This is the first linkage analysis reported in this species using RAPD markers and single tree megagametophytes. The importance of these results is founded on the use of a genetic map in the study of population biology, evolutionary studies, and its potential application in molecular breeding and marker-assisted selection. Linked markers can form reference points, which will assist in further gene mapping. Evidently the genetic map will assist in the identification of implicated genomic areas in the natural or artificial selection process.


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### References


ARAVANOPOULOS, F. A.: Analysis of genetic linkage in *Salix exigua* NUTT. Silv. Genet. **47**: 127–131 (1998). — ARAVANOPOULOS, F. A., ZSUFFA, L. and CHONG, K. X.: Inheritance analysis of isoenzyme genes in *Salix eriocephala* MICHX. J. Hered. **85**: 381–388 (1994). — BAILEY, N. T. J.: Introduction to the mathematical theory of genetic linkage. Clarendon Press, Oxford (1961). — BARTELS, H.: Genetic control of multiple esterases from needles and macrogametophytes of *Picea abies*. Planta **99**: 283–289 (1971). — BEAVIS, W. D. and GRANT D.: A linkage map based on information from four $F_2$ populations of maize (*Zea mays* L.). Theor. Appl. Genet. **82**: 636–644 (1991). — COLQUHOUN, D.: Lectures on biostatistics. Clarendon Press, Oxford (1971). — DEVEY, M. E., FIDDLER, T. A. LIU, B. H., KNAPP, S. J. and NEALE, D. B.: An RFLP linkage map for loblolly pine based on a three-generation outbred pedigree. Theor. Appl. Genet. **88**: 273–278 (1994). — DOYLE, J. J. and DOYLE, J. L.: Isolation of plant DNA from fresh tissue. Focus **12**: 13–15 (1990). — ECHT, C. S. and NELSON, C. D.: Linkage mapping and genome length in eastern white pine (*Pinus strobes* L.). Theor. Appl. Genet. **94**: 1031–1037 (1997). — FURMAN, B. J., GRATTAPAGLIA, D., DVORAK, W. S. and O'MALLEY, D. M.: Analysis of genetic relationships of Central American and Mexican pines using RAPD markers that distinguish species. Molecular Ecology **6**: 321–331 (1997). — GERBER, S. and RODOLFE, F.: Estimation and test for linkage between markers: a comparison of LOD score and chi-square test in a linkage study of maritime pine (*Pinus pinaster* Ait.). Theor. Appl. Genet. **88**: 293–297 (1994). — GOMEZ, A., ARAVANOPOULOS, F. A., ALIA, R. and BUENO, M. A.: *Pinus halepensis* RAPD markers: linkage and genetic diversity. In: Proc. Intern. Congress "Applications of biotechnology to forest trees" Sept. 22–25, 1999, Vitoria-Gasteiz, pp. 39–40 (1999). — GRATTAPAGLIA, D. and SEDEROFF, R.: Genetic linkage maps of *Eucalyptus grandis* and *E. urophylla* using a pseudotestcross: mapping strategy and RAPD markers. Genetics **137**: 1121–1137 (1994). — HALDANE, J. B. S.: The combination of linkage values and the calculation of distance between the loci of linked factors. J. Genet. **8**: 299–309 (1919). — KONDO, T., TERADA, K., HAYASHI, E., KURAMOTO, N., OKAMURA, M. and KAWASAKI, H.: RAPD markers linked to a gene for resistance to pine needle gall midge in Japanese black pine (*Pinus thunbergii*). Theor. Appl. Genet. **100**: 391–395 (2000). — KOSAMBI, D. D.: The estimation of map distances from recombination values. Ann. Eugen. Lond. **12**: 172–175 (1944). — KUBISIAK, T. L., NELSON, C. D., NANCE, W. L. and STINE, M.: RAPD linkage mapping in a longleaf pine x slash pine F1 family. Theor. Appl. Genet. **90**: 1119–1127 (1995). — LI, C. X. and YEH, F. C.: Construction of a framework map in *Pinus contorta* subsp. *latifolia* using random amplified polymorphic DNA markers. Genome **44**: 147–153 (2001). — MORTON, N. E.: The detection and estimation of linkage between genes for elliptocytosis and the Rh blood type. Am. J. Hum. Genet. **8**: 80–96 (1956). — NELSON, C. D., NANCE, W. L. and DOUDRICK, R. L.: A partial genetic linkage map of slash pine (*Pinus elliottii* Engelm. var. *elliottii*) based on random amplified polymorphic DNAs. Theor. Appl. Genet. **87**: 145–151 (1993). — OWEN, A. R. G.: The theory of genetic recombination. Adv. Genet. **3**: 117–157 (1950). — PARAN, I., KESSELI, R. and MICHELMORE, R. W.: Identification of restriction fragment length polymorphism and randomly amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce using near-isogenic lines. Genome **34**: 1021–1027 (1991). — PLOMION, C., BAHRMAN, N., DUREL, C. E. and O'MALLEY, D. M.: Genomic mapping in *Pinus pinaster* (maritime pine) using protein markers. Heredity **74**: 661–668 (1995). — SOKAL, R. R. and ROHLF, F. J.: Biometry. W. H. Freeman and Co., New York (1981). — STRAUSS, S. H. and CONKLE, M. T.: Segregation, linkage and diversity of allozymes in knobcone pine. Theor. Appl. Genet. **72**: 483–493 (1986). — TULSIERAM, L. K., GLAUBITZ, J. C., KISS, G. and CARLSON, J.: Single tree genetic linkage mapping in conifers using haploid DNA from megagametophytes. BioTechnology **10**: 686–689 (1992). — VOWDEN, C. J., RIDOUT, M. S. and TOBUTT, K. R.: LINKEM: A program for genetic linkage analysis. J. Hered. **85**: 249–250 (1995). — WOLFF, K.: Analysis of allozyme variability in three *Plantago* species and a comparison to morphological variability. Theor. Appl. Genet. **81**: 119–126 (1994).


