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Genetic analysis of inbreeding depression in plus tree 850.55 of *Pinus radiata* D. Don. I. Genetic map with distorted markers

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Abstract A genetic map of *Pinus radiata* plus tree 850.55 was constructed using megagametophytes of S_1 seeds. The map contained 19 linkage groups, with 168 RAPD and four microsatellite markers. The total map length was 1116.7 cM (Kosambi's function) and was estimated to cover 56% of the genome. Of the 172 markers, 59 (34%) were distorted from the expected 1:1 ratio in megagametophytes ($P < 0.05$). We show that if the distortion is caused by a single viability gene or by sampling error, the estimate of recombination frequency in megagametophytes of selfed seeds would not be affected.

Key words Genetic linkage map · Segregation distortion · Megagametophyte · *Pinus radiata*

Introduction

Radiata pine (*Pinus radiata* D. Don) is one of the more economically important forest tree species in the world, and particularly so in the southern hemisphere where breeding programs have been ongoing for almost 50 years (Balocchi 1997). These programs provide the majority of seed that is used to establish commercial plantations, usually by outcrossing trees of high breeding value.

Radiata pine, like most other conifers, displays inbreeding depression upon selfing, manifested as reduced growth rate and seed set (reviewed by Williams and Savolainen 1996). In several tree species, segregation distortion of genetic markers has been found in progeny arising from inbreeding (Fowler 1964, 1965; Cheliak 1987; Hedrick and Muona 1990).

Reasons for segregation distortion in plants are not well understood but are thought to be related to factors such as chromosome loss (Kasha and Kao 1970), genetic isolating mechanisms (Zamir and Tadmor 1986), or viability genes (Hedrick and Muona 1990; Beavis and Grant 1991; Lin et al. 1992; Liedl and Anderson 1993; Bradshaw and Stettler 1994; Cheng et al. 1996; Xu et al. 1997). With respect to viability genes, selection may be at either the gametic level (Lin et al. 1992; Liedl and Anderson 1993; Yanagihara et al. 1995; Cheng et al. 1996; Xu et al. 1997) or the zygotic level (Hedrick and Muona 1990; Bradshaw and Stettler 1994). Non-biological factors such as scoring errors (Deve xy et al. 1994; Plomion et al. 1995) or sampling errors (Plomion et al. 1995; Echt and Nelson 1996) may also result in segregation distortion.

The availability of DNA marker systems has facilitated the development of moderate-dense linkage maps, therefore enhancing the power to detect segregation distortion. There have been numerous reports of segregation distortion from linkage experiments in plants, including forest trees (e.g., Helentjaris et al. 1986; McCouch et al. 1988; Nelson et al. 1993; Pillen et al. 1993; Bradshaw and Stettler 1994; Cheng et al. 1996; Xu et al. 1997).

In conifers, the use of the haploid megagametophyte of conifer seeds provides an efficient approach for constructing genetic maps of individual trees (Tulsieram et al. 1992; Nelson et al. 1993, 1994; Binelli and Bucci 1994; Plomion et al. 1995; Echt and Nelson 1997) and for detecting segregation distortion in the maternal parent. This mapping strategy is analogous to that for backcrosses with the linkage phase unknown (Nelson et al. 1993). However, the effect of distortion due to viability genes and sampling error on linkage analysis using megagametophytes has not yet been determined.

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In backcross populations, the classic estimate of recombination frequency between two markers is equal to the proportion of recombinants, which is identical to the maximum likelihood estimate (MLE). If a single viability gene (zygotic selection) is linked with the two markers, the estimate of recombination frequency is the same as when there is no viability disturbance (Bailey 1961). This result has not been extended to the use of conifer megagametophytes.

In this paper, we demonstrate that, under standard assumptions, the conventional maximum likelihood estimate of recombination is unbiased when genotypic data is obtained from megagmetophytes, even when segregation distortion is present. Furthermore, we describe a linkage map of a high-value radiata pine tree, 850.055, that was constructed from megagametophyte genotypes of seed produced by artificial self pollination. Here, deviations from expected Mendelian ratios were frequently found.

