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Linkage Maps of *Eucalyptus globulus* Using RAPD and Microsatellite Markers

By P.C. BUNDOCK[1]), M. HAYDEN[2]) and R.E. VAILLANCOURT

Co-operative Research Centre for Sustainable Production Forestry and School of Plant Science, University of Tasmania, GPO Box 252-55, Hobart, Tasmania 7001, Australia

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Abstract

The construction of linkage maps based on RAPD markers using an F_1 intraprovenance cross in *Eucalyptus globulus* subsp. *globulus* is reported. Twenty-one microsatellite loci originating from *E. globulus* and four other *Eucalyptus* species were added to the RAPD maps. Linkages between microsatellites previously reported for *E. grandis*/*E. urophylla* were found to be conserved in *E. globulus* allowing confident assignment of homology for several linkage groups between maps of these species. Homology was also identifiable between most linkage groups of the two *E. globulus* parents based on microsatellites and RAPD loci segregating from both parents. At a LOD score threshold of 4.9 the male parent has 13 linkage groups covering 1013 cM with 101 framework markers ordered at LOD 3.0. The female parent has 11 linkage groups covering 701 cM with 97 framework markers. On the female map there were more regions of segregation distortion than expected and genetic mechanisms to explain distorted segregation are discussed. Several linkages that arise between pairs of *E. globulus* linkage groups as the LOD score is reduced are supported by interspecific homologies identified using microsatellite loci.

Key words: SSR, blue gum, genomic map, genetic map, segregation distortion.

[1]) Present address: Centre for Plant Conservation Genetics, Southern Cross University, PO Box 157, Lismore, NSW 2480, Australia

Please address correspondence to:

PETER BUNDOCK, Centre for Plant Conservation Genetics, Southern Cross University, PO Box 157, Lismore, NSW 2480, Australia.

Phone: (02) 66 203581

Int Ph: +61 2 66 203581

Fax: (02) 66 269129

Int Fax: +61 2 66 269129

e-mail: pbundock@scu.edu.au

[2]) Present address: PBI Private Bag 11, University of Sydney, Cobbitty, NSW 2570, Australia

Introduction

In temperate regions *Eucalyptus globulus* is the most significant hardwood tree species grown as a source of fibre for the manufacture of paper products, whilst in tropical regions *E. grandis, E. urophylla* and their hybrids are the most commonly planted *Eucalyptus* for pulpwood (ELDRIDGE et al., 1993). Worldwide there has been considerable effort to select and breed genetically superior *Eucalyptus* trees and it has been recognised that molecular markers have potential application in both breeding and deployment (eg. WILLIAMS, 1995; KERR et al., 1996; DALE and CHAPARRO, 1996; GRATTAPAGLIA, 1997). Consequently linkage maps composed of DNA based markers have been constructed and published for several species of *Eucalyptus*. The crosses used for generating the segregating mapping populations were either interspecific F_1s (GRATTAPAGLIA and SEDEROFF, 1994; VERHAEGEN and PLOMION, 1996; MARQUES et al., 1998) or an F_2 cross using grandparents from widely disjunct populations (BYRNE et al., 1995). This would virtually ensure that parent trees would be genetically distant from one another. Using genetically distant parent trees is likely to increase the efficiency of mapping. However since *Eucalyptus globulus* is generally grown as a pure species, intraspecific (and also intraprovenance) crosses are required in most breeding programs. An AFLP map of *E. globulus* has been published, however it was based on an interspecific F_1 cross with *E. tereticornis* (MARQUES et al., 1998). In this paper we report on the first maps published for *Eucalyptus globulus* based on a pure species cross and the first published *Eucalyptus* maps using an intraprovenance cross.

Like the previously published maps for *E. grandis* and *E. urophylla* (GRATTAPAGLIA and SEDEROFF, 1994; VERHAEGEN and PLOMION, 1996), the *E. globulus* maps in this study are based on RAPD markers. Although RAPD markers are extremely useful for map construction, especially using an F_1 cross, they have limited transferability because they are dominant markers with only two alleles. Co-dominant markers such as microsatellite loci on the other hand, are expensive to isolate but have much broader transferability and are also potentially fully informative in crosses other than an F_1. Including microsatellite markers as a significant component of *Eucalyptus* genomic maps should increase the informativeness, transferability and reliability of these maps – factors important to their future applicability. BYRNE et al. (1996) reported that four microsatellite loci isolated from *E. nitens* were amplifiable and polymorphic in several other species in the genus, demonstrating the potential transferability of microsatellites between species of *Eucalyptus*. BRONDANI et al. (1998) list primer sequences used to amplify twenty microsatellite loci that originate from *E. grandis* and *E. urophylla* and also show the location of these loci on RAPD maps from a cross between these two species. These primer sequences have been used in the present study to add *E. grandis*/*E. urophylla* microsatellite loci to the RAPD maps of *E. globulus*. In addition microsatellite loci originating from *E. globulus*, *E. nitens* and *E. sieberi* have been mapped in this cross. This has aided the identification of linkage group homology between the two *E. globulus* parental RAPD maps, and enabled the identification of homology between the *E. globulus* linkage groups reported in this paper and the *E. grandis* and *E. urophylla* linkage groups of BRONDANI et al. (1998).

Materials and Methods

Plant Material and DNA Extraction

The mapping population consists of a single full-sib family of 165 progeny from an *E. globulus* subsp. *globulus* intraprovenance cross, carried out by CSIRO Division of Forestry and North Forest Products (Australia) and planted at a number of field sites (VAILLANCOURT et al., 1995a). Both parents originate from King Island which is located in Bass Strait between continental Australia and Tasmania. The male parent (G164) is located at a distance of several kilometres from the mother tree of the female parent (KI2) and on this basis the two parents would not be expected to be closely related (SKABO et al., 1998). The female parent is an open pollinated progeny planted in a seed orchard.

Two grams of frozen leaf material was ground to a smooth powder in liquid nitrogen using a mortar and pestle. The DNA was extracted using the CTAB extraction protocol of DOYLE and DOYLE (1990). The CTAB extraction buffer was modified by the addition of polyvinylpyrolidone (PVP-40, Sigma) at 2% (w/v). Two volumes of ice-cold ethanol (~65% final concentration), rather than isopropanol, was found to precipitate higher quality DNA for PCR. By routinely adding a phenol, phenol-chloroform extraction step, readily PCRable DNA was almost always obtained. DNA was quantified using a Hoefer DNA Fluorometer (TKO 100) and Hoechst 33258 dye.

