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Detection of a major gene for resistance to fusiform rust disease in loblolly pine by genomic mapping

(random amplified polymorphic DNA markers/host–pathogen interaction/forest pathology)

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ABSTRACT **Genomic mapping has been used to identify a region of the host genome that determines resistance to fusiform rust disease in loblolly pine where no discrete, simply inherited resistance factors had been previously found by conventional genetic analyses over four decades. A resistance locus, behaving as a single dominant gene, was mapped by association with genetic markers, even though the disease phenotype deviated from the expected Mendelian ratio. The complexity of forest pathosystems and the limitations of genetic analysis, based solely on phenotype, had led to an assumption that effective long-term disease resistance in trees should be polygenic. However, our data show that effective long-term resistance can be obtained from a single qualitative resistance gene, despite the presence of virulence in the pathogen population. Therefore, disease resistance in this endemic coevolved forest pathosystem is not exclusively polygenic. Genomic mapping now provides a powerful tool for characterizing the genetic basis of host pathogen interactions in forest trees and other undomesticated organisms, where conventional genetic analysis often is limited or not feasible.**

Fusiform rust, a disease of southern pines (*Pinus* sp.), is the most economically important tree disease in commercial forestry in the United States (1). This disease is caused by the endemic rust fungus *Cronartium quercuum* (Berk.) Miyabe ex. Shirai f. sp. *fusiforme* (hereafter referred to as *Cqf.*). Several oak species (*Quercus* sp.) serve as the alternate hosts for *Cqf.* Loblolly pine (*Pinus taeda* L.) and slash pine (*Pinus elliottii* Engelm. var. *elliottii*) are the major economically important hosts. On pine, branch and stem galls result from *Cqf.*-induced cell proliferation in developing woody tissues. Stem galls reduce wood quality, weaken trees (disposing them to storm breakage), and cause mortality, especially in seedlings (2). The pathogen has little known effect on oak.

Deployment of rust-resistant pine planting stock is considered the only feasible means of controlling the disease in commercial forest plantations (3, 4), and appropriate use of resistant plantations could increase grower revenues by 36–192 million dollars per year (ref. 5, F. W. Cubbage personal communication). Although phenotypic selection for resistance and breeding among recognized resistant trees (4) have increased the supply of resistant seedlings, the genetic basis of the host–pathogen interaction in fusiform rust disease was not understood despite 37 years of research (1) that included numerous quantitative genetic analyses.

Disease resistance in forest trees must be durable (effective long-term resistance) for individuals to survive centuries or even millennia. In contrast, disease resistance in crop plants is often not durable (6, 7). Qualitative, gene-for-gene (8) resistance mechanisms are known for diseases of several crop species (9). In a gene-for-gene system, a discrete resistance gene in the host blocks infection by strains of the pathogen, except for strains carrying a specific virulence gene. Individual, major, discrete resistance genes are frequently overcome within a few years in agronomic crops, where the pathogen's asexual repeating stage is present on the economically important host (10).

Genetic variation for host resistance in endemic forest pathosystems typically appears continuous. Few discrete resistance genes are known in forest trees, and inheritance of disease resistance in forest trees has been commonly explained by polygenic models, where resistance is controlled by many genes, each with a small additive effect (7, 11). One example of major gene resistance in a forest tree is the sugar pine gene for resistance to the nonendemic white pine blister-rust pathogen. This resistance gene was recognized years ago by Kinloch *et al.* (12) and recently mapped by Devey *et al.* (13). Indeed, the scarcity of evidence for simply inherited resistance in trees implied support for a model of polygenic inheritance of resistance (4, 7, 11). Polygenic resistance to pests and pathogens was considered more likely to be durable in long-lived forest trees (14), and genetic analysis using inbred lines to detect discrete resistance genes was precluded by the long generation times and the high genetic load typical of forest trees. Alternatively, the durability and complexity of endemic forest pathosystems could also result from interactions of multiple pathotype-specific resistance genes in the host with corresponding avirulence genes in the pathogen (15, 16).