# Construction of Genome Map for *Eucalyptus camaldulensis* DEHN


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### Abstract

A linkage genome map for *Eucalyptus camaldulensis* DEHN was constructed using segregation data from a 92 full-sib progeny. The linkage map was based on Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP) and nuclear Microsatellite (SSR) markers. Marker data were analyzed for linkage using mapping software JOINMAP version 2.0. Linkage analysis resulted in 168 markers covering 1236 cM of the genome. The number of linkage groups determined was equivalent the haploid number of chromosomes (n=11) of *Eucalyptus*. This map can be used to define regions of the genome that are associated with important traits in *E. camaldulensis* and other Eucalyptus species such as wood and oil quality.



*Key words: Eucalyptus camaldulensis*, genome map, linkage, molecular, SSR, RFLP, River red gum



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### Introduction

*Eucalyptus camaldulensis* DEHN is one of several *Eucalyptus* native from Australia, hardy, fast-growing evergreen tree,

growing 24–40 m tall with pendulous branches. *Eucalyptus* has become by far the most widely planted hardwoods in the tropical and subtropical regions of the world (ELDRIDGE *et al.,* 1993; RANI and RAINA, 1998). *Eucalyptus* has been widely planted in areas with Mediterranean climates. It is one of the few forest trees introduced and grown successfully in Egypt (ABOU-GAZIA and ABOUELKHAIR, 1989) and it is well suited for various topographical, climatic and soil conditions (ABOU-GAZIA and EL-BAHA, 1989).

The DNA amount in picograms (pg) per haploid geneome (C-value) of *E. camaldulensis* was 0.7 pg as estimated in meristematic zones of root and shoot tips (RANI and RAINA, 1998). Similarly, it was reported that the C-value for *E. grandis* was 0.6 pg (GRATTAPAGLIA and BRADSHAW, 1994). The genome size of *Eucalyptus* is relatively very small as compared to gymnosperm forest species (AHUJA, 2001). For example, the nuclear DNA amounts in genus Pinus ranged from 18.9 (O'BRIEN *et al.,* 1996) to 29.6 (WAKAMIYA *et al.,* 1993) and Douglas-fir that has approx 25-34 pg DNA per haploid nucleus (INGLE *et al.,* 1975; CARLSON *et al.,* 1991).

RAPD (Random Amplified Polymorphic DNA) based on polymerase chain reaction (PCR) markers provide additional tools for the genetic analysis of forest trees that circumvents factors which complicate a standard RFLPs approach (Restriction Fragment Linkage Polymorphisms). The relatively small size of the *E. camaldulensis* genome suggests that RAPD approach provides a quick and efficient means of identifying DNA sequence based polymorphisms at a very large number of loci using very small amounts of DNA. Generating a sufficiently saturated RAPD-based genetic map in Eucalyptus, which is anchored to informative markers, may prove to be a quicker and less time consuming than exclusive RFLPs analyses (GRATTAPAGLIA, 2000). However, RAPDs are DNA dominant markers and are mostly used for single tree or family genome mapping (AGRAMA and CARLSON, 1995). RAPD markers have allowed a significant advance in the ability to generate linkage maps quickly. Maps of RAPD markers were reported for loblolly pine (GRATTAPAGLIA *et al.,* 1991; SEWELL *et al.,* 1999), white spruce (TULSIERAM *et al.,* 1992), Arabidopsis (REITER *et al.,* 1992), peach (CHAPARRO *et al.,* 1994) and Douglas-fir (AGRAMA *et al.,* 1995; KRUTOVSKI *et al.,* 1998). RFLP techniques provide a potentially infinite number of variable markers for the development of genetic linkage maps. In tree species RFLPs have been used to construct genetic linkage maps in *Pinus taeda* (DEVEY *et al.,* 1994; GROOVER *et al.,* 1994), *Populus tremuloides* (LIU and FURNIER, 1993), *Cryptomeria japonica* (MUKAI *et al.,* 1995) and *Pseudotsuga menziesii* (JERMSTAD *et al.* 1998; 2001). RFLPs provide orthologous markers for aligning the linkage information from each parental data set to produce a sex-average map (DEVEY *et al.,* 1994). Simple sequence repeats (SSRs), also known as microsatellites, are class of molecular markers based on tandem repeats of short (2–6 bp) DNA sequences (LITT and LUTTY, 1989). These repeats are highly polymorphic, even among closely related cultivars, due to mutations causing variation in the number of repeating units (BROWN *et al.,* 1996). Different alleles can be detected at a locus by the PCR, using conserved DNA sequences flanking the SSR as primers. However, SSR markers are potential to be used in tree breeding, because it is a fast and simple technique compared with AFLPs, RFLPs or isozymes (VAN DER NEST *et al.,* 2000). Occurrence and characteristics of nuclear microsatellites has been reported in several forest tree species (BRONDANI *et al.,* 1998).