Materials and methods

Plant materials and DNA extraction

Three hundred and seventy-eight selfed seeds of plus tree 850.55 were germinated singly in containers. Of the sown seeds 87 failed to germinate, leaving a total of 291 seeds that germinated successfully. Of these 291 seedlings 76 died within 1 month after germination; the remaining 215 seedlings survived for more than 1 year. Megagametophytes were collected from germinated seeds and stored at -20°C until DNA extraction. Genomic DNA from megagametophytes of the 215 surviving individuals and 76 dead individuals was extracted using a Fast Prep FP 120 machine (Savant) and the Bio 101 Kit H. Of the 215 DNA samples from megagametophytes of surviving seedlings, 198 were chosen for map construction based on the quality of the DNA.

Polymerase chain reaction (PCR) and random amplified polymorphic DNAs (RAPDs)

RAPD PCR was performed as previously described (Richardson et al. 1995), using 2.5 m*M* $MgCl_2$, 10 m*M* TRIS-HCl (pH 8.3), 50 m*M* KCl, 0.4 m*M* primer (Operon or UBC primers), 15 ng genomic DNA, 0.8 unit of *Taq* DNA polymerase, 0.2 m*M* of each of dNTPs, and deionized water (up to 25 µl) with a 50 µl oil overlay. The PCR thermal cycle consisted of: 1 cycle of 94°C for 3 min /37°C for 1 min / 72°C for 2 min, followed by 39 cycles of 94°C/37°C/72°C (1 min each), then 1 cycle at 72°C for 8 min. PCR was conducted in 96-well polycarbonate plates in a Techne PHC-3 thermal cycler. PCR products were separated by electrophoresis through a 1.5% agarose gel in 1 × TBE buffer. Gels were stained in 0.25 mg/ml ethidium bromide for 1 h, de-stained for 0.5 h, then photographed under UV light.

One hundred and twenty-five RAPD primers with segregating markers in plus tree 850.55 were initially selected (unpublished data). These primers were tested on DNA from needles and 6 megagametophytes of plus tree 850.55 to ensure that the expected bands were present and segregated. A total of 198 polymorphic markers were detected by 94 primers.

The names of the RAPD markers denote the supplier (U = UBC; otherwise, Operon) and primer name, followed by the size of the PCR fragment. The last letter in the marker name indicates the marker quality, classified as A, B or C, according to band intensity and background of the null allele, with A being best and C worst. The case of marker names shows their linkage phase. For example, ai05800a is a good marker, with a band fragment size of 800 bp, amplified by Operon primer AI05; U327600B is a marker of medium quality, with a PCR fragment size of 600 bp, amplified by primer UBC327; the null allele of ai05800a and band allele of U327600B are in coupling phase.

Four previously published microsatellite markers were also utilized (Fisher et al. 1996; Smith and Devey 1994), giving a total of 202 markers.

The DNA of 198 megagametophytes of surviving seedlings was screened with all 202 markers. Segregation distortion, as measured by departure from the expected 1 : 1 ratio, was tested with chi-square analysis for each marker.

Linkage analysis

Because the mapping strategy using megagametophytes of conifers is identical to that for backcrosses with phase unknown, the data file was modified so that both coupling and repulsion phases could be recognized (Nelson et al. 1993). A linkage map was constructed using MAPMAKER (Lander et al. 1987) version 3.0. Markers were assigned to linkage groups with LOD > 5 and $\theta < 0.25$. The markers in each group were ordered using the "order" command, with interval support LOD > 3 and error detection on (base threshold LOD > 1, net error threshold LOD > 5). The resulting linkage order was checked using "ripple" command (LOD threshold = 3). Unsupported markers were listed at the side of their linkage group (Plomion et al. 1995). Map distance was calculated using Kosambi's function.

Effects of viability genes on linkage analysis

Suppose a viability gene L is linked with two markers A and B, alleles A/B/L and a/b/l are in coupling phase, and the viabilities of the diploid genotype *LL*, *lL* and *ll* are 1, 1 − hs and 1 − s, respectively, where s is the selection coefficient and h is the degree of dominance. After selection, the expected genotype frequencies of megagametophytes of selfed seeds can be inferred. The effects of degree of dominance (h) and selection coefficient (s) on the estimation of recombination frequency are indicated by the combined frequencies of recombinants (aB and Ab) (see Appendix). It is shown that neither s nor h affect recombination rates, and thus do not affect the linkage analysis.

