged from 6.7 to 10.8 cM (Table 2).

As expected, the distribution of markers in the maps of the F<sub>1</sub> hybrid was highly biased towards the donor linkage phase in each backcross (the *E. globulus* linkage phase in the backcross to *E. grandis* and vice versa). In the paternal map of the F<sub>1</sub> hybrid (the *E. grandis* backcross), 216 (63%) of the testcross markers mapped to the donor (*E. globulus*) linkage phase, while in the maternal map

Fig. 3 (continued)

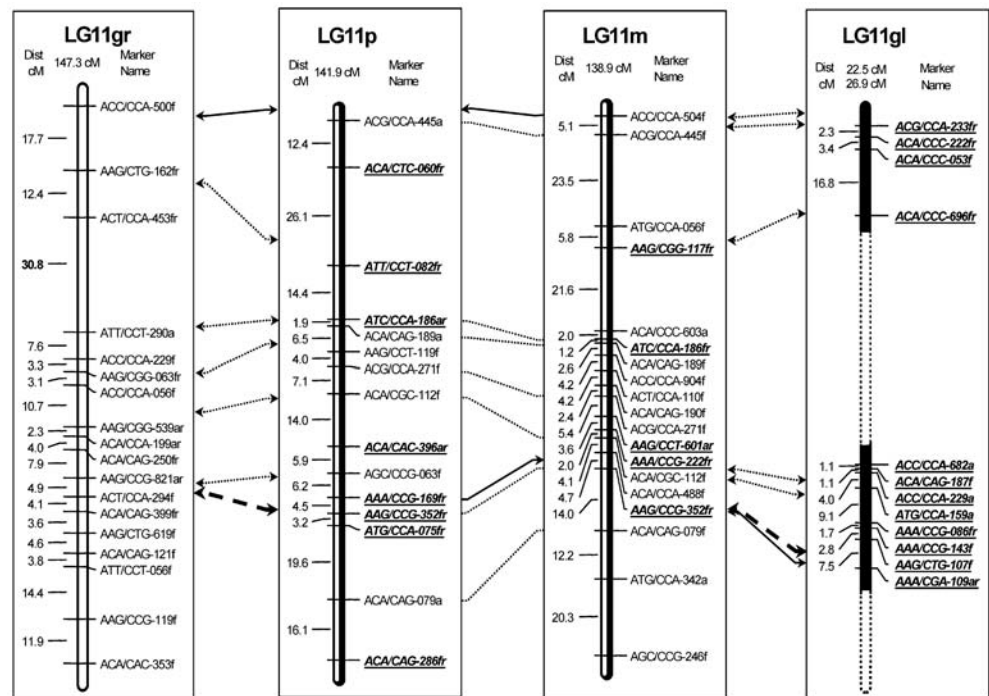


Table 2 Summary of framework maps constructed using testcross AFLP markers

Description <sup>a</sup>	<i>E. grandis</i>		<i>E. globulus</i>	
	Maternal map	F <sub>1</sub> hybrid Paternal map	Maternal map	Paternal map
Testcross marker inventory				
No. of framework markers selected	138 (48%)	169 (46%)	209 (46%)	141 (57%)
No. of mapped accessory markers <sup>b</sup>	116 (41%)	173 (47%)	202 (44%)	95 (38%)
No. of testcross fragments not mapped <sup>c</sup>	31 (11%)	23 (7%)	46 (10%)	11 (5%)
No. of unlinked testcross fragments at LOD 7.0	11 (4%)	6 (2%)	10 (2%)	5 (2%)
Framework maps (11 linkage groups each)				
Average linkage group size (cM)	118	132	120	128
Average framework marker spacing (cM)	10.5	9.2	6.7	10.8
Map length based on classical estimate of <i>r</i> (cM)	1,335	1,448	1,318	1,405
Map length based on Bailey's estimate of <i>r</i> (cM)	1,249	1,369	1,235	1,357
Estimation of genome length				
Hulbert estimate of genome length (cM)	1,331	1,469	1,411	1,273
Remington estimate of genome length (cM)	1,184	1,325	1,251	1,112
Framework map coverage <sup>d</sup>				
Map coverage ( <i>c</i> × 100%) at <i>d</i> = 20 cM	98.4%	99.0%	99.7%	98.8%
Map coverage ( <i>c</i> × 100%) at <i>d</i> = 10 cM	87.4%	90.0%	94.8%	89.1%