Genetic linkage maps were constructed for *E. grandis* and *E. urophylla* using RAPDs (GRATTAPAGLIA and SEDEROFF, 1994; VERHAEGEN and PLOMION, 1996; VERHAEGEN *et al.,* 1997), RFLP, RAPD and isozyme markers (BYRNE *et al.,* 1995) and SSR (BRONDANI *et al.,* 1998), *E. nintens* using RFLP, RAPD and isozyme gene markers (BYRNE *et al.,* 1997), and in *E. gloulus* and *E. terticornis* using AFLP (MARQUES *et al.,* 1998). However, a linkage map of the *E. camaldulensis* (n=11) constructed using molecular markers has never been reported. In this present paper, we report on construction of linkage genetic map for *E. camaldulensis* based on a two-way pseudotestcross strategy (RITTER *et al.,* 1990), which has been successfully applied in various outcrossing species such as apple (CONNER *et al.,* 1997), grape (LODHI *et al.,* 1995), *Eucalyptus* (GRATTAPAGLIA and SEDEROFF, 1994) and pear (WEEDEN *et al.,* 1994). The main objective of the development of this map using RAPDs, RFLPs and SSRs is to contribute to the genomic characterization of *E. camaldulensis* in particular for localizing genes and quantitative trait loci (QTLs) controlling wood and oil quality.

## Materials and Methods

### *Mapping population and DNA extraction*

A single full-sib family of *Eucalyptus camaldulensis* DEHN was used to generate a molecular based genetic linkage map. The family consisted of 92 individuals and resulted from the cross between two trees 7046-34 (female) and 10574-18 (male). Leaves were frozen directly after collection from plants grown at Ismalia and Menoufia Agricultural Research Stations, ARC, Egypt. DNA extraction was carried out as described by KEIL and GRIFFIN (1994).

### *RAPD, RFLP and SSR procedures*

The RAPD protocol (WILLIAMS *et al.,* 1990) was optimized for *Eucalyptus* DNA amplification (BYRNE *et al.,* 1995). One hundred and twenty random 10-mer primers of OP-A to -N (Operon Technologies) and 142 RFLP probes (BYRNE *et al.,* 1995; 1997) were used in this study. For RFLP, genomic DNA of each individual was digested with one of the restriction enzymes *Eco*RI, *Hind*II, or *Bam*HI. Digeted DNA was fractionated on 0.8 % agarose gels. After electrophoresis, the gels were denatued, neutralized and Southern-blotted onto uncharged nylon membranes (MSI Magnagraph). DNA probes were labeled by Polymerase chain reaction (PCR) amplification with 2.5 % digoxigenin-dUTP (Boehringer-Mannheim). The probes were detected using the antidigoxgenin-alkaline phosphatase-AMPPD chemiluminescent protocol described in details by AGRAMA *et al.* (1999). Microsatellite marker analysis was performed using twenty EMBRA primer pairs developed by BRONDANI et al. (1998). The amplification was done using PCR reactions as described by the author (BRONDANI *et al.,* 1998), except 55 °C was used for primer annealing. The SSR and RAPD reaction products were evaluated for polymorphisms on 3 % Metaphor agarose gels (FMC Products, Rockland, ME, USA) and 2 % agarose gels, respectively. Gels were stained in TBE buffer containing 1 μg $mL^{-1}$ ethidium bromide for 30 to 60 minutes. Gel images and marker data were processed using Quantity One Software v. 4.0.1 (Bio-Rad Laboratories, Hercules, CA USA). The SSR and RAPD bands were sized and then binary coded by 1 or 0 for their presence or absence in each genotype.

### *Segregation analysis and construction of linkage groups*

Each primer or RFLP probe was screened against DNA from the two parents and 6 DNA samples from the progeny. Those markers that revealed clear unambiguous polymorphic loci were then used in the subsequent segregation analysis with DNA from all 92 full sib progeny. Maps were constructed according to the tow-way pseudotestcross strategy (RITTER *et al.,* 1990; GRATTAPAGLIA and SEDEROFF, 1994). Segregation type

codes for dominant RAPDs were designated as *"ab* x *aa"* (maternally informative); *"aa* x *ab"* (paternally informative); and *"ab x ab"* (intercross) according to JOINMAP 2.0 (STAM and VAN OOIJEN, 1995). The codominant RFLPs and microsattellite SSRs revealed three types of segregation: *ab* x *aa, aa* x *ab,* and the *ab* x *cd* and *ab* x *ac* (fully informative) in which four alleles segregate at one locus resulting 1:1:1:1 segregation. RFLP, RAPD and SSR markers are present in one parent and absent in the other or vice versa, and expected to segregate 1:1 in full-sib generation. RAPDs which are present in both parents, as a result of heterozygosity on both sides, segregating 3:1 in the progeny. A $\chi^2$-test ($\alpha = 0.05$) was performed for each of the polymorphic bands generated to determine whether the segregation ration of present/absent was significantly different from either ration 1:1 or 3:1.

Two separate data sets were obtained, one for each parent. The data was analyzed using the software program JOINMAP Version 2.0 (STAM, 1993; STAM and VAN OOIJEN 1995). The genetic model for inheritance in pseudotestcross is analogous to the parental phase. Two sets of data were used to assign loci to different groups of male and female parents using LOD score threshold of 3.0. In the first step of mapping procedure, the two data sets of each parent were formed according to inheritance patterns of the markers and ordering of markers within a linkage group was performed separately for the male and female loci segregating 1:1. In the second step, markers heterozygous in both parents were included (*ab* x *ab* type) RFLP and SSR. Finally, in third step, the 3:1 segregating RAPD loci were integrated into linkage groups only if they did not conflict with the marker order constructed in step two (SEWELL *et al.,* 1999; SEFFELDER *et al.,* 2000). A consensus genome linkage analysis was performed using all 92 full-sib segregation data using LOD of 4.1. Genetic map distances between the ordered loci were estimated using the mapping function of KOSAMBI (1944) and expressed in centiMorgam (cM). Maps figures were constructed with the computer program DrawMap version 2.0 (VOORRIPS, 2001).