To demonstrate that viability genes had no effect on linkage analysis, we constructed and compared different maps using (1) megagametophytes of surviving seedlings, (2) megagametophytes of dead seedlings, and (3) both. Contingency chi-square tests were performed to test the difference of interval recombination frequencies among maps.

Analysis of co-migrating RAPD bands

Five bands which showed strong segregation distortion were examined to determine if the distortion could be caused by co-migrating bands. To do so, the PCR fragments were purified from RAPD agarose gel using DNA Purification Kits (Bio-Rad, Calif., USA) following the manufacturer's instructions. The purified fragments were re-amplified with the original RAPD primer under the same conditions as those described above, digested with *Mse*, then separated in a 2% agarose gel. If the sum of lengths for the digested products was greater than the length of the DNA in the original band, co-migrating bands were indicated.

Results

Linkage analysis

All 198 RAPD and four microsatellite markers were initially included for linkage analysis using MAPMAKER (Lander et al. 1987), ignoring segregation distortion. Markers were assigned to 19 linkage groups with $\theta < 0.25$ and LOD > 5. Six markers unlinked with any group were also detected, and these were not included in the map. The markers in each group were ordered using the "order" command, with interval support LOD > 3 and error detection on (base threshold LOD > 1, net error threshold LOD > 5). The resulting linkage order was checked using command "ripple" (LOD threshold $= 3$). Un-supported markers were listed at the side of their linkage group. The resulting map, shown in Fig. 1, contained 168 RAPD and four microsatellite markers, covering 1116.7 cM. The longest linkage group, Group 1, was 136.4 cM. It had 28 markers and hosted a locus with a lethal allele responsible for seedling death (Kuang et al. 1998). Using the method of Chakravarti et al. (1991) we calculated the total genome length of radiata pine to be 1979 cM (Kosambi's), with a 95% confidence interval of 1830–2148 cM. Therefore, the map covered approximately 56% of the genome.

The causes of segregation distortion and their effects on linkage analysis

Of the 172 markers ultimately mapped, 59 were distorted from a 1:1 ratio in the megagametophytes of selfed seeds ($P < 0.05$). Of these, 49 were clustered in 10 discrete regions; 10 were scattered in the linkage groups (Fig. 1).

Scoring errors

The un-supported markers, which were listed at the side of linkage groups, were likely to have many scoring errors. If an un-supported marker was distorted but its linked marker in the framework was not, the segregation distortion was most likely caused by scoring problems. Five of the un-supported markers were chosen to test for co-migrating polymorphic bands. Two markers, U256700B (138 megagametophytes with band allele versus 47 with null allele) and U322850B (122 with band allele versus 44 with null allele), were found to be composed of several different fragments (co-migrating polymorphic bands).

Sampling error

Of the 172 markers 9 were expected to be distorted ($P < 0.05$) by chance (type-I errors, ignoring linkage). Which distorted markers were due to sampling error in this experiment is not clear. If the distortion is due to sampling error, the estimation of recombination frequency between markers is unbiased (not shown). When the markers are distorted at a very high level (e.g., $P < 0.001$), it is unlikely that their distortion is by chance.