<sup>a</sup> All map distances are in cM Kosambi

<sup>b</sup> The number of testcross fragments placed in framework-marker intervals using the bin mapping procedure of MapPop

<sup>c</sup> Unlinked testcross fragments (at LOD 7.0) are included in the number of testcross fragments not mapped

<sup>d</sup> The Hulbert estimate of genome length was used to estimate map coverage (% of genome within *d* cM of a framework marker)

(the *E. globulus* backcross) 297 (72%) mapped to the *E. grandis* linkage phase. The greater bias in the maternal map can most likely be ascribed to the presence of hemizygous marker loci in the *E. grandis* homologs of the F<sub>1</sub> hybrid.

The total observed length of each parental framework map is listed in Table 2. The observed map lengths of the F<sub>1</sub> hybrid (1,235 cM to 1,448 cM) corresponded well with

that reported for *E. grandis* in previous studies (Grattapaglia and Sederoff 1994; Verhaegen and Plomion 1996). However, the map length of the *E. grandis* backcross parent (1,249 cM to 1,335 cM) was somewhat smaller than that reported earlier. The map length of the *E. globulus* backcross parent was approximately the same as that recently reported for another individual of the same

**Table 3** Summary of testcross markers occurring in small clusters

Description	<i>E. grandis</i> map	F <sub>1</sub> Hybrid paternal map	F <sub>1</sub> Hybrid maternal map	<i>E. globulus</i> map
Total no. of small clusters (<5 cM)	18	28	43	14
Average no. of markers per cluster	4.7	5.5	5.6	4.9
Total no. of markers in small clusters	84 (33%)	154 (45%)	240 (58%)	69 (28%)
<i>E. grandis</i> linkage phase <sup>a</sup>	–	59 (recurrent)	174 (donor)	–
<i>E. globulus</i> linkage phase <sup>a</sup>	–	95 (donor)	66 (recurrent)	–

<sup>a</sup> Phase assignment was arbitrary from one linkage group to the next in the maps of the *E. grandis* and *E. globulus* backcross parents

species based on an intraspecific cross (Thamarus et al. 2002).

We observed several pairs of neighboring framework markers in each map with large differences in number between the two recombinant marker classes (Ab and aB), especially in regions with a steep increase in distortion from one marker to the next. The classical estimate of recombination used by MAPMAKER was higher than that of Bailey's unbiased estimate (Bailey 1949) for all these marker intervals. Re-calculation of map distances using Bailey's estimate resulted in an overall decrease of only 5% in the total lengths of the parental framework maps (Table 2), although individual framework intervals were adjusted up to 86% by this procedure (data not shown).

The Remington estimate of total genome length was on average 11% lower than that of the Hulbert estimate (Hulbert et al. 1988; Chakravarti et al. 1991). It also agreed well with the total observed map lengths based on Bailey's estimate of recombination fraction, except in the case of the *E. globulus* map, where the Remington estimate was significantly smaller than the observed map length (Table 2). We used the Hulbert estimate of genome length to obtain conservative estimates of genome coverage for the framework genetic maps. On average, 99% of loci in the four parental maps were within 20 cM of a framework marker, while 87% to 94% of loci were within 10 cM of a framework marker (Table 2).

The genome-size estimate of the F<sub>1</sub> hybrid based on the maternal testcross set was 4% smaller than that based on the paternal set (Table 2). The observed length of the maternal map was also 9% smaller than that of the paternal map. This suggested a slightly lower overall rate of recombination during maternal gamete formation in the F<sub>1</sub> hybrid tree. A total of 82 framework markers were shared between the paternal and maternal maps of the F<sub>1</sub> hybrid. These markers flank 71 shared framework marker intervals. A paired *t*-test of the difference in the length of shared map intervals suggested significant differences in the recombination rate in several regions of the maps ( $P = 0.037$ ), although the overall mean of shared intervals was not significantly different ( $P = 0.457$ ). At least three regions located on linkage groups 7, 10 and 11 had adjacent marker intervals with lower recombination rates in the maternal map than the paternal map of the F<sub>1</sub> hybrid (Fig. 3).