Molecular Marker Assays

Ten-mer RAPD primers were obtained from the University of British Columbia (UBC; Dr. J. B. HOBBS, c/o Biotechnology Laboratory, Wesbrook Building, 6174 University Boulevard, Vancouver, B.C. V6T1Z3) and Operon Technologies Inc. (OP; 1000 Atlantic Ave., Alameda, CA 94501, USA). Four hundred UBC RAPD primers comprising sets 1, 2, 3 and 5 and OP kit B were screened to identify those that amplified strong, reliable and polymorphic RAPD bands. DNA from the two parents and six progeny were used for the screening of the RAPD primers.

Amplification conditions are based on those of WILLIAMS et al. (1993). RAPD reactions (20 µL) were composed of the following components: 50 mM KCl, 10 mM Tris-HCl pH 9.0 @ 25°C, 0.1% Triton X-100, 200 µM dNTPs, 3 mM $MgCl_2$, 150 µg/mL Bovine Serum Albumin (BSA), 0.25 µM RAPD primer, 1.6 units Taq DNA Polymerase and 20 ng genomic DNA and overlaid with 30 µL of mineral oil prior to amplification. An MJ Research Inc. PTC-100 programmable thermal controller was used for amplification using the following cycling profile: 94° – 2 min, [94° – 1 min, 35° – 1 min, 72° – 2 min] x 40, 72° – 5 min, 10° – hold. Reactions were electrophoresed in 1.5% agarose gels at 22 volt.hours/cm in 1 x TBE buffer. Fluorescence from ethidium bromide (incorporated into the gel at 0.2 µg/mL) was used to photograph the RAPD bands using Polaroid 665 film. Polymorphic bands were scored manually from the Polaroid negative. RAPD markers inherited from one parent only and segregating in an apparent 1:1 pattern were classed as originating from either the male or the female parent, creating two separate data sets depending on the parent of origin. A third data set was created for those RAPD markers that originated from both parents and had a 3:1 segregation pattern.

All of the microsatellite loci that were used in this study were originally isolated as dinucleotide repeats. There were three sources of microsatellite primer sequences: EMCRC (*Eucalyptus M*icrosatellites from *C*o-operative *R*esearch *C*entre for Sustainable Production Forestry) loci originate from *Eucalyptus globulus* DNA enriched for microsatellite sequences; CSIRO primer sequences were obtained from Dr. GAVIN MORAN at CSIRO Forestry and Forest Products and were isolated from *E. nitens* and from *E. sieberi;* and the EMBRA microsatellites were cloned from *E. grandis* and *E. urophylla* and originate from Dr. DARIO GRATTAPAGLIA's lab in Brazil. The primer sequences for the EMBRA loci are those of BRONDANI et al. (1998) and the coding of loci is the same as in that publication.

The primer design for the EMCRC and CSIRO microsatellite loci are subject to confidentiality agreements which disallows their publication here. The reaction conditions used for the EMBRA microsatellites were essentially those of BRONDANI et al. (1998) with the following modifications: 0.1% Triton X-100, 0.1 mg/mL BSA, no DMSO and annealing at 57°C. All amplifications were carried out on an MJ Research Inc. PTC-100 Thermal Cycler. All amplifiable loci were screened for their potential to be mapped on both parental maps with sizing of alleles carried out on polyacrylamide gels using an Applied Biosystems automated DNA sequencer. Reaction product fragment sizes were calculated using GENESCAN software based on an internal standard. Metaphor (FMC) agarose gels [3.5% (w/v) in 1 x TBE buffer] were used to separate microsatellite reaction products for scoring all microsatellite loci in the progeny. Depending on the size of the microsatellite and the size difference between alleles, the running of the gels varied from 20 volt.hours/cm at 4° to 16 volt.hours/cm at room temperature. Ethidium bromide (0.4 µg/mL) was incorporated into the gels which were scored from Polaroid (665) photographs of the fluorescing PCR products.

A number of different enzymes were tested for their ability to be detected using frozen mature leaf tissue as a source and starch gel electrophoresis for separation with only one polymorphism reliably detectable. The methods used for detecting enzyme activity and starch gel electrophoresis were based on those of MORAN and BELL (1983).

Linkage Analysis

All loci segregating 1:1 from both parents were tested for evidence of linkage to each 3:1 segregating locus using a chi-squared goodness of fit test. Only those progeny that were found to be homozygous absent at the 3:1 locus were used in the test where a 1:1 segregation pattern would be expected for an unlinked (1:1) marker. Linkage is indicated where a significant departure from the 1:1 pattern arises, in this study where $\alpha < 1 \times 10^{-4}$.

The program MAPMAKER Version 3.0b (UNIX) was used to determine linkage groupings and ordering of markers within linkage groups for loci segregating in a 1:1 pattern (LANDER et al., 1987; LINCOLN et al., 1992). Data for loci segregating 1:1 was entered as F_2 backcross data and markers were scored as either present (H), absent (A) or undetermined (–) for each offspring. Because MAPMAKER recognises linkage in coupling phase only, each marker needed to be represented by an original and also an inverse (repulsion phase) form in the data set. This allowed linkages in repulsion to be recognised by MAPMAKER as de facto linkages in coupling to inverse markers.

The LOD score threshold for declaring linkage using MAPMAKER was calculated based on the maximum number of independently segregating ('unlinked') positions expected in the genome and the required type I error. This is based on the expected number of linkage groups, an estimate of the genome size and a prior definition of linkage in map units. The expected number of linkage groups is 11 since cytological observations suggest this to be the haploid number of chromosomes in *Eucalyptus* (POTTS and WILTSHIRE, 1997). An upper estimate for the size of the *Eucalyptus* genome from other mapping studies is approx. 1500 cM. Linkage can be defined arbitrarily as two markers being less than 50 cM (Kosambi) apart. The number of 'independently segregating positions' is thus the maximum number of positions that are 50 cM or more apart in the genome (ie. 41). There are 820 unique pairwise linkage tests that can be made between these 41 positions. For a probability of Type I error of 0.01 for the genome as a whole, the appropriate LOD score threshold is $-\log_{10}(820/0.01) = 4.9$. Thus a LOD score threshold of 4.9 for declaring linkage between markers was used with the "group" command of MAPMAKER with a consequent estimated probability of Type I error of 0.01 for each linkage map. Since the maximum recombination fraction at which linkage is declared will vary according to the number of progeny which have been scored in common for a marker pair, the recombination fraction parameter was left as non-restrictive (ie. r = 0.49) when using the "group" command of MAPMAKER.