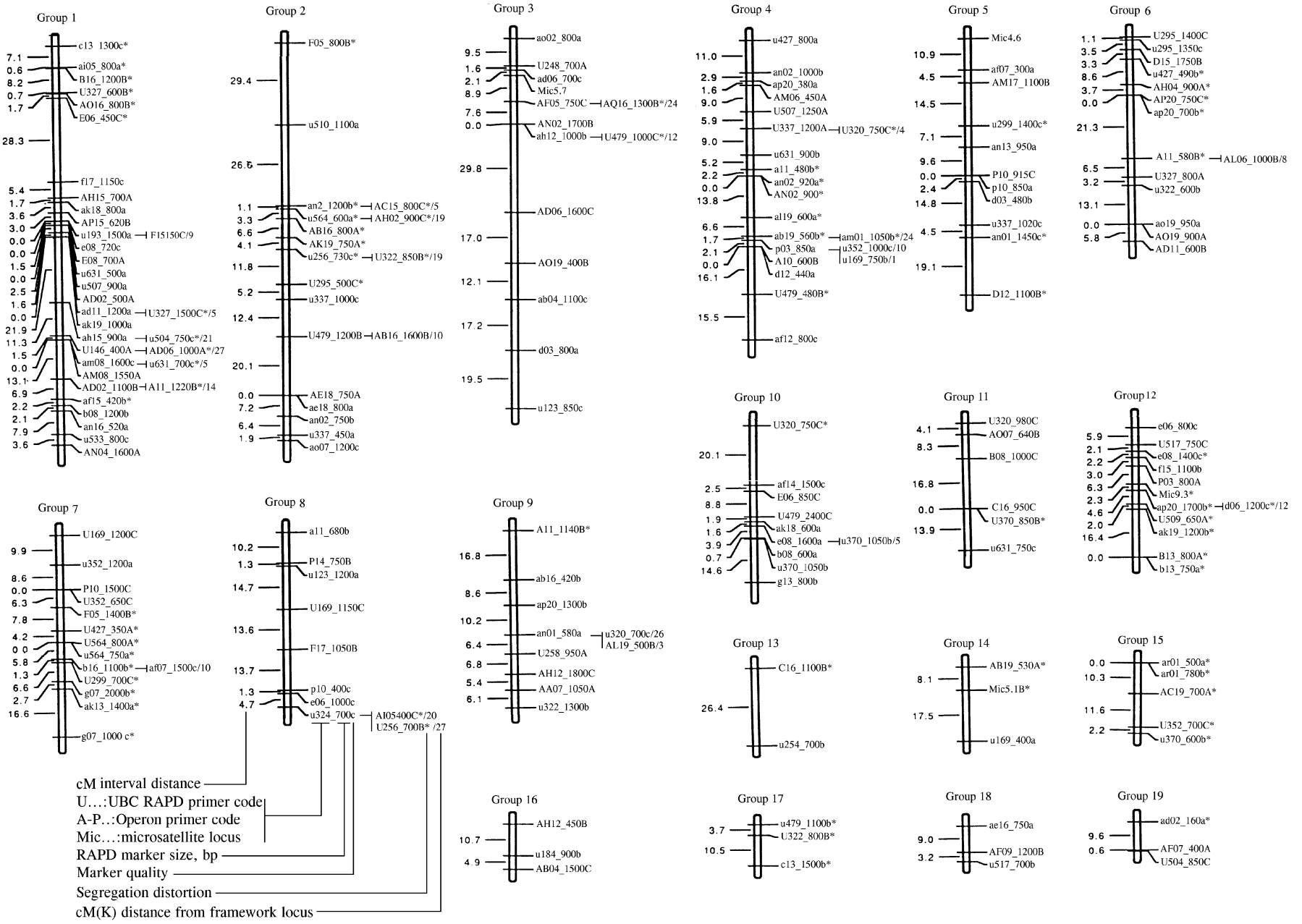
Faint bands, when unable to be scored explicitly, were scored as "data missing". If most of the unscored individuals belong to one genotype, false distortion might occur. However, this type of distorted marker should not show more double recombinants than expected, and its flanking markers should show a normal segregation ratio. The ten distorted markers that did not occur in clusters might be caused by this type of sampling error. Like distortion by chance, this type of distortion also does not affect linkage analysis (not shown).

Viability genes

Segregation distortion of DNA markers may also have a biological basis. Gametic or zygotic selection will cause skewed segregation of viability genes, and the markers that are highly linked with them. There is no evidence for gametic selection in conifers, and gametic selection should not cause the reduced seed set, germination, and survival following germination which are evident in this population. In this study, ten discrete distorted regions were identified. Their distortion might be caused by viability genes acting in zygotic selection. However, we could not exclude the possibility that some of them were caused by sampling errors.