#### *Bin mapping of accessory markers*

Given the framework map data and an expected error rate of 2%, MapPop was able to place an average of 85% of the remaining testcross fragments in framework marker intervals (Table 2). The testcross fragments that were not successfully placed by MapPop included fragments with true map positions outside of terminal framework markers and fragments with scoring error in excess of 2%.

#### *Marker clustering*

We tested for clustering of framework and accessory markers in the four parental maps by dividing each parental map (with linkage groups arranged end-to-end) into 50 arbitrary intervals of equal size based on the maptable output of MapPop. For each interval, the observed count of framework and accessory markers was compared to the expected marker count under the Poisson distribution. As has been found in other mapping studies, the *EcoRI/MseI*-generated marker loci were found to be non-randomly distributed in the three genomes studied here (data not shown). However, closer inspection of the distribution of markers in the maps of the F<sub>1</sub> hybrid (Table 3) revealed the presence of many small clusters of marker loci with an excess of markers in the same phase of linkage, i.e. with the same species origin. Small clusters were defined as regions with three or more markers in a moving window of 5.0 cM and a maximum interval of 2.0 cM anywhere within the cluster. Putative allelic AFLPs were counted as a single locus for the purpose of defining small clusters. The total number of markers in small clusters differed greatly between the paternal and maternal map of the F<sub>1</sub> hybrid (Table 3). The apparent excess of 86 tightly clustered testcross fragments in the maternal map corresponded well with the overall excess of 92 testcross fragments in this map relative to the paternal map of the F<sub>1</sub> hybrid (Table 1). Furthermore, 45% more testcross markers occurred in small clusters in the donor (*E. grandis*) linkage phase of the maternal map than in the donor (*E. globulus*) linkage phase of the paternal map (Table 3). These results are consistent with the presence of approximately 20% hemizygous marker loci in the *E. grandis* chromosomes of the F<sub>1</sub> hybrid.

## Comparative maps of *E. grandis*, *E. globulus* and the F<sub>1</sub> hybrid

The double pseudo-backcross mapping approach (Fig. 2) allowed us to construct comparative maps of 11 syntenic linkage groups of *E. grandis*, *E. globulus* and the F<sub>1</sub> hybrid (Fig. 3). The relatively large proportion of shared testcross fragments in the paternal and maternal maps of the F<sub>1</sub> hybrid provided many positions where these two maps could be connected. The order of 82 shared framework markers did not differ between the two independently constructed linkage maps of the F<sub>1</sub> hybrid (Fig. 3).

Due to the low power to map the dominantly scored intercross markers relative to a framework of testcross markers, we did not attempt to include these markers in the parental framework maps. Instead, we determined the distance and approximate position of each intercross marker relative to the nearest framework marker. Only 55.5% and 60.8% of intercross fragments were successfully located on both parental maps at LOD 5.0 and  $\theta = 0.2$  in the *E. grandis* and *E. globulus* backcross families, respectively. However, the locations of these intercross markers were colinear within each linkage group (Fig. 3). A substantial number of intercross markers were also mapped with much higher confidence as testcross markers in the alternative backcross family (indicated with solid arrows, Fig. 3). This aided the comparative mapping effort by providing independently obtained, high-confidence map locations for these intercross fragments.

## Discussion

Comparative genetic mapping of the well-diverged genomes of *E. grandis* and *E. globulus* will provide the opportunity to study the architecture of genetic differentiation between these two important tree species. In this study, we aimed to produce such a comparative genetic framework for a series of detailed, whole-genome analyses of differentiation in fitness traits and in commercially important traits such as growth, rooting ability and wood quality.