Previous breeding efforts to improve the level of fusiform-rust resistance in loblolly pine have assumed a polygenic basis for resistance and followed quantitative breeding models (4); however, Kinloch and Walkinshaw (17) proposed on the basis of infection percentages and reciprocal specificities with single gall isolates of the fungus that host resistance to fusiform rust in slash pine followed a gene-for-gene model. Studies evaluating phenotypic specificity of interactions of different fungal isolates of *Cqf.* with southern pines are consistent with a small

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Abbreviations: *Cqf.*, *Cronartium quercuum* (Berk.) Miyabe ex. Shirai f. sp. *fusiforme*; SAI, single-aeciospore isolate; RAPD, random amplified polymorphic DNA; LOD, logarithm of odds; MLE, maximum likelihood estimate.

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number of genes controlling resistance to fusiform rust disease (18, 19).

Methods of genetic analyses resulting from the development of restriction fragment length polymorphism (RFLP) and PCR-based marker systems have led to the genetic dissection of quantitative traits, including growth and yield in a variety of forest trees (20–22). In our laboratory, random amplified polymorphic DNA (RAPD) (23) markers have been used to obtain genomic maps of individual forest trees (24, 25) and to dissect quantitative traits (21). The genetic analysis of disease resistance in forest trees is similar to complex trait analysis in medical science, where both major gene segregation and environmental effects contribute to trait variation within families (26). In this report, we provide genetic evidence for discrete resistance genes in an endemic coevolved forest pathosystem by identifying and mapping a major gene in loblolly pine that confers pathotype-specific resistance to fusiform rust disease.

MATERIALS AND METHODS

Host Plant Materials. All plants used in greenhouse tests were progeny of loblolly pine genotype 10-5 or progeny from half-sib daughters of 10-5. Tree 10-5 was selected at age 32 from a naturally regenerated forest in 1958 by the North Carolina State University: Industry Cooperative Tree Improvement Program. Progeny of 10-5 consistently show an intermediate proportion of gall-free individuals in screening trials for fusiform-rust resistance (27). Tree 10-5 and three resistant daughters of 10-5 (resulting from wind pollination of 10-5) were crossed with pollen donor 4-6664, a genotype that transmits no significant resistance to its progeny (28). Thus, the inheritance of resistance from genotype 10-5 can be followed over three generations (Fig. 1). Tree 10-5 was the seed parent of family A, and the three half-sib daughters (resulting from wind pollination of 10-5) were the seed parents of families B, C, and E. Seeds of families A, B, C, and E were cold stratified and germinated in vermiculite. Germinating seedlings were later transplanted into the greenhouse and inoculated with *Cqf.* At the time of germination, haploid megagametophytes were harvested for DNA isolation and marker genotype analysis. Some additional wind-pollinated progeny of 10-5 were studied in two 7-year-old progeny tests located in Barnwell County and Collecton County, South Carolina. Vegetative shoot tips and young needles were harvested from the field tests in March 1994 to provide DNA for correlating markers with the presence or absence of fusiform rust galls in field trees.

Fungal Materials, Inoculations, and Phenotypic Assessments. Greenhouse-grown pine seedlings (8–9 weeks old) were inoculated with basidiospores of *Cqf.* following the concentrated basidiospore spray method of Matthews and Rowan (29) at 45,000 spores per ml. The basidiospores originated from two single-aeciospore isolates (SAIs) designated NC 2-36 and SC 20-21, which were used to control inoculum genotype. Production of SAIs followed the methods of Kuhlman and Matthews (30). Briefly, a single dikaryotic ($N + N$) aeciospore obtained from a sporulating aecial gall was inoculated onto a young northern red oak (*Quercus rubra* L.) leaf to yield upon infection a uredinial pustule. Other oak seedlings were repeatedly reinfected with the resulting dikaryotic asexual urediniospores. Haploid basidiospores were obtained after meiosis from telial columns (clumped teliospores) formed on infected oak leaves (Fig. 2). Greenhouse-inoculated pine seedlings were assessed for presence or absence of rust galls at 9 months for correlation with genetic markers. Trees at the two field locations were not artificially inoculated but showed significant incidence of fusiform rust disease resulting from natural ambient inoculum. Trees were assessed for gall presence or absence at the time that tissues were harvested for DNA sampling (age 7 years). The presence of a single gall was sufficient to phenotypically classify a tree as susceptible.