**Results**

RAPD primers, RFLP probes and SSR pair primers markers were screened against the two parents and 6 DNA samples from the progeny to identify those which revealed polymorphic loci. The markers that produce well-defined polymorphic bands were selected if they were consistently and readily observed. Of 120 RAPD primers, screened 21 produced monomorphic patterns, while 10 produced no amplification or weak products considered unreliable for use in mapping. For the 89 remaining primers, 285 RAPD bands were automatically detected with Bio-Rad Quantity One image analyzer. This represented an average 3.2 bands per effectively amplifying primer. Eighty-eight (31%) of RAPD bands were polymorphic. The number of potential loci segregating in the 1:1 and 3:1 ratio were 63 and 25, respectively. The size of the bands ranged between 185 bp and up to 2800 bp. However 73% of the band size ranged from 400 to 1000 bp.

RFLPs were detected with 142 probes. Eighty-four of them were genomic DNA and the remaining 58 were cDNA. Forty-nine probes were discarded because they were unsuccessfully cross-hybridized, complex and uninterpretable, or gave indistinct or weak signals. Using these 93 probes produced 121 RFLPs (an average of 1.3 markers per probe). A single locus was detected by seventy of the probes. Eighteen probes detected two loci each and five gave three loci. Fifty-two of the probes detected monomorphic bands in addition to the segregating RFLP, indicating that they hybridized to more than one locus. Detection of more than one locus in these 52 clones was more frequent in genomic probes (69%, 36 probes) than in cDNA probes (31%, 16 probes).

PCR amplifications with SSR primers yielded in most cases two bands represented one locus on Metaphor agarose gel. Eighteen of the 20 SSR markers loci were polymorphic between the two parents. All these markers loci were heterozygous in both parents and segregated in a fully informative fashion either three or four different and clearly interpretable segregating alleles. Because of the mating design, these marker loci were mapped on both parental maps allowing the determination of synteny between homologous linkage groups in the two parents.

Summary of the segregation type and marker type of loci analyzed were presented in *Table 1*. Two data sets, 7046-34-female and 10574-18-male, contained 156 and 143 genetic markers, respectively, of which 119 (76.3%) and 107 (74.8%) were linked at LOD ≥ 3.0. Among these markers, 90 intercross and fully informative (25 RAPDs, 47 RFLPs and 18 SSRs) loci were common to the two parental maps, and thus were used to identify linkage group homologous between mapping female and male linkage groups (*Table 1*). The maps generated by JOINMAP, cover 1028 and 1149 cM (KOSAMBI mapping function) for female and male parents, respectively. The parental estimate for genome length was greater than the maternal estimate. The order of markers is quite well conserved between the two maps.

*Table 1.* – Segregation type and marker type of loci utilized in genomic map.

| Segregation type[a] | Number of markers analyzed | Number of markers mapped |
|---|---|---|
| MI (ab x aa) | 84 | 61 |
| PI (aa x ab) | 71 | 49 |
| IC (ab x ab) | 25 | 19 |
| FI (ab x ac or ab x cd) | 47 | 39 |
| Total | 227 | 168 |
| *Marker type* | | |
| RAPDs | 88 | 73 |
| RFLPs | 121 | 81 |
| SSRs | 18 | 14 |
| Total | 227 | 168 |

[a] MI: maternally informative; PI: parentally informative; IC: intercross; FI: fully informative.

A total of 227 markers were analyzed for linkage and resulted in a genomic linkage map (*Figure 1*) contains 168 (73 RAPDs, 81 RFLPs, and 14 SSRs) genetic markers. Fifty nine loci were not assigned to any linkage group. In the first analysis, a total of 14 linkage groups were mapped in the full-sib progeny. There are three groups consist of only three framework markers each. At LOD 4.30, thirteen linkage groups form as a consequence of groups 13 and 9 coalescing and formatting a new LG9 covering 121.3 cM whilst at LOD 4.1, groups 12 and 14 formed two single linkage groups (LG 3 and LG4) with group 3 and 4, respectively. Thus at LOD 4.1, eleven linkage groups were formed which is the observed haploid number of chromosomes in *E. camaldulensis*, and covered 1236 cM (K) of the genome.

## Discussion

The first step in genome mapping is the right selection of the progenitors of the mapping population (CERVERA *et al.,* 2000). This selection is based on choosing the genetically divergent parents in order to maximize heterozygosity level in the progeny. The two *Eucalyptus camaldulensis* DEHN parental trees in this study were included in an evaluation experimental trial in 1987 (ABOU-GAZIA and EL-BAHA, 1989). The results indicated contrast phenotypes between the two parents 7046 and 10574 which reflects the success in choosing the parents to produce the full-sib progeny. A single full-sib family comprising 60-100 individuals is sufficient for quite accurate genetic mapping (CERVERA *et al.,* 2000).