### Linkage mapping in a wide interspecific pedigree

Linkage mapping in wide interspecific crosses presents a number of challenges. Wide crosses often result in distorted genotypic ratios in mapping progeny, which can lead to biased estimates of recombination and increased numbers of false linkages (Lorieux et al. 1995; Liu 1998). Highly diverged regions of chromosomes may also result in suppression of recombination in the genomes of F<sub>1</sub> hybrids (Chetelat et al. 2000). Apart from these specific problems, de novo mapping of large numbers of markers in outbred pedigrees also suffer from the general problem of map expansion associated with

genotypic errors in dense genetic maps (Lincoln and Lander 1992).

Previous mapping studies in *Eucalyptus* reported higher proportions of distorted markers in interspecific crosses (Grattapaglia and Sederoff 1994; Marques et al. 1998) than intraspecific crosses (Byrne et al. 1995; Thamarus et al. 2002). However, the proportion of distorted markers observed in this study (30%) was approximately twice that observed in a previous study that was also based on a cross between species in two different sections of the subgenus *Symphomyrtus* (Marques et al. 1998). The amount of transmission ratio distortion observed in the F<sub>1</sub> hybrid was similar to that observed in interspecific hybrids of other plant species (Xu et al. 1997; Whitkus 1998; Ky et al. 2000), but the relatively high levels of distortion observed in the backcross parents were unexpected. The most likely explanation for this observation is that genetic variability exists within *E. grandis* and *E. globulus* for genetic factors that affect hybrid fitness. This implies that the genetic architecture of postzygotic isolation may vary to some extent among different crosses of these two species.

Evaluation of the bias in map distances introduced by distorted genotypic ratios, by using the method of Bailey (1949) to re-calculate recombination rates, revealed that very few marker intervals were affected by distorted segregation. Map distances were only markedly biased where a sharp increase or decrease in the level of distortion occurred from one marker to the next. In some of these cases, re-calculation of the recombination distance using Bailey's method resolved apparent discrepancies in the length of shared marker intervals in different parental maps (Fig. 3). We were not able to use Bailey's estimates of recombination to repeat the multi-point mapping and ordering of all the framework markers, because this method was not supported by MAPMAKER and manual ordering would not be feasible for such a large number of framework intervals.

A general reduction in recombination rate is often observed in hybrid genomes due to suppression of recombination in diverged parts of the parental genomes (Williams et al. 1995; Kreike and Stiekema 1997; Chetelat et al. 2000). No overall suppression of recombination was observed in the F<sub>1</sub> hybrid relative to the *E. grandis* and *E. globulus* backcross parents. In fact, the length estimates of the hybrid genome (Hulbert and Remington estimates) were 10% higher than that of the backcross parents. Length differences were indeed observed between some linkage groups in the F<sub>1</sub> hybrid and the backcross parents, but these differences could in most cases be ascribed to differences in map coverage. The most pronounced difference in recombination rate occurred on linkage group 9 where an approximate 4-fold difference was observed between similar regions of the maps of the F<sub>1</sub> hybrid and the *E. globulus* backcross parent (Fig. 3). It is not clear what caused this phenomenon, because no such increase was seen in the same map region of the *E. grandis* backcross parent.

We avoided map expansion by using the framework mapping approach proposed in the early stages of human genetic mapping (Keats et al. 1991). This remains the best approach for de novo map construction in outbred plant species (Remington et al. 1999), because the maximum number of markers that can be confidently ordered in any map is limited by the number and distribution of recombination breakpoints sampled in the particular mapping population. The bin mapping approach of Vision et al. (2000) further aided our framework mapping effort by providing an efficient method to locate large numbers of accessory markers in framework marker intervals.