For ordering of markers within each linkage group a subgroup of six or less markers were chosen that were well spaced from one another and had a minimum of missing data. The most likely marker orders for the subgroup were found using the "compare" command of MAPMAKER with a LOD 3.0 threshold for alternative marker orders. The "build" command of MAPMAKER was used to identify any remaining markers in a linkage group that could be added to the order established for the initial subgroup of markers at LOD 3.0. Framework marker orders were used as the basis for data checking using the error detection system in MAPMAKER and discernible errors corrected. Error correction did not affect marker ordering as tested using the "ripple" command of MAPMAKER on the corrected data sets. The "build" command was used after error correction to add any previously unplaced framework markers to the corrected data sets at LOD 3.0 and to add accessory markers at LOD 2.0. Several markers which had missing data and that significantly inflated the length of a linkage group due to dubious double crossovers were excluded from the framework and placed as accessories. Accessory markers were located on the framework map alongside the nearest framework marker. Unplaced microsatellite loci in a linkage group were assigned to the most closely linked framework marker using the "near" command of MAPMAKER.

Test for Segregation Distortion

All loci classified as segregating 1:1 were tested for distortion from this expected ratio using a chi-squared goodness of fit test. Loci segregating from one parent with significant departure from a 1:1 pattern at $\alpha = 0.05$ were compared with expected ratios for 2:1 and 3:1 segregation. To determine the frequency of segregation distortion in the genomes of the two parents, the number of regions (rather than markers) expected to have distorted segregation was estimated for each map. (A region is defined as a group of linked markers or a lone mapped marker.) Estimates are based on the number of 'independently segregating positions', which is the size of each linkage group in cM divided by 50 rounded upwards and summed for the entire map. The expected number of regions with distorted segregation is the number of these 'independently segregating positions' multiplied by the threshold value used for declaring distortion as significant (eg. 0.05, 0.01). Since distortions from a 1:1 ratio can alter the probability of linkage between two markers, pairs of framework markers with strong distortion and with weak linkages were checked for any extreme alterations to their probability of linkage. Two point LOD scores for these marker pairs were also recalculated based on an adjusted θ value for no linkage.

Results

Screening and Scoring of Molecular Markers

Sixty-five RAPD primers out of 400 were selected for use in genotyping, approximately one out of every six screened. The primers amplified 326 RAPD markers segregating in an appar-

(a) Map of the Male Parent

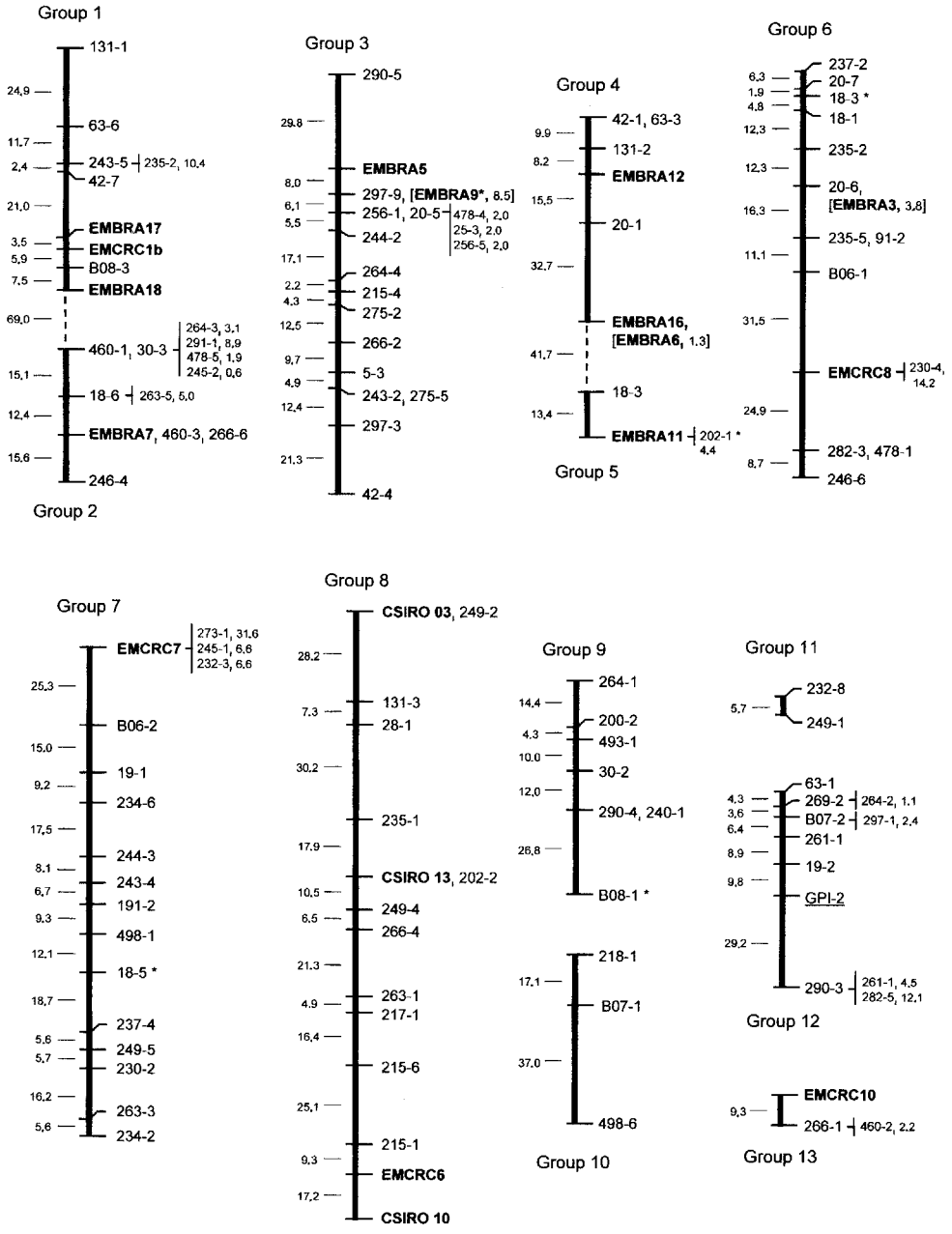

Table 1. – The source, amplifiability and informativeness of microsatellite loci used for mapping.