DNA Isolation and RAPD Marker Analysis. Genomic DNA for RAPD marker analysis was isolated from haploid megagametophytes from seedlings inoculated in greenhouse experiments and from diploid vegetative tissues (shoot apices and needles) from trees in field experiments. DNA was extracted essentially according to Doyle and Doyle (31). RAPD reactions followed Williams *et al.* (23) and genetic mapping was carried out as described by Plomion *et al.* (24) and Grattapaglia and Sederoff (25).

RESULTS AND DISCUSSION

Genomic Mapping. We used genomic mapping and bulk segregant analysis approaches to investigate the genetic basis of fusiform-rust resistance in our 10-5 pedigree (Fig. 1). This involved the study of seedlings inoculated with SAIs of *Cqf.* and analysis of DNA obtained from their haploid megagametophytes. Our first objective was to obtain moderate density genomic maps from a second and a third generation family of the 10-5 pedigree. In conifers, a heterozygous locus segregates 1:1 into meiotic products (Fig. 3) which can be genotyped directly from the megagametophytes present in mature seed. Each megagametophyte contains a haploid genotype of one of the four products of a single meiosis and represents the same genotype that is the maternal contribution to the embryo in that seed. Cosegregation analysis for linkage is easily carried out for any two segregating RAPD markers from the geno-

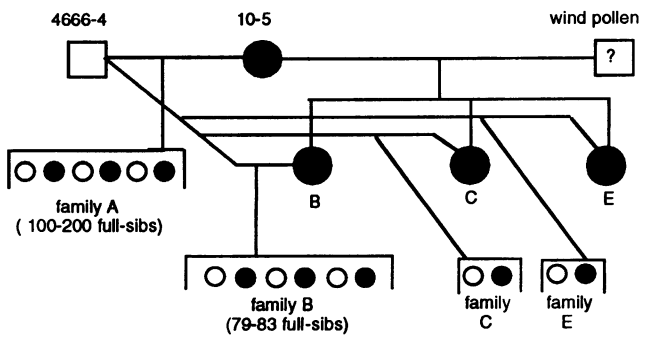

FIG. 1. Pedigree of loblolly pine showing study families A, B, C, and E obtained by crossing seed parents A, B, C, and E with pollen donor 4666-4. Squares are pollen parents. Large circles are seed parents. Resistant individuals have solid symbols.

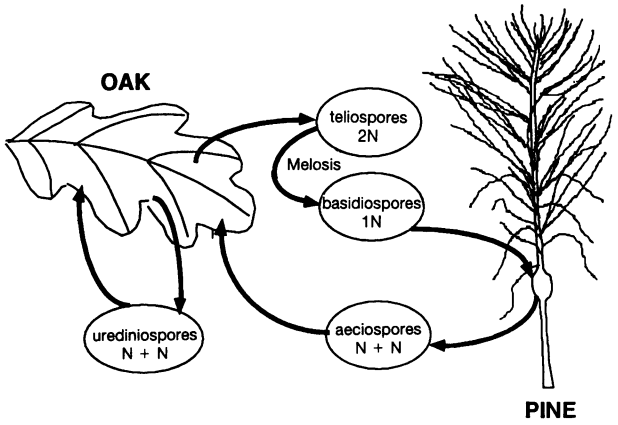

FIG. 2. Life cycle of *Cqf.* showing the various spore forms and spore movement from pine to oak and vice versa. Variation in *Cqf.* inocula placed on pine seedlings was controlled through production of single aeciospore isolates as described in text.