#### Genomic distribution of hemizygous marker loci

The physical size of the *E. grandis* genome was estimated to be approximately 20% greater than that of the *E. globulus* genome (Grattapaglia and Bradshaw 1994). Furthermore, these estimates were consistent with the estimated genome sizes of other species in the same sections (*Latoangulatae* and *Maidenaria*), which suggest that genome-size differentiation accompanied genetic differentiation among members of these two sections. The distribution and nature of the “extra” DNA in the *E. grandis* genome relative that of the *E. globulus* genome may provide important insights into genome evolution in *Eucalyptus*. The large number of AFLP loci genotyped in this wide interspecific pedigree allowed us to differentiate between two alternative hypotheses for genome-size differentiation between *E. grandis* and *E. globulus*. The genome-size difference may be the result of a small number of gross chromosomal changes such as large duplications or deletions. In this case, large clusters of co-segregating markers would be observed in the maps of the F<sub>1</sub> hybrid. Alternatively, the difference in genome size may be the result of many dispersed regions of genome expansion such as would result from small duplications and insertions. In this case, many small clusters of markers, perfectly linked in coupling, should be observed in the maps of the F<sub>1</sub> hybrid, particularly in the backcross to *E. globulus*. Our results were consistent with the second hypothesis (i.e. dispersed genome expansion) between *E. grandis* and *E. globulus*. We observed an excess of testcross AFLPs in the maternal map of the F<sub>1</sub> hybrid and, in particular, the *E. grandis* linkage phase of this map. Furthermore, this excess corresponded with an excess of marker loci that occurred in small clusters dispersed throughout the *E. grandis* linkage phase (Table 3).

#### Colinearity of the genetic maps of *E. grandis*, *E. globulus* and their F<sub>1</sub> hybrid

More than 1,200 unique AFLP marker loci were characterized in this interspecific backcross pedigree. Approximately 30% of these AFLPs segregated in testcross and/or intercross configuration in both backcross families and

could therefore be used to align the genetic maps of the three parental trees. The framework-mapping criteria used for construction of the individual parental maps resulted in very stable marker orders. The order of shared testcross markers in the independently constructed paternal and maternal maps of the F<sub>1</sub> hybrid agreed perfectly. This result is consistent with previous reports of stable marker orders for 98% of shared marker intervals in maps with a LOD interval support of 3.0 or more (Plomion et al. 1995). With population sizes of greater than 180 individuals, interval support was much greater than 3.0 for the majority of marker orders in our maps, even in relatively dense map regions.

We did not obtain evidence for gross rearrangement of genetic material between the genomes of *E. grandis*, *E. globulus* and the F<sub>1</sub> hybrid. Large rearrangements, such as the inversions observed in comparative maps of tomato and potato (Tanksley et al. 1992), or translocations, would have resulted in severe suppression of recombination and large blocks of co-segregating markers in the linkage maps of the F<sub>1</sub> hybrid. No crossover was observed between the locations of intercross markers in the maps of the F<sub>1</sub> hybrid and the backcross parents, or between the two backcross parents, although the placement of these markers had much lower statistical support than that of the testcross markers. It is possible that small rearrangements exist between these two genomes and that they would be detected if more intercross markers were mapped. Co-dominant markers such as microsatellite markers will improve the power to map intercross loci in the backcross families (Broniani et al. 1998) and will improve the alignment of the maps of the pure-species parents with those of the F<sub>1</sub> hybrid.

#### Utility of the pseudo-backcross mating design and comparative maps

Interspecific mapping studies in *Eucalyptus* have to-date focused on the F<sub>1</sub> hybrid generation and produced linkage maps of the pure-species parents (Grattapaglia and Sederoff 1994; Verhaegen and Plomion 1996; Marques et al. 1998). In contrast, our study was based on segregation in the F<sub>2</sub> hybrid generation and produced linkage maps of an F<sub>1</sub> hybrid and two backcross parents. Mapping in second-generation hybrid progeny offered several important advantages over previous approaches. First, the increased level of heterozygosity of the F<sub>1</sub> hybrid resulted in a higher proportion of segregating markers and more efficient use of marker genotyping resources. Second, the use of a shared parent (the F<sub>1</sub> hybrid) resulted in an increased proportion of shared polymorphisms, which facilitated comparative mapping of the three parental genomes with dominantly scored AFLP markers. Third, because the F<sub>1</sub> hybrid should be heterozygous at all loci that differentiate the parental genomes, and because these loci all segregate in the backcross progeny, this pedigree is an excellent experimental framework for the analysis of genetic differenti-

ation. We have used this feature to perform a comparative QTL analysis of chemical and physical wood properties in this mapping pedigree (unpublished results). Fourth, this mapping approach provided the opportunity to study the fertilization ability of recombinant gametes produced by the F<sub>1</sub> hybrid and the viability of backcross progeny that inherited these gametes. We have analyzed the genomic pattern of transmission ratio distortion observed in the parental linkage maps and used this information to locate genetic factors that result in differential transmission of heterospecific alleles in the two backcross families (Myburg et al. 2003).