Source	No. tested	No. amplifiable	Heterozygosity in *E. globulus* parents		
			Neither	*One*	*Both*
EMBRA[a]	20	15	4	4	7
EMCRC[b]	12	11	4	5	2
CSIRO[c]	10	9	2	4	3
total	42	35	10	13	12

[a]) EMBRA = *Eucalyptus grandis / E. urophylla* – BRONDANI et al. (1998)
[b]) EMCRC = *E. globulus*
[c]) CSIRO = *E. nitens / E. sieberi*

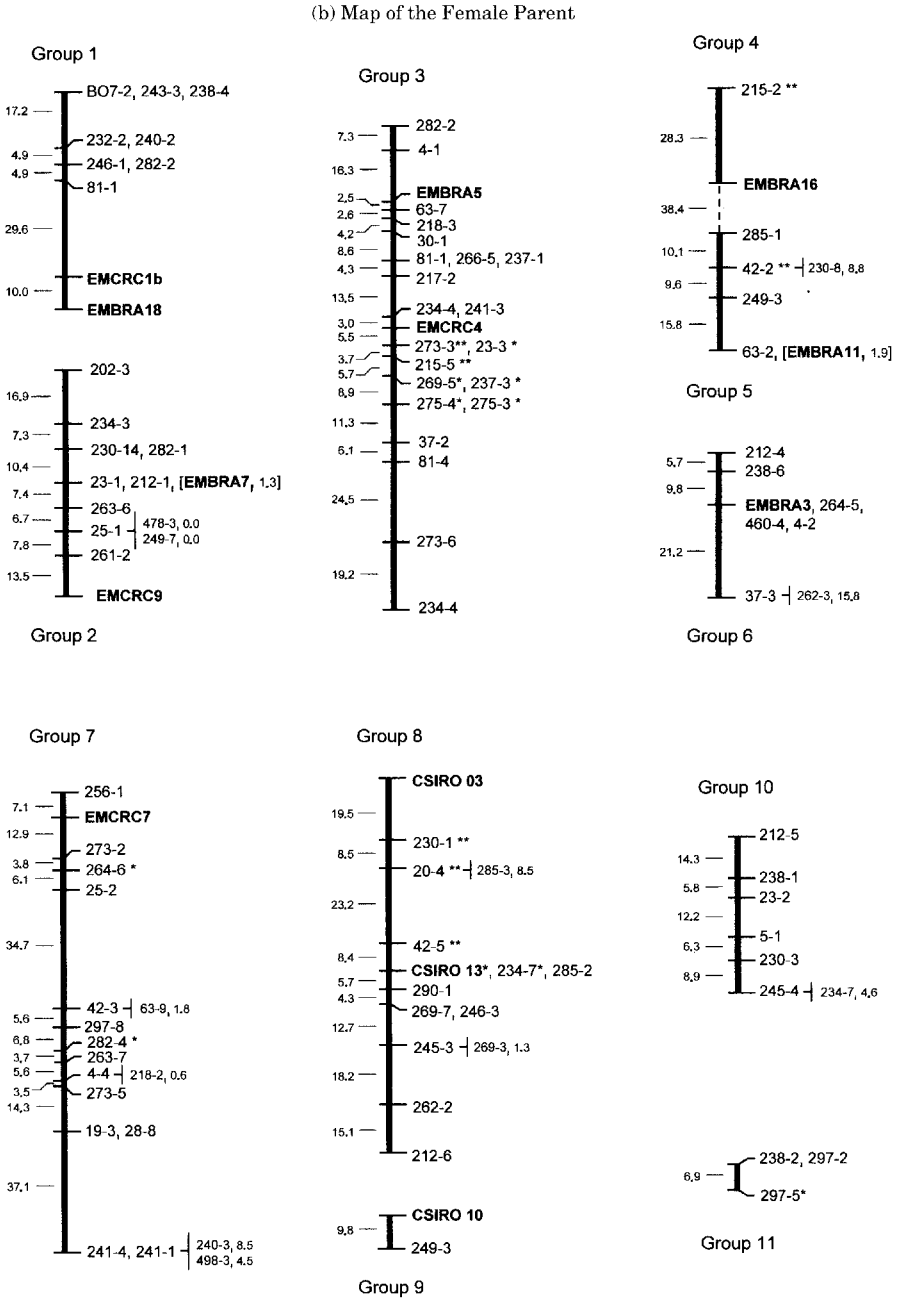

Figure 1. – Linkage maps of *Eucalyptus globulus* using RAPD and microsatellite markers. Framework marker orders were determined using the program MAPMAKER at a LOD threshold of 3.0. Microsatellite loci are in bold text. Microsatellites that are not framework or accessory markers are bracketed and listed next to the nearest framework marker in the linkage group. RAPD loci amplified from UBC primers are listed by the UBC number followed by the ranked size of the amplified band from large to small. RAPD loci amplified with OP primers are listed using the OP designation followed by the ranked size of the amplified band. The isozyme locus, GPI-2 is underlined. The distance between framework markers is in Kosambi centimorgans on the left-hand side of each linkage group. Markers that mapped to the same position are listed separated by a comma. Accessory markers that were placed at LOD 2.0 are located in smaller text alongside the nearest framework marker with the two point distance in centimorgans from this marker. Loci which had distorted segregation at $\propto$ 0.05 are followed by a single asterisk, those with distortion at the $\propto$ 0.01 level are followed by a double asterisk. A dashed line between linkage groups indicates linkage at a reduced LOD score threshold. (*a*) Map of the male (G164) parent, (*b*) Map of the female (KI2) parent.

ent 1:1 pattern with 173 inherited from the male parent and 153 inherited from the female parent, resulting in five markers segregating 1:1 per primer. Twenty loci were scored as being heterozygous in both parents and segregating in an apparent 3:1 pattern.