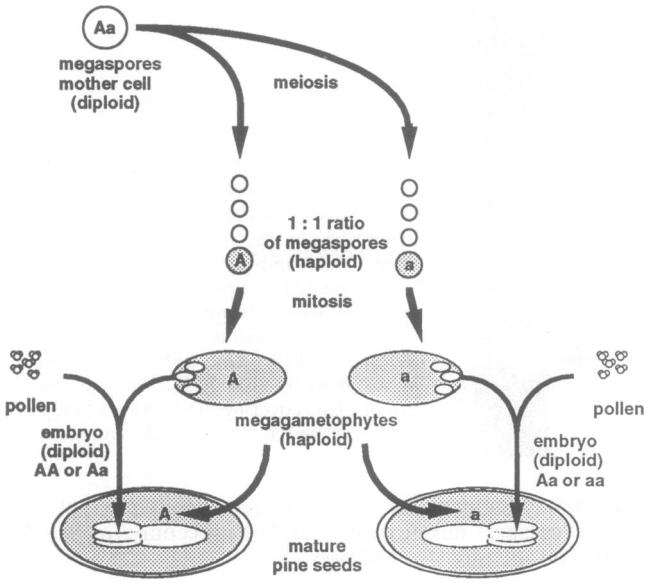

FIG. 3. Pine megagametophyte biology. RAPD markers used for mapping were obtained by genotyping haploid megagametophytes. The megagametophyte is the nutritive tissue surrounding the developing embryo and is derived from the same megaspore that gives rise to the maternal gamete. Thus, the genotype of the megagametophyte is identical to the contribution of the seed parent to the diploid seedling. A heterozygous RAPD marker locus segregates 1:1 in haploid megagametophytes of conifers.

types of a set of megagametophytes; thus, it was possible to construct linkage maps of the seed parents 10-5 and 152-231.

Working with progeny sets inoculated with *Cqf.* SAI NC 2-36, segregating RAPD markers amplified from megagametophyte DNA were used to construct genomic maps for the parent of family A (10-5) and the parent of family B (152-231). The genomic maps will be available electronically through the Genome Database for Forest Trees (http://s27w007.pswfs.gov/). We used 91 ten-base oligonucleotide primers to identify 314 segregating polymorphic DNA fragments in family A and 60 primers for 232 segregating polymorphic DNA fragments in family B. Markers from both family A and family B were assigned to linkage groups and ordered by using a matrix correlation algorithm in MAPMAKER MACINTOSH 2.0 [logarithm of odds (LOD) > 5, $\theta < 0.25$].

The genus *Pinus* has 12 chromosomes with ≈ 2.5 chiasmata per bivalent (32). These cytological data suggest a total map length of approximately 1500 centimorgans (cM). A comprehensive map of maritime pine (*Pinus pinaster* Ait.), constructed from 463 markers had 12 linkage groups and a map length of 1860 cM (33). The map of tree 10-5 had a map length of 1727 cM, with four unlinked markers. The total map length for 10-5 could be greater than 2000 cM, based on the method of Hubert *et al.* (34) for estimating map length from marker recombination data. Assuming a random distribution of markers and an estimated map length of 2087 cM, on the basis of Hulbert's methods, at least one marker should be located within 30 cM of approximately 95% of the 10-5 genome.

Association of Markers with Disease Phenotype. A segregating locus in the seed parent could explain a substantial portion of the resistance found in the progeny of 10-5. The segregation of such a locus should be evident if the test seedlings were inoculated with an avirulent isolate of the pathogen (*Cqf.*) and the presence or absence of the disease accurately reflected the resistant/susceptible genotypes. Mendelian inheritance, however, had not been previously obtained for fusiform rust disease in loblolly pine, either due to the limitations of the methods used to assay the host–pathogen interaction or because the genetic basis of host resistance could be polygenic. Genetic marker analysis can distinguish between these alternatives.

Some RAPD markers in the megagametophytes should cosegregate with disease phenotypes in the corresponding seedlings if that trait is controlled by a resistance gene inherited from the seed parent and the challenging inoculum is avirulent for this gene (Fig. 4). Our genomic maps should provide sufficient coverage to make it likely that an association would be observed between one or more markers and resistance genes, provided the number of resistance genes is small. Our first combination of host and pathogen genotypes assayed when using the pathogen isolate NC 2-36 and progeny from the resistant hosts 10-5 and 152-231 showed no significant association of any specific marker with the presence or absence of disease, even though there was nearly a 1:1 ratio of infected vs. uninfected individuals (54.8% infection) in family A. Cosegregation between markers and phenotype was evaluated by using MAPMAKER MACINTOSH, where a minimum LOD value of 3.0 was necessary to identify a significant marker-phenotype association.