The pseudo-backcross mapping approach should be extremely useful in other genera where conventional F<sub>2</sub> intercross or backcross approaches cannot be applied due to problems with inbreeding depression or self-incompatibility. In these genera, pseudo-backcrosses may be the only feasible way to analyze the segregation of genetic material in second-generation hybrids. We have shown that this mating design, in combination with high-throughput marker analysis of pseudo-backcross progeny, provides an efficient approach to rapidly construct comparative linkage maps that can be used to study the colinearity of the genomes of F<sub>1</sub> hybrids and their parental species.

Finally, the comparative maps presented here establish synteny and colinearity between linkage groups of representatives of the sections *Latoangulatae* (*E. grandis*) and *Maidenaria* (*E. globulus*). Previous mapping studies by Marques et al. (1998) included members of the sections *Maidenaria* (*E. globulus*) and *Exsertaria* (*E. tereticornis*), whereas that of Grattapaglia et al. (1994) and Verhaegen and Plomion (1996) included members of the *Latoangulatae* (*E. grandis* and *E. urophylla*). Intra-specific maps have also been constructed for the *Maidenaria* species *Eucalyptus nitens* (Byrne et al. 1995) and more recently for *E. globulus* (Bundock et al. 2000; Thamarus et al. 2002). The possibility therefore now exists to construct comparative maps of all three commercially important sections of the subgenus *Symphomyrtus*. This will be best achieved by mapping a common set of microsatellite markers (Brondani et al. 2002) and gene-based markers (Gion et al. 2000; Thamarus et al. 2002) in different interspecific mapping pedigrees. Such an approach will provide valuable information on genome evolution in *Eucalyptus*, and will provide a powerful framework for comparative analysis of postzygotic reproductive barriers and other quantitative traits of commercial importance in this genus.

**Acknowledgements** The authors wish to thank representatives of Forestal Oriental SA, Uruguay, and Forestal y Agrícola Monte Aguila S.A., Chile, for providing the plant materials used in this study. We express our gratitude to Pablo Santini and co-workers of Shell Uruguay Renewables S.A. for the maintenance of the backcross families and collection of leaf samples for DNA extraction, Steve Verryn of CSIR, South Africa, for leaf material of tree G50, and Jane Harbard of Shell Forestry Technical Services, U.K., for the controlled pollinations that produced the backcross families. We thank Derek Harkins, Arthur Johnson and David O'Malley for valuable discussions, encouragement and technical

advice; and David Remington and Claus Vogl for helpful suggestions during the preparation of this manuscript. This work was supported by funding from the North Carolina State University Forest Biotechnology Industrial Associates Consortium and by the National Institutes of Health (Grant GM45344-06). A.A.M. was funded by the Fulbright Program and the National Research Foundation of South Africa.