The results of screening the microsatellite loci are summarised in *table 1.* From the 35 amplifiable loci, 25 were heterozygous in one or both parents with the male parent heterozygous at 21 loci and the female parent at 16 loci. Of the 11 EMBRA loci scored in common with *E. grandis* and *E. urophylla,* the

male *E. globulus* parent was heterozygous at 10 and the female parent heterozygous at 8 loci. This compares with the parent trees in BRONDANI et al. (1998) where the *E. grandis* parent is heterozygous at 10 and the *E. urophylla* parent at all 11 of the loci scored in common. Although seven CSIRO microsatellites were found to be potentially mappable, only the three fully informative loci (ie. segregating from both parents) were assayed for all progeny and used in the linkage analysis. All EMCRC and EMBRA microsatellites that were found to be segregating from one or both parents were used for genotyping and linkage analysis. All of the 21 microsatellite loci that were scored for segregation in the progeny originate from species of *Eucalyptus* in the subgenus *Symphyomyrtus* except for CSIRO-03 which is from *E. sieberi* belonging to the subgenus *Monocalyptus*. The male parent was found to be heterozygous for 18 of these loci and the female parent heterozygous at 14 loci with 11 loci heterozygous in both parents. Of the total of 32 microsatellite markers obtained from the 21 loci only one (EMBRA 10) remained unlinked at LOD 4.9. Of the three enzyme systems found to have scorable activity using frozen adult leaf material (MDH, AAT and GPI) only glucosephosphate isomerase 2 (GPI-2, E.C. 5.3.1.9) was polymorphic. Ninety-one progeny were scored for polymorphism at GPI-2 which was heterozygous in the male parent only. Twenty percent of markers were scored for segregation in 94 progeny only with 35% scored in a non-overlapping set of 71 progeny and 43% assayed across all 165 progeny.

Linkage Maps

For the male parent (G164) a total of 192 loci segregating 1:1 were used in the initial linkage analysis. Thirteen linkage groups were defined over a range of LOD score values from 4.25 to 4.95 with 15 markers (7.6%) remaining unlinked. There were 101 framework markers ordered with 19 accessory markers added to the framework map (*Figure 1a*). The 101 framework markers map to 90 positions covering a total distance of 1013 cM (Kosambi mapping function). There are 77 intervals between markers on the linkage groups with an average size of 13 cM and a maximum size of 37 cM. Linkage group frameworks varied in size from two positions covering 5.7 cM (Group 11) to 13 positions covering 195 cM (Group 8). Three groups (5, 11 and 13) consist of only two framework markers each. At LOD 4.20 twelve linkage groups form as a consequence of Groups 4 and 5 coalescing and forming a new interval of 41.7 cM whilst at LOD 4.0, Groups 1 and 2 form a single linkage group with a new interval of 69.0 cM (dashed lines on *Figure 1a*). Thus at LOD 4.0, eleven linkage groups form, which is the observed haploid number of chromosomes in *Eucalyptus*.

For the female parent (KI2) 167 loci segregating 1:1 were used in the analysis. These formed into 11 linkage groups from LOD score thresholds ranging from 4.35 to 6.15 with 15 markers (approx. 9%) remaining unlinked at LOD 4.9. There were 97 framework markers at 75 positions on the linkage map of 11 groups with 11 accessory markers (*Figure 1b*). The framework spans 64 intervals covering 701 cM with an average interval size of 11 cM and a maximum interval size of 37.1 cM (Kosambi mapping function). Three groups (4, 9 and 11) are composed of two framework positions only. The largest group (Group 3) has 18 framework positions and covers 148 cM with the smallest group (Group 11) being 6.9 cM. Using a LOD of 4.3 with the group command of MAPMAKER, ten linkage groups form rather than 11, with Groups 4 and 5 coalescing to form a new interval of 38.4 cM (dashed line *Figure 1b*).

Linkage Group Homology

The presence of microsatellite loci mapped in both parents indicates there is homology between eight linkage groups from the male parent with nine linkage groups from the female parent (*Table 2*). Groups 1 through to 8 are proposed as homologous between parents, with Group 9 of the female parent homologous with one end of Group 8 of the male parent. Homology with four linkage groups of the *E. grandis*/*E. urophylla* maps of BRONDANI et al. (1998) is also suggested based on the sharing of EMBRA microsatellite loci (*Table 2*). In the four cases where more than one EMBRA microsatellite was found on an *E. globulus* linkage group, conservation of linkage of EMBRA loci was found between *E. globulus* and *E. grandis*/*E. urophylla*. Of the 20 RAPD loci segregating in a 3:1 pattern, 11 were found to link ($\alpha = 0.0001$) to framework loci segregating 1:1 in both of the parental maps. This indicated homology between five pairs of linkage groups of the two parents (*Figure 2*). Homology is also indicated for each of these pairs of linkage groups on the basis of sharing of microsatellite loci (*Figure 2*). There is also strong evidence for linkage of a 3:1 locus (226-3) to Group 10 of the male parent and an unlinked marker of the female parent.

Evidence for Linkage Based on Homology

In several instances EMBRA microsatellite loci that are present on one group of the *E. grandis* and/or *E. urophylla* maps of BRONDANI et al. (1998) are present on two *E. globulus* linkage groups (*Table 2*). Linkage Group 9 of the *E. urophylla* map of BRONDANI et al. (1998) has microsatellite loci which occur on both linkage Group 1 (EMBRA 17 and 18) and linkage Group 2 (EMBRA 7) of the male *E. globulus* parent (*Table 2* and *Figure 1a*). Similarly linkage Group 1 of both the *E. grandis* and *E. urophylla* maps has loci which occur on Group 4 (EMBRA 6, 12 and 16) and Group 5 (EMBRA 11) of the male *E. globulus* parent (*Table 2* and *Figure 1a*). It has been noted that at reduced threshold LOD scores linkage was detected between these two pairs of groups in the male parent (see Linkage Maps above). The evidence thus suggests that Groups 1 and 2 belong to a single linkage group and that Groups 4 and 5 similarly belong in a single group and that the arrangement of the EMBRA loci on these groups has been conserved between species.

Linkage Groups 1 and 2 of the female parent also share microsatellite loci (EMBRA 18 and 7) with Group 9 of *E. urophylla* and linkage Groups 4 and 5 have microsatellite loci (EMBRA 16 and 11) that map to Group 1 of both the *E. grandis* and *E. urophylla* maps (*Table 2, Figure 1b*). At a reduced threshold LOD score linkage is detected between Groups 4 and 5 of the female parent (see Linkage Maps above) but not, however, between Groups 1 and 2. Again the evidence supports conservation in the arrangement of EMBRA loci on Groups 4 and 5. Linkage between Groups 1 and 2 in the female parent can be inferred from the linkage between the homologous groups in the male parent and from the arrangement of EMBRA microsatellites in both the male parent and *E. urophylla*.