Bulk Segregant Analysis. In a second series of tests, we used bulk segregant analysis (35) to expedite the search for markers associated with the rust disease. Bulked samples sharing a common phenotype can be used to identify markers closely linked to the gene responsible for the phenotype. Both the resistant and susceptible bulks should contain approximately equal amounts of DNA template for marker alleles that are not closely linked to a resistance locus; therefore, most RAPD markers will be amplified identically in both bulks. The RAPD markers linked to a major resistance gene, however, should be intense in one of the bulked samples, but faint or absent in the other.

Bulk segregant analysis is particularly useful with RAPD markers because many RAPD primers can be screened for polymorphism between pairs of bulked samples with high efficiency. In family A, megagametophyte DNAs from 16 galled plants were combined to make a bulked sample for individuals susceptible to a different *Cqf.* SAI inoculum, SC 20-21. A similar family A "resistant" bulked sample was prepared from megagametophytes corresponding to gall-free individuals. A total of 60 primers were used in the screening of the bulked samples. Most showed no differential effect between susceptible and resistant bulks; however, several

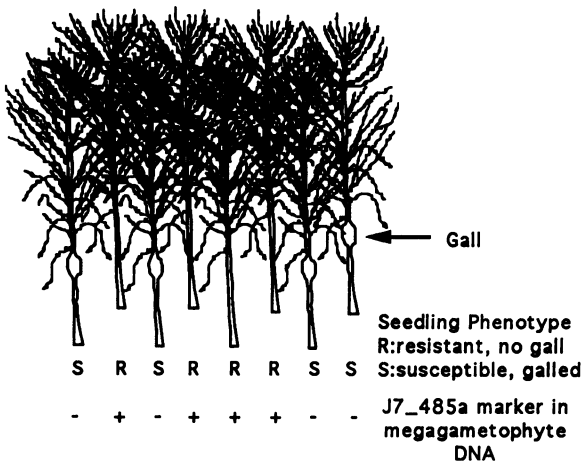

FIG. 4. Cosegregation analysis to determine marker–trait association. RAPD markers should cosegregate with the presence or absence of disease in the corresponding seedlings if the trait is controlled by a discrete gene inherited from the seed parent and the challenging inoculum is avirulent to this gene. Individual megagametophytes were genotyped for markers that were polymorphic in pooled DNA samples. For a tightly linked marker locus (such as J7-485A), the marker will cosegregate with disease phenotype, whereas an unlinked marker will not be associated with disease phenotype.

markers from linkage group 9 showed striking differences between resistant and susceptible bulks (Fig. 5).

Similar but smaller scale bulking and cosegregation studies were conducted with family C megagametophyte DNA and seedlings challenged with inoculum SC 20-21. Across families A, B, C, and E, more than a dozen markers from linkage group 9 (obtained by bulking or by examination of the maps previously constructed for parents A and B) were found to have a significant association (LOD > 3) with resistance to SAI inoculm SC 20-21. Cosegregation data for six of these markers and disease phenotype studied in two or more families are shown in Table 1. Marker J7-485A has the strongest association with resistance and is shown segregating in a family C progeny set (Fig. 6). All markers associated with resistance in all of the families tested from the 10-5 pedigree (Fig. 1) reside in the same linkage group (group 9) and map near marker J7-485A. Across this pedigree, the genomic region associated with J7-485A explains dominant resistance inherited from genotype 10-5 over three generations and meets the operational definition of a resistance gene. This chromosomal region is designated *Frl*, based on its genetic properties, but it is not defined at the molecular level.

Mapping Region *Frl*. Precise mapping of the *Frl* locus was not straightforward because the *Frl* resistance allele did not appear to correspond exactly with "no gall", the absence of disease (Fig. 7). Assuming a simple two-locus model for the cosegregation of the disease phenotype (heritability 1.0) with marker J7-485A, the recombination fraction between *Frl* and J7-485A was 0.14. However, there was a significant departure from the expected 1:1 ratio for the trait ($P < 0.01$) and also for the marker ($P < 0.05$). Furthermore, there was a significant excess of individuals that lacked the J7-485A marker but did not develop the disease ($P < 0.001$). No significant marker–disease phenotype association was found in other regions of the genome. We used a maximum likelihood approach to determine if the disproportionately large number of seedlings lacking both the marker and disease was better explained by "escapes" during the inoculation process or by chance alone. We obtained a maximum likelihood estimate (MLE) for the uneven distribution of recombinants occurring due to chance alone by the following:

$$\ln L(\theta|a,b,c,d) = a\ln(1-\theta) + b\ln\theta + c\ln\theta + d\ln(1-\theta), \quad [1]$$

where a, b, c, and d are the numbers of individuals in each of the four classes of association (Fig. 7.) and θ is the estimated recombination fraction.

