USDA-Forest Service	1. Number	2. Station
	SRS-4153	Southern Research Station
RESEARCH WORK UNIT DESCRIPTION	3. Unit Location	
Ref: FSM 4070	Saucier, Mississippi	

4. Research Work Unit Title

Southern Institute of Forest Genetics

5. Project Leader (Name and address)

Robert L. Doudrick, Project Leader, RWU FS-SRS-4153, Southern Institute of Forest Genetics, Harrison Experimental Forest, 23332 Highway 67, Saucier, MS 39574-9344

6. Area of Research Applicability	7. Estimated Duration
Southeastern United States, National, International	5 Years

8. Mission

To discover the principles of heredity that operate in southern forests and demonstrate how these principles may be applied to sustain and enhance forest quality and productivity.

9. Justification and Problem Selection

Southern pine forests provide a variety of benefits to a diverse public. Both commodity and non-commodity constituencies must be served. The Southern Institute of Forest Genetics (SIFG) is uniquely positioned to meet the needs of both groups of clients. Our more traditional forest genetics research primarily supports commodity groups by providing fundamental information useful to tree improvement programs. This research includes studies on the suitability of seed sources, the mode of inheritance of traits of interest, and population management strategies. Increased emphasis on biodiversity, particularly on publicly owned lands, requires that we expand this traditional research to include non-commodity species. This emphasis also requires that we understand the mechanisms by which evolutionary processes act and interact to shape, maintain and dissipate variation in species and multi-species associations that comprise forest communities. Problem 1 is to elucidate the genetics of traits and populations and the forces that influence variation among and within forest species to facilitate breeding, management and conservation efforts.

The genetic controls of disease are particularly complex. To understand these controls, one must know the individual and population genetics of both the host and pathogen, and their interactions. Problem 2 is to elucidate the genetic processes involved in host-pathogen systems.

After nearly 40 years of selection and breeding, there are more than one billion genetically improved southern pine seedlings planted each year. Genetic improvement is a continuing process, however, and programs in the South are only now beginning the third cycle of recurrent selection and seed orchard establishment. Classical breeding methods have been effective, but innovative strategies are now needed for breeding advanced generations. These strategies must be more efficient and effective, yet maintain genetic diversity for long-term breeding to cope with an uncertain future.

Signature	Title	Date
Recommended:		
	Assistant Director for Research	
	Assistant to Staff Director	
	Staff Director	
Approved		
	Station Director	
Concurred		
	Deputy Chief for Research	

9. Justification and Problem Selection (continued):

Uncertainty exists because of the possibility of changes in the environment, consumer demands or economic conditions and lack of understanding of the fundamental basis for trait(s) inheritance. Element (a), Problem 1 is to develop population management strategies for southern pine ecosystems that will maintain or enhance genetic diversity in the long-term and provide the basis for genetic improvement in the short-term.

The emphasis on ecosystem diversity makes an improved understanding of the biology, evolution and genetics of the southern pine species fundamental to the development of appropriate strategies for both commercial utilization and the ecological maintenance of the southern pine ecosystems. Information about levels of genetic variability within and among populations of species in forest ecosystems and the patterns in which it is distributed across geographic landscapes is essential to the development of effective genetic improvement and gene conservation programs as well as endeavors involved in managing biodiversity. Of no less importance to the efficacy of these enterprises is a fundamental understanding of the mechanisms by which evolutionary processes act and interact to shape, maintain, and dissipate variation in species and multi-species associations that are components of forest communities. Element (b), Problem 1 is to elucidate the genetic principles and evolutionary forces that influence genetic variation within and among forest species.

The ability to understand the evolution and genetics of forest tree species and to develop appropriate strategies both for their commercial use and maintenance of biodiversity will be greatly enhanced by developing a fundamental understanding of their genomic organization. Element (c), Problem 1 is to elucidate the genomic organization of forest species.

Managing or ensuring the continuance of forest tree populations and their associated ecosystems requires an understanding of how pathogen populations will affect those species and ecosystems. Furthermore, a knowledge of the genetic structure of forest tree host populations and their associated pathogen populations is essential to developing strategies for the management of forests at risk to disease. Two fundamentally different host-pathogen systems must be elucidated: exotic, incipient, or non-coevolved host-pathogen systems Element (a), Problem 2 and endemic or coevolved systems Element (b), Problem 2.

10. Approach to Problem Solution

Problem 1. Research is needed to elucidate the genetics of traits and populations and the forces that influence variation among and within forest species to facilitate breeding, management and conservation efforts

Problem 1, Element (a). Research is needed to develop population management strategies for southern pine ecosystems that will maintain or enhance genetic diversity in the long-term and provide the basis for genetic improvement in the short-term.