Distortion caused by zygotic selection due to a single viability gene does not affect the estimate of recombination frequency between markers in megagametophytes of selfed seeds. This is demonstrated in the appendix. There, it is shown that for a viability gene L present between markers A and B (A–L–B), the classical estimate of recombination frequency between the markers is $r_1 + r_2 - 2r_1r_2$, which is the same as when there is no viability disturbance. The estimate of the recombination frequency is independent of selection coefficient or degree of dominance of the viability gene. This is also true when the viability gene is located on either side of the two markers (not shown).

An example that a single viability gene does not affect linkage analysis in megagametophytes of selfed seeds is shown in Fig. 2. A lethal allele responsible for seedling death was discovered previously (Kuang et al. 1998). After selection under this gene, the surviving seedlings lacked homozygotes of the lethal allele, whereas all dead seedlings were homozygotes for the lethal allele. The linkage map constructed using the megagametophytes of surviving seedlings is shown in Fig. 2a, and the linkage map using megagametophytes of dead seedlings is shown in Fig. 2c. Markers in both


Group 1
c13_1300c*
ai05_800a*
B16_1200B*
U327_600B*
AO16_800B*
E06_450C*
f17_1150c
AH15_700A
ak18_800a
AP15_620B
u193_1500a — F15150C/9
e08_720c
E08_700A
u631_500a
u507_900a
AD02_500A
ad11_1200a — U327_1500C*/5
ak19_1000a
ah15_900a — u504_750c*/21
U146_400A — AD06_1000A*/27
am08_1600c — u631_700c*/5
AM08_1550A
AD02_1100B — A11_1220B*/14
af15_420b*
b08_1200b
an16_520a
u533_800c
AN04_1600A
Group 2
F05_800B*
u510_1100a
an2_1200b* — AC15_800C*/5
u564_600a* — AH02_900C*/19
AB16_800A*
AK19_750A*
u256_730c* — U322_850B*/19
U295_500C*
u337_1000c
U479_1200B — AB16_1600B/10
AE18_750A
ae18_800a
an02_750b
u337_450a
ao07_1200c
Group 3
ao02_800a
U248_700A
ad06_700c
Mic5.7
AF05_750C — AQ16_1300B*/24
AN02_1700B
ah12_1000b — U479_1000C*/12
AD06_1600C
AO19_400B
ab04_1100c
d03_800a
u123_850c
Group 4
u427_800a
an02_1000b
ap20_380a
AM06_450A
U507_1250A
U337_1200A — U320_750C*/4
u631_900b
a11_480b*
an02_920a*
AN02_900*
al19_600a*
ab19_560b* — am01_1050b*/24
p03_850a — u352_1000c/10
A10_600B — u169_750b/1
d12_440a
U479_480B*
af12_800c
Group 5
Mic4.6
af07_300a
AM17_1100B
u299_1400c*
an13_950a
P10_915C
p10_850a
d03_480b
u337_1020c
an01_1450c*
D12_1100B*
Group 6
U295_1400C
u295_1350c
D15_1750B
u427_490b*
AH04_900A*
AP20_750C*
ap20_700b*
A11_580B* — AL06_1000B/8
U327_800A
u322_600b
ao19_950a
AO19_900A
AD11_600B
Group 7
U169_1200C
u352_1200a
P10_1500C
U352_650C
F05_1400B*
U427_350A*
U564_800A*
u564_750a*
b16_1100b* — af07_1500c/10
U299_700C*
g07_2000b*
ak13_1400a*
g07_1000 c*
Group 8
a11_680b
P14_750B
u123_1200a
U169_1150C
F17_1050B
p10_400c
e06_1000c
u324_700c — AI05400C*/20
U256_700B* /27
Group 9
A11_1140B*
ab16_420b
ap20_1300b
an01_580a — u320_700c/26
AL19_500B/3
U258_950A
AH12_1800C
AA07_1050A
u322_1300b
Group 10
U320_750C*
af14_1500c
E06_850C
U479_2400C
ak18_600a
e08_1600a — u370_1050b/5
b08_600a
u370_1050b
g13_800b
Group 11
U320_980C
AO07_640B
B08_1000C
C16_950C
U370_850B*
u631_750c
Group12
e06_800c
U517_750C
e08_1400c*
f15_1100b
P03_800A
Mic9.3*
ap20_1700b* — d06_1200c*/12
U509_650A*
ak19_1200b*
B13_800A*
b13_750a*
Group 13
C16_1100B*
u254_700b
Group 14
AB19_530A*
Mic5.1B*
u169_400a
Group 15
ar01_500a*
ar01_780b*
AC19_700A*
U352_700C*
u370_600b*
Group 16
AH12_450B
u184_900b
AB04_1500C
Group 17
u479_1100b*
U322_800B*
c13_1500b*
Group 18
ae16_750a
AF09_1200B
u517_700b
Group 19
ad02_160a*
AF07_400A
U504_850C
cM interval distance
U...:UBC RAPD primer code
A-P...:Operon primer code
Mic...:microsatellite locus
RAPD marker size, bp
Marker quality
Segregation distortion
cM(K) distance from framework locus