The MLE was compared with that from an alternative model which incorporated parameters both for recombination fraction (θ) and for an excess of disease-free phenotypes that were due to "escapes" during the inoculation procedure (ρ). The log likelihood for this is as follows:

$$\ln L(\theta,\rho|a,b,c,d) = a\ln[(1-\theta)+\rho\theta] + b\ln[\theta+\rho(1-\theta)] + c\ln[(1-\rho)\theta] + d\ln[(1-\rho)(1-\theta)], \quad [2]$$

where a, b, c, d, and θ are defined above and ρ is the proportion of escapes. To obtain the MLE from this model, it was necessary to maximize the likelihood over values of two parameters, θ and ρ. This alternative model, accounting for escapes, fit the data better than did the model with θ alone. By using the alternative model and the data in Fig. 7, the likelihood is maximized at $\theta = 0.02$ and $\rho = 0.22$ (Fig. 8). The likelihood ratio test statistic is 47.984 (approximates a χ^2 distribution with one degree of freedom) for testing $\rho = 0.22$ against $\rho = 0.00$. While quantification of escapes was important in this study, experiments now in progress (H.V.A. and E.G.K., unpublished data) using a higher density of basidiospores indicate that the frequency of escapes can be reduced. By using the 0.02 joint MLE for the recombination fraction between *Frl* and J7-485A, the position of *Frl* in linkage group 9 is shown in Fig. 9.

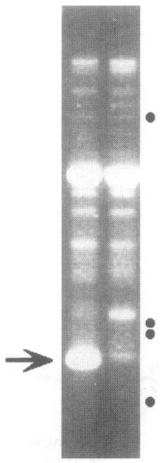

FIG. 5. An agarose gel from the bulk segregant analysis of family A (parent tree 10-5) showing marker J7-485A (arrow) as polymorphic in the pooled megagametophyte DNA samples. The left lane contains a pooled sample of megagametophyte DNA from 16 nongalled seedlings. The right lane contains a sample from diseased seedlings. Dots indicate molecular size markers (1018, 517, 506, and 396 bp). Markers covering the entire 10-5 map were screened by using bulk segregant analysis.

Markers Associated with *Frl* Predict Resistance in the Field. The marker J7-485A, identified in previous greenhouse experiments, was used to evaluate marker–phenotype association in 7-year-old, field-grown, wind-pollinated progeny of tree 10-5 subjected to natural, local inoculum at two South Carolina locations. Cosegregation between marker J7-485A (observed in diploid DNA from vegetative tissues) and the

Table 1. Cosegregation of RAPD markers with disease resistance

Marker	Family	Sample	+ marker, no gall	− marker, no gall	+ marker, with gall	− marker, with gall	LOD	Correlation
A19-500A	A,B	179	71	32	12	64	10.1	0.51
H20-400C	A,B	179	71	30	9	69	12.8	0.56
A9-625A	A,B	179	69	32	8	70	12.7	0.55
J4-550A	A,B	179	65	37	10	67	9.2	0.47
F13-780	A,B	177	63	37	10	67	8.9	0.47
J7-485A*	A,B,C,E	386	168	52	3	163	49.3	0.72

Cosegregation data are presented for several markers linked with disease phenotype (gall vs. no gall) in families A and B. For marker J7-485A, data are also given for families C and E. For marker F13-780c the dominant allele was linked in repulsion with the "no gall" phenotype, whereas the other markers were linked in coupling to each other and to the absence of galls. The data for F13-780c were recoded in coupling for the format of this table. LOD scores of 4 approximate a P value of 0.0001. Combined LOD scores are totaled across families. + marker, no gall designates those progeny carrying the plus allele at the marker locus but showing no sign of infection. Similarly, + marker, with gall indicates those progeny carrying the plus allele but showing signs of infection.