Outbreeding species show high level of genetic diversity and the F1 progeny segregates for markers and traits. The concept of F1 generation in wood plants can be assimilated to that of an F2 or backcross in an annual selfing crops (TULSIERAM *et al.,* 1992). The informative 1:1 configuration (testcross locus confi-

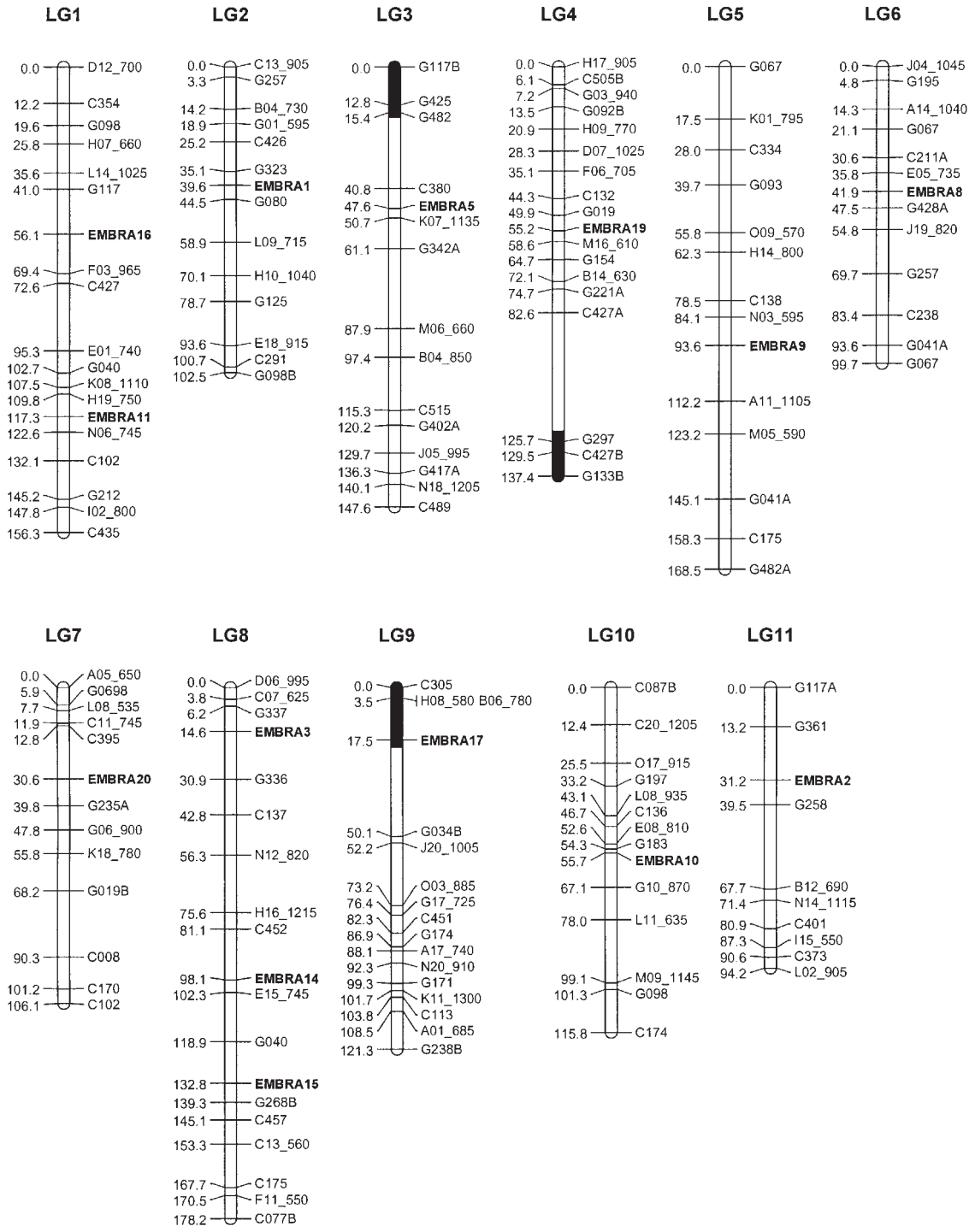


*Figure 1.* – Genomic linkage map of *E. camaldulensis.* Marker identification is given to the *right* of the bars. Cumulative Kosambi distance (cM) of markers along groups is indicated to the *left* of the bars. Microsatellite markers are the markers in bold. RAPD loci amplified from OPERON primers are listed using the OP designation followed by their size in base pair. The map contains 168 loci, covering a total of 1236 cM. The sold segments were integrated on LG 3 and 4 with LOD score 4.1 and LG 9 with 4.2

guration) appears to be quite common in matings between highly heterozygous outcrossed wood species. The term "two-way pseudo-testcross" mapping strategy was introduced to define a mapping strategy based on selection of markers heterozygous in one parent and homozygous null in the other parent and therefore segregating 1:1 in their progeny as in a testcross (GRATTAPAGLIA *et al.,* 1994). The informative RAPDs in both parents represented approximately 28.4 % of total segregating RAPD loci. The use of RAPDs markers in peusdo-testcross configuration is a general strategy for the construction of genetic linkage maps in outbred forest trees. We applied this strategy in combination with the RFLP, RAPD and SSR assay to construct the first genome linkage map for *E. camaldulensis*. With 168 genetic molecular markers, this map is an excellent resource from which markers may be selected for future mapping projects within *E. camaldulensis* and for comparative studies among other eucalyptus (e.g., BYRNE *et al.,* 1995). In this study, RFLP probes were non-radioactive detected according to chemiluminescent protocol which applied successfully to analysis of enhanced-axillary-branching in *E. camaldulensis* (RANI and RAINA, 1998).