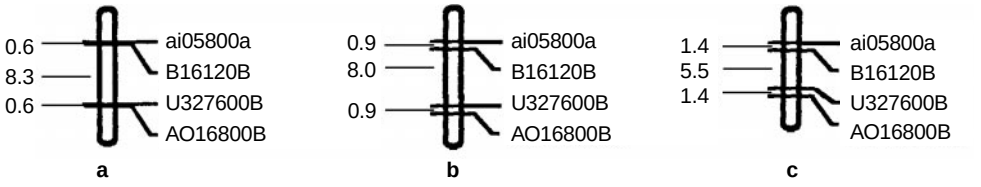


Fig. 2a–c Segregation distortion due to a single viability gene does not affect linkage analysis. Linkage group was constructed using megagametophytes of surviving seedlings (**a**), all seedlings (**b**), and dead seedlings (**c**)

data sets were highly distorted ($P < 0.0001$), but in favor of different alleles. When these two data sets were combined, markers showed normal segregation, and the resulting linkage group is shown in Fig. 2b. As shown in Fig 2, these maps are consistent in marker order. Contingency chi-square test indicates that the interval recombination frequencies are not significantly different among these maps.

Discussion

A genetic map with 19 linkage groups (pines have 12 chromosomes) was constructed for radiata pine plus tree 850.55 using megagametophytes of selfed seeds. This map has 168 RAPD and four microsatellite markers, covering 1116.7 cM. This map is consistent with a previous map which shared 38 markers, except that the linkage order between markers an 01580a and U258100 A in linkage group 9 was reversed. Using the method of Chakravarti et al. (1991), we estimated the total genome length of radiata pine to be 1979 cM (K), with a 95% confidence interval of 1830–2148 cM. This is a typical estimate for pine species (Echt and Nelson 1997).

Of the 172 markers 59 were distorted from the expected 1 : 1 segregation ratio ($P < 0.05$). Fifty-one of them were clustered in ten discrete regions; 8 were scattered elsewhere. Segregation distortion might result from sampling or scoring errors or from lethal/sublethal genes. Gametic selection may also cause segregation distortion. However, gametic selection should not result in inbreeding depression. There is no evidence for self-incompatibility in conifers prior to fertilization (Hagman and Mikkola 1963).

We showed that the estimation of recombination frequency was consistent for markers which were distorted in magagametophytes of S_1 seeds due to sampling error or a single viability gene. It has been shown that the estimation of recombination frequency is biased in backcross and F_2 mapping populations if there are two viability genes controlling the segregation of markers (Lorieux et al. 1995a,b). That is also true for megagametophytes of S_1 seeds. However, the effects of two (or more) linked deleterious genes on linkage analysis in megagametophytes of S_1 seeds are beyond the scope of this paper. We assumed that a maximum of one viability gene was responsible for each discrete distorted region in the construction of the map presented here. In practice, it is difficult to distinguish whether one or several highly linked viability genes are responsible for the segregation distortion of markers in one region. If two linked deleterious alleles are in coupling phase, their effects on segregation are similar to those of a single viability gene. If they are in repulsion phase, their effects on segregation are similar to those of a single overdominant gene (pseudo-overdominance). Overdominant and pseudo-overdominant viability genes will change the segregation ratio of diploid genotypes, but they may not disturb the segregation ratio in megagametophytes. Like a dominant viability gene, a single overdominant viability gene does not affect the estimation of recombination frequency between nearby markers (data not shown).