*The same 179 samples examined for other markers in the table yielded a marker–resistance LOD association of 17.4 for the J7-485A marker.

presence or absence of galls was evaluated as before with MAPMAKER MACINTOSH (minimum LOD for significance > 3.0). The J7-485A marker was again associated with the absence of disease at both locations (LOD 5.98, $n = 123$ for Colleton County and LOD 3.56, $n = 48$ for Barnwell County). In diploid DNA samples from foliage of wind pollinated 10-5 progeny at these field locations, the J7-485A marker segregated close to 1:1, suggesting that both the marker and *Frl* are rare in the pollen pool and were inherited from 10-5.

Evidence for Fungal Specificity. We have also found heterogeneity for pathogen virulence in different SAIs. Basidiospore inoculum from an SAI may contain a mixture of virulent and avirulent haploid basidiospores, as the originating aeciospore for the SAI contained two nuclei. An inoculum that contains equal proportions of virulent and avirulent basidiospores would probably perform like a virulent inoculum, given the large spore numbers used in the concentrated basidiospore spray system. An avirulent basidiospore inoculum would arise from an aeciospore homozygous for avirulence. For seedlings challenged with inoculum from SAI NC 2-36, no significant associations (LOD > 3) were detected between 314 and 232 markers and disease phenotype for families A and B, demonstrating that SAI NC 2-36 is virulent to *Frl*, whereas SAI SC 20-21 is avirulent to *Frl*. In a subsequent inoculation of family A progeny with SAI NC 2-36, 81% developed galls, indicating the lower infection level in the earlier inoculation was unlikely to be due to a different major resistance gene evading detection due to incomplete map coverage.

Implications. On the basis of the age of parent tree 10-5, *Frl* resistance has existed (in 10-5 and its progeny) for at least 68 years prior to this study, yet *Frl* still confers effective resistance in the field despite the existence of virulent pathogen genotypes. Therefore, the inheritance of long-term resistance in the loblolly pine–fusiform rust endemic coevolved forest pathosystem is not exclusively polygenic.

Our results also have implications for breeding for disease resistance in forest trees. The association of *Frl* with field resistance in progeny of 10-5 in an area from which pathogen virulence for *Frl* has been detected via greenhouse screening of 10-5 progeny (U.S. Department of Agriculture Forest Service Resistance Screening Center, Asheville, North Carolina, unpublished data), suggests effective resistance could be achieved by using major resistance genes. Deployment of mixtures of major genes, each with different pathotype specificities, has the potential to reduce disease incidence in forest plantations in a manner similar to that achieved for coffee leaf rust with the coffee tree composite cultivar "Colombia" (36). The production of seed or plants for such mixtures is possible by using existing forest seed orchard and vegetative production technologies in association with genetic markers. The combined use of these technologies could augment traditional quantitative approaches to resistance breeding and lead to further reductions of disease in plantations.

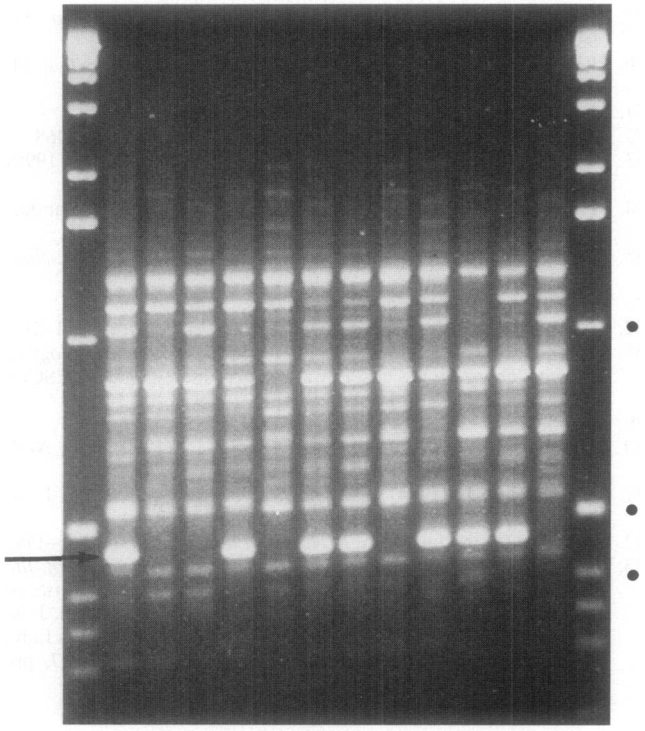

FIG. 6. Agarose gel showing segregation of marker J7-485A (arrow) in a family C progeny set. DNA samples from 12 progeny are flanked by 1-kb DNA ladder size standards (GIBCO/BRL). Size markers in base pairs (1018, 506, and 396) are denoted by dots.