The three CSIRO microsatellites map to one group in the male parent (Group 8) but to two groups (Groups 8 and 9) in the female parent (*Table 2, Figure 1a, 1b*). Since the linkages in Group 8 of the male parent are of high likelihood and assuming the distribution of microsatellites is the same between the two parents it is likely that Groups 8 and 9 of the female parent belong to one linkage group. If all the linkages inferred from homology in both the *E. globulus* parents are taken as correct then the male parent would have eleven linkage groups and the female parent eight.

Segregation Distortion

There are six markers with segregation distortion at $\alpha = 0.05$ segregating from the male parent. These map to five regions

Table 2. – Homology of linkage groups between *E. globulus* parent trees and between *E. globulus* and *E. urophylla / E. grandis* based on the mapping of shared microsatellite loci.

Microsatellite locus	Male parent linkage group	Female parent linkage group	*E. urophylla/ E. grandis* group[a]
EMCRC1b	1	1	–
EMBRA17	1	–	9
EMBRA18	1	1	9
EMBRA7	2	2	9
EMBRA5	3	3	5
EMBRA9	3	–	5
EMBRA6	4	–	1
EMBRA12	4	–	1
EMBRA16	4	4	1
EMBRA11	5	5	1
EMBRA3	6	6	8
EMCRC7	7	7	–
CSIRO03	8	8	–
CSIRO13	8	8	–
CSIRO10	8	9	–

[a]) *E. urophylla/E. grandis* linkage group no. from BRONDANI et al. (1998)

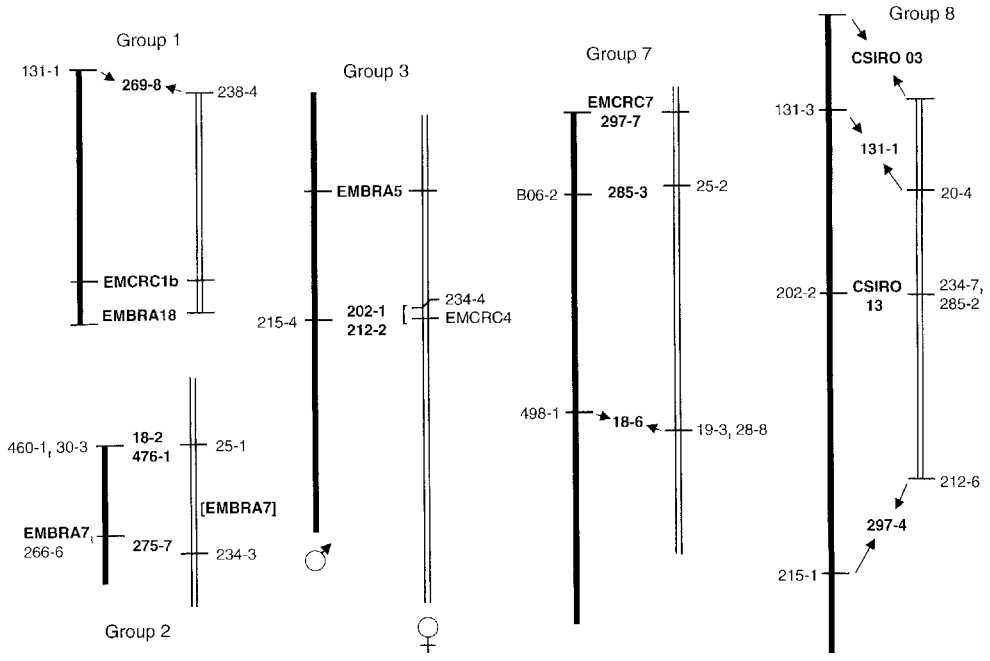

Figure 2. – Linkage of 3:1 segregating RAPD markers to loci on both parental maps. Linkage group homology inferred from 3:1 loci is supported in each case by the mapping of one or more fully informative microsatellite loci. RAPD loci segregating 3:1 are located between each pair of homologous linkage groups and numbered in bold text along with framework microsatellite loci. Framework RAPD loci which were most strongly linked to each 3:1 locus are shown in plain text. Otherwise coding of RAPD and microsatellite loci is as per *figure 1.*

and include three framework markers (*Figure 1a*, asterisked markers). With 28 'independently segregating positions' on the map of the male parent (see Materials and Methods), 1.4 regions are expected to have distorted segregation at $\alpha = 0.05$. There is thus approximately three and a half times the number of regions expected to have distortion at $\alpha = 0.05$. There were no markers with segregation distortion at $\alpha = 0.01$ segregating from the male parent. (If it is assumed that segregation patterns for each marker are derived from random and independent events, then the expected number of markers with

segregation distortion at $\alpha = 0.05$ is 9.6 and based on this method of assessment there would be fewer markers observed to have distorted segregation than expected.)

In the overall data set of the female parent there are twenty-three markers with distorted segregation at $\alpha = 0.05$. These map to seven regions and include 17 framework markers (*Figure 1b*, asterisked markers). The female parental map of 701 cM has a total of 19 'independently segregating positions' with 0.95 regions expected to have segregation distortion at $\alpha = 0.05$. There are thus seven times the expected number of regions with distorted segregation at $\alpha = 0.05$. There are eight markers overall with distorted segregation at a threshold of $\alpha = 0.01$. These map to four regions with three being framework markers on linkage Group 8 and one framework marker on each of Groups 3, 4 and 5 (*Figure 1b,* double asterisks). Since there are 0.19 regions expected to have distorted segregation at $\alpha = 0.01$ there are approx. 21 times the expected number of regions with segregation distortion at $\alpha = 0.01$. (Assuming that segregation of each marker results from random and independent events, then 8.35 markers would be expected to have segregation distortion at $\alpha = 0.05$ and 1.67 markers at $\alpha = 0.01$. Using this assumption there are 2.8 times the expected number of loci with distortion at $\alpha = 0.05$ and 4.8 times the expected number of loci with distortion at $\alpha = 0.01$.).