The linkage data from two mapping parents contained 90 orthologous markers that were common at the two mapping parents. By this, it is possible to determine homologies of linkage groups in the two maps or integrate the two maps into one (BEAVIS and GRANT, 1991). A survey of 60 mapped RAPD markers in 10 full sib families of *Eucaluptus urophylla* indicated that an average of 64 % of RAPD markers should be readily transferable and informative among linkage maps for individual trees from the same population (GRATTAPAGLIA, 2000). Linkage analysis of the full-sib progeny resulted in a map of eleven linkage groups covering 1236 cM of *E. camaldulensis* genome. For all the *Eucalyptus* maps constructed to date, regardless of the type of molecular marker and pedigree structure employed, the number of linkage groups determined was always equivalent or closely approached the haploid number of chromosomes (GRATTAPAGLIA, 2000). The total map length has been estimated between 1156 cM for *E. urophylla* to 1620 cM for *E. grandis* (GRATTAPAGLIA *et al.,* 1994), 1462 cM in *E. nintens* (BYRNE *et al.,* 1995) and between 1277 cM and 1133 cM for framework markers of male and female parent in *E. globules*, respectively. However, our genome coverage was in the average of pervious published maps in other eucalyptus species (BUNDOCK *et al.,* 2000).

Different genetic maps could be constructed in related or unrelated families, thereby generating multiple genome maps using different markers covering information on specific traits in a single species. For obtaining maximum genetic information within an individual map, it is possible to construct consensus genome maps in a species (AHUJA, 2001). Consensus maps have been constructed for a number of plant species, including *Arabidopsis thaliana* (HAUGE *et al.,* 1993; MEINKE *et al.,* 1998), *Brassica oleracea* (KINIAN and QUIROS, 1992), *Zea mays* (BEAVIS and GRANT, 1991), and *Secale cereale* (PHILIP *et al.,* 1994). A consensus genome map in forestry tree was constructed in *Pinus taeda* (SEWELL *et al.,* 1999) and *Eucalyptus* nitens (BYRNE *et al.,* 1995) based on RFLPs, RAPDs, and isozymes by integrating linkage data from two unrelated three-generation outbred pedigrees. The current trend in genome mapping is to combine both cytogenetic and molecular genome maps to evolve the integrated genetic maps in plants, including trees (AHUJA, 2001). The map forms a basis for defining regions of the genome and location of quantitative trait loci for wood and oil quality and other economic traits in *E. camaldulensis* and other Eucalyptus species. The identification and manipulation of QTLs specific to particular species emerge an important component of selection for quantitative traits by marker assisted breeding in half-sib or full-sib tree improvement programs (STUBER *et al.,* 1999).

## References


ABOU-GAZIA, H. A. and ABOUELKHAIR, K. S.: Effect of salinity on seed germination of some *Casuarina* species and *Eucalyptus camaldulensis* DEHN. Alex. J. Agric. Res. **34**: 99–110 (1989). — ABOU-GAZIA, H. A. and EL-BAHA, A. M.: Performance of ten-year-old *Eucalyptus camaldulensis* DEHN. Provenances in Egypt. Alex. J. Agric. Res. **34**: 85–97 (1989). — AGRAMA, H. A., BROOME, J. and CARLSON, J. E.: Genetic linkage maps for Douglas-fir and markers for QTLs. In: J. Lavereau (ed.), Proceeding of the Twenty-fifth meeting of the Canadian Tree Improvement Association, p 38. University of Victoria, Canada, Aug. 28 – Sept. 1, 1995, part I, (1995). — AGRAMA, H. A. and CARLSON, J. E.: DNA markers for selecting high wood density in Douglas-fir. In: Lavereau, J (ed) Proc of 25th Canadian tree Improvement Association/Western Forest Genetics Association p 90, Hugh John Flemming Forestry Centre, Frederiction, New Brunswick, Canada, (1995). — AGRAMA, H. A., MOUSSA, M. E., NASER, M. E., TAREK, M. A. and IBRAHIM, A. H.: Mapping of QTL for downy mildew resistance in maize. Theor. Appl. Genet. **99**: 519–523 (1999). — AHUJA, M. R.: Recent advances in molecular genetics of forest trees. Euphytica **121**: 173–195 (2001). — BEAVIS, W. D. and GRANT, D.: A linkage map based on information from $F_2$ population of maize (*Zea mays* L). Theor. Appl. Genet. **82**: 636–644 (1991). — BRONDANI, R. P. V., BRONDANI, C., TARCHINI, R. and GRATTAPAGLIA, D.: Development, characterization and mapping of microsattellite markers in *Eucalyptus grandis* and *E. urophylla*. Theor. Appl. Genet. **97**: 816–827 (1998). — BROWN, S. M., HOPKINS, M. S., MITCHELL, S. E., SENIOR, M. L., WANG, T. Y., DUNCAN, R. R., GONZALEZ-CANDELAS, F. and KRESOVICH, S.: Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum [*Sorghum bicolor* (L.) Moench]. Theor. Appl. Genet. **93**: 190–198 (1996). — BUNDOCK, P. C., HAYDEN, M. and VAILANCOURT, R. E.: Linkage maps of *Eucalyptus* globules using RAPD and Microsatellite markers. Silvae Genetica **49**: 223–232 (2000). — BYRNE, M., MURRELL, J. C., ALLEN, B. and MORAN, G. F.: An integrated genetic linkage map for eucalyptus using RFLP, RAPD and isozyme markers. Theor. Appl. Genet. **91**: 869–875 (1995). — BYRNE, M., MURRELL, J. C., QWEN, J. V. and WILLIAMS, E. R.: Mapping of quantitative trait loci influencing frost tolerance in *Eucalyptus niens*. Theor. Appl. Genet. **95**: 975–979 (1997). — CARLSON, J. E., TULSIERAM, L. K., GLAUBITZ, V. W. K., KAUFFELDT, C. and RUTLEDGE, R.: Segregation of random amplified DNA markers in F1 progeny of conifers. Theor. Appl. Genet. **89**: 758–766 (1991). — CERVERA, M. T., PLOMION, C. and MALPICA, C.: Molecular markers and genome mapping in wood plants. In: Jain SM and Minocha SC (Eds.), Molecular biology of woody plants, Vol. 1, pp 375–394. Kluwer Academic Publishers, Dordercht, (2000). — CHAPARRO, J. X., WERNER, D. J., O'MALLEY, D. M. and SEDEROFF, R. R.: Targeted mapping and linkage analysis of morphological, isozyme and RAPD markers in peach. Theor. Appl. Genet. **87**: 805–815 (1994). — CONNER, P. J., BROWN S. K. and WEEDEN, N. F.: Randomly amplified Polymorphic DNA-based genetic linkage maps of three apple cultivars. J. Am. Soc. Hort. Sci. **122**: 350–359 (1997). — DEVEY, M. E., FIDDLER, T. A., LIU, B. H., KNAPP, S. J. and NEALE, D. B.: An RFLP linkage map for loblolly pine based on a three generation outbred pedigree. Theor. Appl. Genet. **88**: 273–278 (1994). — ELDRIDGE, K. G., DAVIDSON, J. and HARWOOD, C.: *Eucalyptus* domestication and breeding. Clarendon, Oxford, UK (1993). — GRATTAPAGLIA, D.: Molecular breeding of *Eucalyptus*. In: Jain SM and Minocha SC (eds.), Molecular biology of woody plants, pp 451–474, Vol. 1. Kluwer Academic Publishers, Dordercht, (2000). — GRATTAPAGLIA, D. and BRADSHAW, H. D.: Nuclear DNA amounts of commercially important *Eucalyptus* species and hybrids. Can. J. For. Res. **24**: 1074–1078 (1994). — GRATTAPAGLIA, D. and SEDEROFF, R.: Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a Pseudo-testcross: Mapping strategy and RAPD markers. Genetics **137**: 1121–1137 (1994). — GRATTAPAGLIA, D., WILCOX, P., CHAPARRO, J. X., O'MALLEY, D. and MCCORD, S.: A RAPD map of loblolly pine in 60 days, abstract 2224. Third International Congress of the International Society for Plant Moleclar Biology. (1991). — GROOVER, A., DEVEY, M., FIDDLER, T., LEE, J., MEGRAW, R., MITCHEL-OLDS, T., SHERMAN, B., VUJCIC, S., WILLIAMS, C. and NEALE, D.: Identification of quantitative trait loci influencing wood specific gravity in an outbreed pedigree of loblolly pine. Genetics **138**: 1293–1300 (1994). — HAUGE, B. M., HANLEY, S. M. and CARTINHOUR, S.: An integrated genetic/RFLP map of the *Arabidopsis thaliana* genome. Plant J. **3**: 745–754 (1993). — INGLE, J., TIMMIS, J. and SINCLAIR, J.: The relationship between satellite deoxyribonucleic acid, ribosomal ribonucleic acid gene redundancy, and genome size in plants. Plant Physiol. **55**: 496–501 (1975). — JERMSTAD, K. D., BASSONI, D. L., JECH, K. S., WHEELER, N. C. and NEALE, D. B.: Mapping of quantitative trait loci controlling adaptive traits in coastal Douglas-fir. I. Timing of vegetative bud flush. Theor. Appl. Genet. **102**: 1142–1151 (2001). —