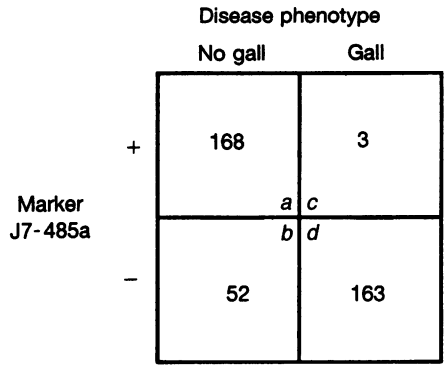

FIG. 7. Contingency table for segregation of marker J7-485A (+ and − are marker alleles) with disease phenotype (no gall vs. gall) for 386 seedlings combined from four families in 10-5 pedigree used to define progeny classes *a*, *b*, *c*, and *d* for the calculation of ρ.

Biotic interactions with pests and pathogens are important for trees and could play a role in generating and maintaining biodiversity in forest ecosystems. Biotic interactions could be especially important for forest trees because longevity makes trees more "apparent" to pests and to pathogens (14). Several mechanisms have been proposed to explain the tremendous biodiversity of tropical forests, including interactions of trees with pathogens and pests that could reduce the numbers of individuals of predominant species through density- or frequency-dependent mortality (37, 38). Similar processes could operate within species to reduce the abundance of common host genotypes, as has been postulated in theoretical models to analyze the maintenance of polymorphism for host–pathogen interactions and the evolutionary advantages of sexual reproduction (39, 40). Studies of host–pathogen interactions in natural plant populations have demonstrated polymorphisms for both resistance and virulence, suggesting a coevolved

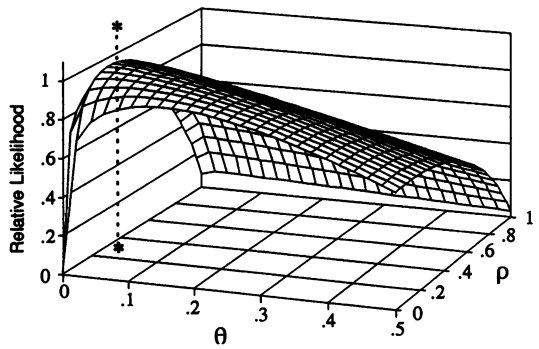

FIG. 8. A three-dimensional plot for Eq. **2**, with relative likelihood as the vertical axis and θ and ρ as the two horizontal axes. *-----* denotes where likelihood is maximized for values of θ and ρ.

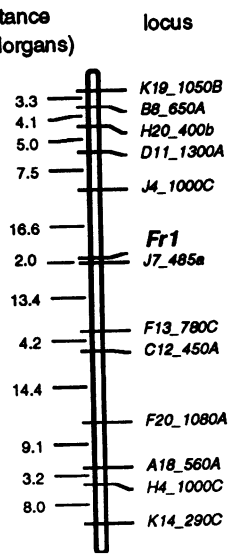

FIG. 9. Framework genetic map (interval support > 3) of linkage group 9 from tree 10-5, showing the location of *Fr1*.

system where neither pathogen nor host completely dominate the other (41, 42). Coevolution of *Pinus–Cronartium quercuum* pathosystems may have continued from as early as the Jurassic to the present (43). Our work also supports the possibility that the durability of forest-tree resistance could result from multiple discrete host–pathogen interactions, as suggested earlier by Kinloch and Stonecypher (15) and Carson and Carson (16). Results of fusiform-rust screening with different tree genotypes (28, 30) and preliminary tests with molecular markers in another pedigree (P.L.W., unpublished data), suggest that additional resistance loci are present in the loblolly pine–fusiform rust pathosystem. More knowledge about forest pathosystems could lead to a better understanding of forest ecosystems and the evolution of trees.

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