For the regions with segregation distortion at $\alpha = 0.01$ in the female parent the region on Group 3 and the region on Group 8 have markers which have been scored on the full array of progeny and there are linkages in repulsion with markers that also have distorted segregation. Thus it is extremely unlikely that the segregation distortion is due to 'unreliability of RAPD markers', at least for these two regions. Lone markers, both of which gave strong unambiguous banding, represent the other two regions with strong distortion in the female parent. It would thus appear very likely that the observed distortions are not artifactual and the excess segregation distortion requires explanation.

All marker loci with significant segregation distortion were found to have segregation ratios between 1:1 and 2:1, as distinct from the 3:1 ratio expected from an unlinked duplication of a locus. None of the linkages involving framework markers with segregation distortion were found to have probabilities (of linkage) which were unduly affected by the distortion. In the most severe case on Group 8 of the female parent (*Figure 1b*), RAPD loci 42-5 and 20-4 are 23.2 cM apart. For two loci with the same degree of distortion, the probability of linkage at this level or closer is 7.8×10^{-10}, which although 70 times larger than two loci segregating in a perfect 1:1 ratio, still provides very strong evidence for linkage. The two point LOD score calculated using $\theta = 0.5$ for no linkage was 11.05 and an adjusted LOD score of 10.96 was obtained using $\theta = 0.455$.

Estimates of Genome Size

Some idea of the completeness of the two maps overall can be gained from the fact that 32 out of 33 microsatellite and allozyme markers were placed into linkage groups with only one marker (EMBRA 10) remaining unlinked at LOD 4.9. For the male parent all 19 of the non-RAPD markers were placed into linkage groups and for the female parent one marker out of 14 remained unlinked at LOD 4.9. A method of estimating the overall length of the genome as detailed in VALLEJOS et al. (1992) was used. The following formula was used for estimation: $G = 2MX/K$ where G is the estimate of genome size, M is the no. of locus pairs, X is the largest estimated map distance value among the K observed no. of locus pairs that are linked with a LOD score of Z or greater (Z = 4.9). This method gave an estimated genome length for the male parent of 1277 cM with 79% of the genome covered by the framework map. The female parent was estimated to have a genome of size 1133 cM with 62% of the genome estimated to be covered by the framework map.

Discussion

Utility of Microsatellite Loci

Microsatellite loci are extremely useful for the identification of linkage group homology and for enabling the integration of linkage information (DIB et al., 1996; DIETRICH et al., 1996). In this study linkage analysis of eleven fully informative microsatellite loci has enabled the identification of homology between eight (pairs of) linkage groups from the RAPD maps of the parents. In addition homology with four linkage groups of *E. grandis/E. urophylla* have been identified based on the mapping of eleven EMBRA microsatellite loci (BRONDANI et al., 1998). In all cases where it could be examined, linkages between EMBRA microsatellites that were found in *E. grandis/E. urophylla* were conserved in *E. globulus*. The close correspondence of microsatellite distributions may be a reflection of the fact that all three of these species belong to the subgenus *Symphyomyrtus,* although *E. globulus* belongs in a different section to *E. grandis* and *E. urophylla*. In total 21 microsatellite loci from five different species of *Eucalyptus* have been placed on the two parental maps detailed here.

A potential outcome of identifying linkage group homology between maps is that the arrangement of loci on one map can be used to infer linkage between groups on the second map. In this study such homology has been used to support several weak linkages found between linkage groups. The two lines of evidence lend support to one another to indicate that these linkages are likely to be correct. Importantly homology was based on a between species alignment of microsatellite loci. This illustrates the potential power of microsatellites to draw upon mapping information from both within and between species to aid in map construction. This information could be used in strategies to search for markers to fill in gaps in a linkage map eg. using bulked segregant analysis (MICHELMORE et al., 1991).

Using homology to infer linkage has had a considerable influence on map construction in this study, impacting on the number of linkage groups and the structure of the maps. If all the linkages inferred from homology in both the *E. globulus* parents are taken as correct then the male parent would have eleven linkage groups and the female parent eight. Cytological studies indicate that n = 11 in *Eucalyptus* (POTTS and WILTSHIRE, 1997). So for the male parent, eleven linkage groups matches the number expected based on chromosome counts. However for the female parent there are three linkage groups less than expected from chromosome counts. It is likely that by scoring additional RAPD markers inherited from the female parent these three linkage groups would be defined, especially since some of the 15 unlinked markers (approx. 9%) probably belong to these three groups.

Mapping in Eucalyptus Using an Intraprovenance Cross

The levels of heterozygosity and variation within a provenance in *E. globulus* are clearly sufficient to allow the construction of RAPD maps from an intraprovenance cross. Since this species is cultivated as a pure species, the construction of such maps from intraspecific and intraprovenance crosses will be required if QTL detection and marker assisted selection is to be carried out as part of *E. globulus* breeding programs. Since the two parents of the cross in this study origi-

nate from the same island provenance, it might have been expected that map construction would be hampered due to low genetic divergence of the parents and a consequent paucity of RAPD loci segregating in a fully informative manner. However if there is low genetic divergence between the two parents in this study it has not proven to be a significant hindrance and has been overcome by using the easy to implement strategy of screening a large number of RAPD primers and selecting a subset which detect a maximum number of polymorphisms. Approximately one in every six primers screened was selected for genotyping, yielding five loci segregating 1:1 on average per selected primer. This compares with GRATTAPAGLIA and SEDEROFF (1994) where half of the screened primers were used for genotyping, yielding an average of 3.7 markers per primer and VERHAEGEN and PLOMION (1996) where almost ¾ of screened primers were used for genotyping, yielding 3.2 markers per primer. In both these cases an *E. grandis* x *E. urophylla* interspecific cross was used.

Segregation Distortion

Commonly, the frequency of segregation distortion expected due to chance is calculated using the total number of markers that have been scored in the data set. However, since linked markers are not independent, a more meaningful method of calculating the degree of expected segregation distortion for linkage maps may be to calculate the expected number of regions with segregation distortion. The number of linkage groups and their sizes determines the expected number of regions. In this study this has been shown to provide a different basis on which to judge the expected extent of segregation distortion. For example in the male parent at $\alpha = 0.05$, the ratio of the observed to expected was 3.5 (somewhat more than expected) whilst for calculations based on the number of markers the ratio of observed to expected is 0.625 (fewer than expected). The method of calculating the extent of segregation distortion can thus have a significant bearing on the assessment of whether excess distortion is occurring.