JERMSTAD, K. D., BASSONI, D. L., WHEELER, N. C. and NEALE, D. B.: A sex-averaged linkage map in costal Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var *'menziesii'*) based on RFLP and RAPD markers. Theor. Appl. Genet. **97**: 762–770 (1998). — KEIL, M. and GRIFFIN, A. R.: Use of random amplified Polymorphic DNA (RAPD) markers in the discrimination and verification of genotypes in *Eucalyptus*. Theor. Appl. Genet. **89**: 442–450 (1994). — KINIAN, S. F. and QUIROS, C. F.: Generation of a *Brassica oleracea* composite RFLP map: linkage arrangements among various populations and evolutionary implications. Theor. Appl. Genet. **84**: 544–554 (1992). — KOSAMBI, D. D.: The estimation of map distances from recombination values. Ann. Eugen. **12**: 172–175 (1944). — KRUTOVSKI, K. V., VOLLMER, S. S., SORENSEN, F. C., ADAMS, W. T., KNAPP, S. J. and STRAUSES, S. H.: RAPD genome maps of Douglas-fir. J. Hered. **89**: 197–205 (1998). — LITT, M. and LUTTY, J. A.: A hypervariable microsattellite revealed by in vitro amplification of a dinucleotide within the cardiac muscle action gene. Am. J. Hum. Genet. **44**: 397–401 (1989). — LIU, Z. and FURNIER, G. R.: Inheritance and linkage allozymes and restriction fragment length polymorphisms in trembling aspen. J. Hered. **84**: 419–424 (1993). — LODHI, M. A., DALY, M. J., YE, G. N., WEEDEN, N. F. and REISCH, B. I.: A molecular marker based linkage map of Vitis. Genome **38**: 786–794 (1995). — MARQUES, C. M., ARAUJO, J. A., FERREIRA, J. G., WHETTEN, R., O'MALLEY, D. M., LIU, B. H. and SEDROFF, R.: AFLP genetic maps of *Eucalyptus* globules and *E. tereticornis*. Theor. Appl. Genet. **96**: 727–737 (1998). — MEINKE, D. W., CHERRY, M., DEAN, C., ROUNSLEY, S. D. and KOORNNEEF, M.: *Arabidopsis thaliana*: a model plant for genome analysis. Science **282**: 662–682 (1998). — MUKAI, Y., SUYAMA, Y., TSUMURA, Y., KAWAHARA, T., YOSHIMARU, H., KONDO, T., TOMARU, N., KURAMOTO, N. and MURAI, M.: A linkage map for sugi (*Cryptomeria japonica*) based on RFLP, RAPD and isozyme loci. Theor. Appl. Genet. **90**: 835–840 (1995). — O'BRIEN, I. E. W., SMITH, D. R., GARDNER, R. C. and MURRAY, B. G.: Flow cytometric determination of genome size in *Pinus*. Plant Science **115**: 91–99 (1996). — PHILIP, P. U., WEHLING, P. and WRICKE, G.: A linkage map of rye. Theor. Appl. Genet. **88**: 243–248 (1994). — RANI, V. and RAINA, S. N.: Genetic analysis of enhanced-axillary-branching-derived *Eucalytus tereticornis* Smith and *E. camaldulensis* DEHN. Plants. Plant Cell Rep. **17**: 236–242 (1998). — REITER, R. S., WILLIAMS, J. G. K., FELDMANN, K. A., RAFALSKI, J. A. and TINGEY, S. V.: Global and local genome mapping in Arabidopsis thaliana by using recombinant inbred lines and random amplified polymorphic DNAs. Proc. Natl. Acad. Sci. USA **89**: 1477–1481 (1992). — RITTER, E., GEBHARDT, C. and SALAMINI, F.: Estimation of recombination frequencies and construction of RFLP linkage maps in plants from crosses between heterozygous parents. Genetics **125**: 645–654 (1990). — SEFFELDER, S., EHRMAIER, H., SCHWEIZER, G. and SEIGNER, E.: Male and female genetic linkage map of hops, *Humulus lupulus*. Plant Breed. **119**: 249–255 (2000). — SEWELL, M. M., SHERMAN, B. K. and NEALE, D. B.: A consensus map for loblolly pine (*Pinus taeda* L.). I. Construction and integration of individual linkage maps from two outbred three-generation pedigrees. Genetics **151**: 321–330 (1999). — STAM, P.: Construction of integrated genetic linkage maps by means of a new computer package JOINMAP. Plant J. **3**: 739–744 (1993). — STAM, P. and VAN OOIJEN, J. W.: JOINMAP, Version 2.0 Software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen (1995). — STUBER, C. W., POLACCO, M. and SENIOR, M. L.: Synergy of empirical breeding, marker-assisted selection, and genomics to increase crop yield potential. Crop Sci. **39**: 1571–1583 (1999). — TULSIERAM, L. K., GLAUBITZ, J. C., KISS, G. and CARLSON, J. E.: Single-tree genetic linkage mapping in conifers using haploid DNA from megagametophyte. Bio/Technology **10**: 686–690 (1992). — VAN DER NEST, M. A., STEENKAMP, E. T., WINGFIELD, B. D. and WINGFIELD, M. J.: Development of simple sequence repeat (SSR) markers in *Eucalyptus* from amplified inter-simple sequence repeats (ISSR) Plant Breed. **119**: 433–436 (2000). — VERHAEGEN, D., PLOMIN, C., GION, J. M., POITEL, M., COSTA, P. and KREMER, A.: Quantitative trait dissection analysis in Eucalyptus using RAPD markers. 1. Detection of QTL in interspcific hybrid progeny, stability of QTL expression across different ages. Theor. Appl. Gent. **95**: 597–608 (1997). — VERHAEGEN, D. and PLOMION, C.: Genetic mapping in *Eucalyptus urophylla* and *Eucalyptus grandis* using RAPD markers Genome **39**: 1051–1061 (1996). — VOORRIPS, R. E.,: MapChart version 2.0: Windows software for the graphical presentation of linkage maps and QTLs. Plant Research International, Wageningen, The Netherlands (2001). — WAKAMIYA, I., NEWTON, R. J. and PRICE, J. S.: Genome size and environmental factors in the genus *Pinus*. Am. J. Bot. **80**: 1235–1241 (1993). — WEEDEN, N. F., HEMMAT, M., LAWSON, D. M., LODHI, M., BELL, R. L., MANGANARIS, A. G., REISCH, B. I., BROWN, S. K. and YE, G. N.: Development and application of molecular marker linkage maps in woody fruit crops. Euphytica **77**: 71–75 (1994). — WILLIAMS, J. G. K., KUBELIK, A. R., LIVAK, K. J., RAFALSKI, J. A. and TINGEY, S. V.: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. **18**: 6531–6535 (1990).