## References

- Bailey NTJ (1949) The estimation of linkage with differential viability, II and III. *Heredity* 3:220–228
- Brondani RPV, Brondani C, Tarchini R, Grattapaglia D (1998) Development, characterization and mapping of microsatellite markers in *Eucalyptus grandis* and *Eucalyptus urophylla*. *Theor Appl Genet* 97:816–827
- Brondani RPV, Brondani C, Grattapaglia D (2002) Towards a genus-wide reference linkage map for *Eucalyptus* based exclusively on highly informative microsatellite markers. *Mol Genet Genomics* 267:338–347
- Brooker MIH, Kleinig DA (1994) Field guide to Eucalypts. Inkata Press, Australia
- Bundock PC, Hayden M, Vaillancourt RE (2000) Linkage maps of *Eucalyptus globulus* using RAPD and microsatellite markers. *Silvae Genet* 49:223–232
- Byrne JM, Murrell JC, Allen B, Moran GF (1995) An integrated genetic linkage map for eucalypts using RFLP, RAPD and isozyme markers. *Theor Appl Genet* 91:869–875
- Chakravarti A, Lasher LK, Reefer JE (1991) A maximum-likelihood method for estimating genome length using genetic linkage data. *Genetics* 128:175–182
- Chetelat RT, Meglic V, Cisneros P (2000) A genetic map of tomato based on BC1 *Lycopersicon esculentum* × *Solanum lycopersicoides* reveals overall synteny but suppressed recombination between these homeologous genomes. *Genetics* 154:857–867
- Eldridge K, Davidson J, Harwood C, Van Wyk G (1993) Eucalypt domestication and breeding. Oxford University Press, Oxford, UK
- Gion JM, Rech P, Grima-Pettenati J, Verhaegen D, Plomion C (2000) Mapping candidate genes in *Eucalyptus* with emphasis on lignification genes. *Mol Breed* 6:441–449
- Gore PL, Potts BM, Volker PW, Megalos J (1990) Unilateral cross-incompatibility in *Eucalyptus*: the case of hybridization between *Eucalyptus globulus* and *Eucalyptus nitens*. *Aust J Bot* 38:383–394
- Grattapaglia D, Bradshaw HD (1994) Nuclear DNA content of commercially important *Eucalyptus* species and hybrids. *Can J For Res* 24:1074–1078
- Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross mapping strategy and RAPD markers. *Genetics* 137:1121–1137
- Grattapaglia D, Bertolucci FL, Sederoff RR (1995) Genetic mapping of QTLs controlling vegetative propagation in *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross strategy and RAPD markers. *Theor Appl Genet* 90:933–947
- Griffin AR, Burgess IP, Wolf L (1988) Patterns of natural and manipulated hybridization in the genus *Eucalyptus* L'Herit – a review. *Aust J Bot* 36:41–66
- Griffin AR, Harbard J, Centurion C, Santini P (2000) Breeding *Eucalyptus grandis* × *globulus* and other interspecific hybrids with high inviability – problem analysis and experience at Shell Forestry Projects in Uruguay and Chile. In: Dungey HS, Dieters MJ, Nikles DG (eds) Hybrid breeding and genetics of forest trees. Proc QFRI/CRC-SPF Symposium, 9–14 April 2000, Noosa, Queensland, Australia, Department of Primary Industries, Brisbane, pp 1–13
- Hulbert SH, Ilott TW, Legg EJ, Lincoln SE, Lander ES, Michelmore RW (1988) Genetic analysis of the fungus, *Bremia*