Scoring errors might be so high for bad markers (e.g., some RAPDs) that distortion appears. Heteroduplex and co-migrating polymorphic bands (Plomion et al. 1995), if not recognized, might have a strongly skewed ratio. For example, two independent segregating bands of similar size will be undistinguishable in the gel, resulting in a ratio of band allele to null allele in megagametophytes of S_1 seeds of 3 : 1 rather than 1 : 1.

Though conifer megagametophytes are an ideal material for genetic mapping, they have a few limitations. First, only PCR-based markers can be used because of the small amount of DNA in megagametophytes. Secondly, megagametophyte genotypes may not be efficient for genetic analysis because the traits of interest are mainly for diploid tissues. Megagametophyte genotypes can only show the maternal contribution, and only the additive effects of quantitative trait loci can be determined (Plomion et al. 1996). Genotyping both megagametophyte and diploid tissues with dominant markers cannot solve this problem completely, but it may provide further information for genetic analysis (Kuang et al. 1998).

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Fig. 1 Genetic linkage map of plus tree 850.55 of *P. radiata*. Asterisks denote that segregation of marker is skewed from a 1 : 1 ratio

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Appendix

Megagametophyte genotype frequency after selection of gene *L*[a]

♂	♀ gamete genotype	AB		Ab	
		A*L*B $(1-r_1)(1-r_2)$[b]	A*l*B r_1r_2	A*L*b $(1-r_1)r_2$	A*l*b $r_1(1-r_2)$
A*L*B	$(1-r_1)(1-r_2)$[b]	$(1-r_1)^2(1-r_2)^2$ [c] 1[d]	$r_1r_2(1-r_1)(1-r_2)$ $1-hs$	$r_2(1-r_1)^2(1-r_2)$ 1	$r_1(1-r_1)(1-r_2)^2$ $1-hs$
A*l*B	r_1r_2	$r_1r_2(1-r_1)(1-r_2)$ $1-hs$	$r_1^2r_1^2$ $1-s$	$r_1r_2^2(1-r_1)$ $1-hs$	$r_1^2r_2(1-r_2)$ $1-s$
A*L*b	$(1-r_1)r_2$	$r_2(1-r_1)^2(1-r_2)$ 1	$r_1r_2^2(1-r_1)$ $1-hs$	$r_2^2(1-r_1)^2$ 1	$r_1r_2(1-r_1)(1-r_2)$ $1-hs$
A*l*b	$r_1(1-r_2)$	$r_1(1-r_1)(1-r_2)^2$ $1-hs$	$r_1^2r_2(1-r_2)$ $1-s$	$r_1r_2(1-r_1)(1-r_2)$ $1-hs$	$r_1^2(1-r_2)^2$ $1-s$
a*L*B	$r_1(1-r_2)$	$r_1(1-r_1)(1-r_2)^2$ 1	$r_1^2r_2(1-r_2)$ 1-*hs*	$r_1r_2(1-r_1)(1-r_2)$ 1	$r_1^2(1-r_2)^2$ $1-hs$
a*l*B	$(1-r_1)r_2$	$r_2(1-r_1)^2(1-r_2)$ $1-hs$	$r_1r_2^2(1-r_1)$ $1-s$	$r_2^2(1-r_1)^2$ $1-hs$	$r_1r_2(1-r_1)(1-r_2)$ $1-s$
a*l*b	$(1-r_1)(1-r_2)$	$(1-r_1)^2(1-r_2)^2$ $1-hs$	$r_1r_2(1-r_1)(1-r_2)$ $1-s$	$r_2(1-r_1)^2(1-r_2)$ $1-hs$	$r_1(1-r_1)(1-r_2)^2$ $1-s$
a*L*b	r_1r_2	$r_1r_2(1-r_1)(1-r_2)$ 1	$r_1^2r_2^2$ $1-hs$	$r_1r_2^2(1-r_1)$ 1	$r_1^2r_2(1-r_2)$ $1-hs$
$\sum$ (after selection)		$[(1-r_1)(1-r_2)(2-hs)+r_1r_2(2-hs-s)]$		$[(1-r_1)r_2(2-hs)+r_1(1-r_2)(2-hs-s)]$	
Female gamete genotype frequency		$\frac{[(1-r_1)(1-r_2)(2-hs)+r_1r_2(2-hs-s)]}{4-2hs-s}$		$\frac{[(1-r_1)r_2(2-hs)+r_1(1-r_2)(2-hs-s)]}{4-2hs-s}$	
Recombination frequency		$r_1+r_2-2r_1r_2$			