# Differences in Flowering Characteristic among Clones of *Cunninghamia lanceolata* (LAMB.) Hook

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**Abstract:**

Using data from a seed orchard of Chinese fir (*Cunninghamia lanceolata* (LAMB.) Hook) located at Chongyang, Hubei, China, we studied the differences in flowering characteristics among clones. The results show that for each flowering characteristics there is no difference among trees within the same clone. The differences in morphological characteristics of male and female cones among clones are clear, but the distributions of these morphological characteristics are not normally distributions. Male cones can be described by diameter, length and the number of microstrobilus, and the female cones by diameter, length and the number of macrosporophyll. Using principal components analysis, their cumulating contribution percentages are 95.8 % and 92.6 % respectively and the contributions of other morphological characteristics are very small and can be ignored. There are obvious differences in male and female cone numbers among both clones and their distribution in the tree crown levels. Using the ratio of female to male cones, the clones can be classed into mainly female clones, female and male clones and mainly male clones. Using flowering percentages of female and male cones among different clones at different dates, clones can be divided into early, mid and late flowering clones. Based on the timing of male and female flowering coincidence, clones can be grouped into early female flowering late male flowering clones, coincident male and female flowering clones and late female flowering early male flowering clones. These flowering characteristics are important in improving or designing a new seed orchard. Only in this way can we be able