- lactucae*, using restriction fragment-length polymorphisms. *Genetics* 120:947–958
- Keats BJ, Sherman SL, Morton NE, Robson EB, Buetow KH, Cartwright PE, Chakravarti A, Francke U, Green PP, Ott J (1991) Guidelines for human linkage maps: an International system for human linkage maps. *Genomics* 9:557–560
- Kreike CM, Stiekema WJ (1997) Reduced recombination and distorted segregation in a *Solanum tuberosum* (2x) × *S. spegazzinii* (2x) hybrid. *Genome* 40:180–187
- Ky CL, Barre P, Lorieux M, Trouslot P, Akaffou S, Louarn J, Charrier A, Hamon S, Noirot M (2000) Interspecific genetic linkage map, segregation distortion and genetic conversion in coffee (*Coffea* sp.). *Theor Appl Genet* 101:669–676
- Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174–181
- Lange K, Boehnke M (1982) How many polymorphic genes will it take to span the human genome? *Am J Hum Genet* 34:842–845
- Lincoln SE, Lander ES (1992) Systematic detection of errors in genetic linkage data. *Genomics* 14:604–610
- Liu BH (1998) Statistical genomics: linkage, mapping and QTL analysis. CRC Press, New York
- Lorieux M, Goffinet B, Perrier X, Deleon DG, Lanaud C (1995) Maximum-likelihood models for mapping genetic markers showing segregation distortion. 1. Backcross populations. *Theor Appl Genet* 90:73–80
- Marques CM, Araujo JA, Ferreira JG, Whetten R, O'Malley DM, Liu BH, Sederoff R (1998) AFLP genetic maps of *Eucalyptus globulus* and *Eucalyptus tereticornis*. *Theor Appl Genet* 96:727–737
- Marques CM, Vasquez-Kool J, Carocha VJ, Ferreira JG, O'Malley DM, Liu BH, Sederoff R (1999) Genetic dissection of vegetative propagation traits in *Eucalyptus tereticornis* and *Eucalyptus globulus*. *Theor Appl Genet* 99:936–946
- Myburg AA, Remington DL, O'Malley DM, Sederoff RR, Whetten RW (2001) High-throughput AFLP analysis using infrared dye-labeled primers and an automated DNA sequencer. *BioTechniques* 30:348–357
- Myburg AA, Vogl C, Griffin AR, Sederoff RR, Whetten RW (2003) Genetics of postzygotic isolation in *Eucalyptus*. Comparative whole-genome analysis of barriers to introgression in a wide interspecific cross of *E. grandis* and *E. globulus*. *Genetics* (in press)
- Plomion C, O'Malley DM, Durel CE (1995) Genomic analysis in maritime pine (*Pinus pinaster*) – comparison of two RAPD maps using selfed and open-pollinated seeds of the same individual. *Theor Appl Genet* 90:1028–1034
- Remington DL, Whetten RW, Liu BH, O'Malley DM (1999) Construction of an AFLP genetic map with nearly complete genome coverage in *Pinus taeda*. *Theor Appl Genet* 98:1279–1292
- Rieseberg LH, Baird SJE, Gardner KA (2000) Hybridization, introgression, and linkage evolution. *Plant Mol Biol* 42:205–224
- Stearns DA, Nicolle D, McKinnon GE, Vaillancourt RE, Potts BM (2002) Higher-level relationships among the eucalypts are resolved by ITS-sequence data. *Aust Syst Bot* 15:49–62
- Tanksley SD, Ganai MW, Prince JP, Devicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992) High-density molecular linkage maps of the tomato and potato genomes. *Genetics* 132:1141–1160
- Thamarus KA, Groom K, Murrell J, Byrne M, Moran GF (2002) A genetic linkage map for *Eucalyptus globulus* with candidate loci for wood, fibre, and floral traits. *Theor Appl Genet* 104:379–387
- Verhaegen D, Plomion C (1996) Genetic mapping in *Eucalyptus urophylla* and *Eucalyptus grandis* using RAPD markers. *Genome* 39:1051–1061
- Verhaegen D, Plomion C, Gion JM, Poitel M, Costa P, Kremer A (1997) Quantitative trait dissection analysis in *Eucalyptus* using RAPD markers. 1. Detection of QTLs in interspecific hybrid progeny, stability of QTL expression across different ages. *Theor Appl Genet* 95:597–608
- Vision TJ, Brown DG, Shmoys DB, Durrett RT, Tanksley SD (2000) Selective mapping: a strategy for optimizing the construction of high-density linkage maps. *Genetics* 155:407–420
- Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP – a new technique for DNA-fingerprinting. *Nucleic Acids Res* 23:4407–4414
- Whitkus R (1998) Genetics of adaptive radiation in Hawaiian and Cook Islands species of *Tetramolopium* (Asteraceae). II. Genetic linkage map and its implications for interspecific breeding barriers. *Genetics* 150:1209–1216
- Williams CG, Goodman MM, Stuber CW (1995) Comparative recombination distances among *Zea mays* L. inbreds, wide crosses and interspecific hybrids. *Genetics* 141:1573–1581
- Xu Y, Zhu L, Xiao J, Huang N, McCouch SR (1997) Chromosomal regions associated with segregation distortion of molecular markers in F<sub>2</sub>, backcross, doubled-haploid, and recombinant inbred populations in rice (*Oryza sativa* L). *Mol Gen Genet* 253:535–545