♂	♀ gamete genotype	aB		ab	
		A*L*B $r_1(1-r_2)$	a*l*B $(1-r_1)r_2$	a*l*b $(1-r_1)(1-r_2)$	a*L*b r_1r_2
A*L*B	$(1-r_1)(1-r_2)$[b]	$r_1(1-r_1)(1-r_2)^2$ 1	$r_2(1-r_1)^2(1-r_2)$ $1-hs$	$(1-r_1)^2(1-r_2)^2$ $1-hs$	$r_1r_2(1-r_1)(1-r_2)$ 1
A*l*B	r_1r_2	$r_1^2r_2(1-r_2)$ $1-hs$	$r_1r_1^2(1-r_1)$ $1-s$	$r_1r_2(1-r_1)(1-r_2)$ $1-s$	$r_1^2r_2^2$ $1-hs$
A*L*b	$(1-r_1)r_2$	$r_1r_2(1-r_1)(1-r_2)$ 1	$r_2^2(1-r_1)^2$ $1-hs$	$r_2(1-r_1)^2(1-r_2)$ $1-hs$	$r_1r_2^2(1-r_1)$ 1
A*l*b	$r_1(1-r_2)$	$r_1^2(1-r_2)^2$ $1-hs$	$r_1r_2(1-r_1)(1-r_2)$ $1-s$	$r_1(1-r_1)(1-r_2)^2$ $1-s$	$r_1^2r_2(1-r_2)$ $1-hs$
a*L*B	$r_1(1-r_2)$	$r_1^2(1-r_2)^2$ 1	$r_1r_2(1-r_1)(1-r_2)$ $1-hs$	$r_1(1-r_1)(1-r_2)^2$ $1-hs$	$r_1^2r_2(1-r_2)$ 1
a*l*B	$(1-r_1)r_2$	$r_1r_2(1-r_1)(1-r_2)$ $1-hs$	$r_2^2(1-r_1)^2$ $1-s$	$r_2(1-r_1)^2(1-r_2)$ $1-s$	$r_1r_2^2(1-r_1)$ $1-hs$
a*l*b	$(1-r_1)(1-r_2)$	$r_1(1-r_1)(1-r_2)^2$ $1-hs$	$r_2(1-r_1)^2(1-r_2)$ $1-s$	$(1-r_1)^2(1-r_2)^2$ $1-s$	$r_1r_2(1-r_1)(1-r_2)$ $1-hs$
a*L*b	r_1r_2	$r_1^2r_2(1-r_2)$ 1	$r_1r_2^2(1-r_1)$ $1-hs$	$r_1r_2(1-r_1)(1-r_2)$ $1-hs$	$r_1^2r_2^2$ 1
$\sum$ (after selection)		$[r_1(1-r_2)(2-hs)+(1-r_1)r_2(2-hs-s)]$		$[r_1r_2(2-hs)+(1-r_1)(1-r_2)(2-hs-s)]$	
Female gamete genotype frequency		$\frac{[r_1(1-r_2)(2-hs)+(1-r_1)r_2(2-hs-s)]}{4-2hs-s}$		$\frac{[r_1r_2(2-hs)+(1-r_1)(1-r_2)(2-hs-s)]}{4-2hs-s}$	
Recombination frequency		$r_1+r_2-2r_1r_2$			

[a] A and B are two markers, and *L* is the viability gene. Linkage order: A–r_1–*L*–r_2–B, where r_1 and r_2 are recombination rates
[b] Gamete genotype frequency. 1/2 is omitted
[c] Diploid genotype frequency. 1/4 is omitted
[d] Viability of the corresponding diploid genotype