In this study it has been found that the number of regions with significantly distorted marker ratios in the map of the female *E. globulus* parent in particular is much greater than would be expected by chance alone. This greater than expected frequency/degree of skewing of Mendelian segregation ratios is not uncommon in plants (ZAMIR and TADMOR, 1986; BRADSHAW and STETTLER, 1994), with *Eucalyptus* being no exception (BYRNE et al., 1994; VAILLANCOURT et al., 1995b; VERHAEGEN and PLOMION, 1996; MARQUES et al., 1998). A number of selection based genetic mechanisms have been suggested to explain these distorted marker ratios. These include incompatibility systems (GEBHARDT et al., 1991), preferential chromosome loss (VAILLANCOURT and SLINKARD, 1992), expression of genetic load (SORENSEN, 1969; BRADSHAW and STETTLER, 1994; VAILLANCOURT et al., 1995b), meiotic drive (GILLET and GREGORIUS, 1992) and haploid expressed deleterious alleles. It is to be noted however that the four regions with strong distortion ($\alpha = 0.01$) in the female parent in this study do not align with regions of distortion in the homologous linkage groups of the male map. This excludes incompatibility as an explanation since incompatibility systems in higher plants usually operate to exclude fertilisation by the male or in the case of rare haplo-homophasic systems would operate to cause distorted segregation in both sexes (GILLET and GREGORIUS, 1992). If genetic load is considered as an explanation for the observed segregation distortion then a deleterious recessive allele must be segregating from the parent with the distortion. The second parent could be either heterozygous or homozygous for the deleterious recessive. However the observed non-alignment of distorted regions excludes the possibility that both parents are heterozygous for a deleterious recessive allele at the same locus since the genetic maps of both parents would be expected to have distorted segregation in the same region. To invoke genetic load as an explanation the male parent in particular would need to be homozygous for deleterious recessive alleles at several loci, which is probably unlikely, as it is a naturally established tree. Genetic load would therefore appear not to be a satisfying explanation for the segregation distortion.

Chromosome loss is also an unlikely explanation for the distortion as it is usually only considered in cases where one parent is an interspecific or intersubspecific hybrid. It is to be noted however that hybridisation is common in the genus *Eucalyptus* (POTTS and WILTSHIRE, 1997), and it is not known if there are small chromosomal rearrangements between and within species. Meiotic drive and haploid expressed deleterious alleles remain as possible explanations for segregation distortion of any region since there is no evidence for selection operating at any particular stage of development. Other models based on post-fertilisation selection, eg. selection for co-adapted allelic combinations, might also explain the skewed segregation ratios.

Conclusion

RAPD maps were readily constructed for two parents of an intraprovenance cross of *E. globulus* demonstrating the utility of RAPD markers for map construction in *Eucalyptus* from intrapopulation crosses. Microsatellite markers that originate from several *Eucalyptus* species have been mapped enabling the identification of homologous linkage groups between *E. globulus* and *E. grandis*/*E. urophylla*. The conservation of linkage of microsatellite loci and transfer of loci between crosses in different species indicates the potential for the construction of a consensus map based on microsatellites. Segregation distortion in the genome was observed to a greater extent than expected by chance and a biological cause is suspected although several of the usual genetic mechanisms to account for the distortion appear to be unlikely.

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Buchbesprechung

Grundlagen und Methoden der Pflanzensystematik. UTB für Wissenschaft: Große Reihe. Von O. SPRING und H. BUSCHMANN. 1998. Verlag Quelle und Meyer, Wiesbaden. ISBN 3-8252-8176-1. 139 Seiten mit zahlreichen Abbildungen und Tabellen. DM 34,80.

Die biologische Systematik bietet wichtige Grundlagen für alle naturwissenschaftlich ausgerichteten Fachgebiete. Diese wiederum haben durch eigenen Erkenntnisgewinn die Entwicklung der Systematik nachhaltig beeinflusst und geprägt. Systematik lässt sich heute als die Wissenschaft definieren, „die sich mit der Vielfalt der Organismen, ihrer Differenzierung, ihrer verwandtschaftlichen Beziehungen und ihrer entwicklungsgeschichtlichen Entstehung befasst". Im ersten Teil des Buches gehen die Autoren auf die historische Entwicklung der Systematik ein und schildern verschiedene Artkonzepte sowie die Prozesse der Artentstehung. Außerdem werden Nomenklatur und Taxonomie einschließlich ihrer Regeln erläutert. Der zweite Teil des Buches gibt einen Überblick über praktische Methoden zur Erfassung von taxonomischen Merkmalen bei Organismen. Hierbei wird ein Bogen gespannt von den eher traditionellen, morphologischen, anatomischen und cytologischen Merkmalen, über strukturelle Merkmale der Chromosomen und über Kreuzungsstudien, über die Periode der Chemosystematik bis hin zur makromolekularen Periode. Die Bedeutung molekularer Techniken zum Nachweis von Proteinen und Nukleinsäuren und ihr Einsatz in der modernen Systematik werden ausführlich dargestellt. In einem letzten Kapitel wird der Frage nachgegangen, wie sich die Fülle der inzwischen vorliegenden Daten von den verschiedensten Merkmalen im Hinblick auf eine phylogenetische Interpretation auswerten lassen. Ein Literaturverzeichnis (8 Seiten), ein Glossar (3 Seiten), eine kurze Übersicht über einige phylogenetische Fachbegriffe, die häufig in der englischsprachigen Literatur verwendet werden, sowie ein Index beschließen das Buch. Die vielen instruktiven Zeichnungen, S/W-Abbildungen und übersichtlichen Tabellen sind didaktisch gut gewählt. Als Einführung in die Systematik kann das Buch empfohlen werden.

B. R. STEPHAN (Grosshansdorf)

Herausgeberin: Bundesforschungsanstalt für Forst- und Holzwirtschaft: Schriftleitung: Institut für Forstgenetik und Forstpflanzenzüchtung, Siekerlandstrasse 2, D-22927 Grosshansdorf — Verlag: J. D. Sauerländer's Verlag, Finkenhofstrasse 21, D-60322 Frankfurt a. M. — Anzeigenverwaltung: J. D. Sauerländer's Verlag, Frankfurt am Main. — Satz und Druck: Graphische Kunstanstalt Wilhelm Herr, D-35390 Giessen
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