Population management strategies for pine ecosystems that will maintain or enhance genetic diversity in the long-term and provide the basis for genetic improvement in the short-term are being adopted to breed advanced generations of genetically improved southern pines. These strategies have inherent risks that should be evaluated as soon as possible to minimize cost and to prevent failures. Alternative strategies for breeding and gene conservation will be evaluated by developing appropriate theoretical models, by computer

simulation, and in experimental populations. A Virginia pine experimental population has been established that will be used to enhance our understanding of the basis of important traits genetic control in small populations.

Because of the long-term nature of tree breeding, the problem of an uncertain future is a particular concern. Environments available to forest enterprises vary over time and traits of interest undergo value changes in response to consumer demand and fluctuating economic conditions. Multiple population systems have been designed for species under different intensities of breeding, but multiple-population theory is not well understood, particularly with respect to genetic variation existing in such a system following long-term selection.

Plant and animal species in ecosystems must have sufficient genetic diversity to respond to changing environments including environmental changes brought about by humans. There is a critical need for the development of methodology and strategies for use in gene conservation programs for forest species. Questions pertaining to size and structure of resource populations are of special importance to gene conservation efforts. These factors also are of significance in the management of biodiversity because they influence levels and types of diversity. For many species, it may be possible to effectively incorporate gene conservation efforts into the breeding system used for those species. Research is needed to develop ways to efficiently combine the two objectives into a single system.

Biochemical genetics can be used to increase our knowledge about the genetics of forest trees and in so doing mapping and studying the genetics of important traits. Biochemical markers may be useful also to increase the efficiency of forest tree breeding programs. Marker-assisted selection (MAS) can be modeled, but verification of the genetic models depends on having appropriately pedigreed populations in which to do experiments.

Accomplishments planned for the next 5 years include:

1. Evaluate alternative multiple-population breeding strategies using computer modeling.
2. Develop computer simulation models for MAS and evaluate the efficacy of MAS in tree breeding programs.
3. Analyze results from the first generation of selection and breeding in a Virginia pine model population.
4. Establish genetic tests of the second-generation base population in the Virginia pine model population.
5. Complete the second generation of selection and breeding in the Virginia pine model population.

Problem 1, Element (b). Research is needed to elucidate the genetic principles and evolutionary forces that influence genetic variation within and among forest species.

Knowledge about phylogenetic relationships among tree species within taxonomic genera is needed to help clarify the picture of genetic associations that are characteristic of forest ecosystems. Analysis of genetic population structure is a necessary step for acquiring information about the evolutionary factors that shape genetic variability within a species. In this regard, studies using both adaptive traits as well as genetically neutral or near-neutral markers are required to obtain complete descriptions of the variability patterns that exist. Investigations to discern population structure within

species, particularly those that have emphasized growth and survival traits in the southern pines, have been research priorities in the SIFG for over four decades. Results have been applied in planting and breeding programs across the southern US. Although the Southwide Southern Pine Seed Source Study (SSPSSS) has been used to investigate geographic variation patterns in the southern pines for over forty years, it now has unique value for providing information about geographic variation patterns for growth and yield traits at or near full rotation age. Moreover, because of its long tenure and resulting exposure to long-term climatic and edaphic factors, the SSPSSS now has assumed increased importance as an instrument for studying variation in adaptive responses to these long-term environmental conditions. Thus, active research involving analysis of data collected in this and similar long-term seed source experiments should continue. In addition, research on population structure using allozyme and DNA genetic markers is needed in the southern pines and other forest species. Such studies have value in that they provide insight about the role that nonselective evolutionary processes have in shaping gene pools. When results of these investigations are compared with those observed for adaptive traits, the findings are a powerful tool for identifying evolutionary factors most effective in molding the genetic makeup of populations.

Exploration through study of theoretical models is an effective adjunct to experimental approaches for obtaining an understanding of evolutionary processes operating in forest populations. Life history evolution in tree populations is complex and not well understood. Natural selection effects change over time and space and there is a need for the development of models that adequately describe these effects and their interaction with other evolutionary influences that shape patterns of genetic variation. Effects produced by density-dependent selection and its joint action with migration and mutation, particularly processes that are involved in maintaining genetic polymorphisms, are especially important, because tree populations, with rare exception are under density regulation. Analyses of evolutionary dynamics and gene action models that influence the complex dynamical behavior that results from density-dependent selection and interspecific competition models is in its infancy and needs further development and expansion.

Information about the phylogenetic relationships that exist among subgeneric taxonomic groups is critical to obtaining a comprehensive assessment of biodiversity and supraspecific genetic associations on a regional scale. Acquisition of this knowledge will permit a complete understanding of the mechanisms of speciation and past evolutionary events that contributed to the development of the observed phylogenetic patterns.

Accomplishments planned for the next 5 years include:

1. Analyze and publish growth and yield results for measurements taken at 35 years and older ages in the SSPSSS. Results will be published for slash, longleaf, loblolly and shortleaf pines.
2. Better define geographic variation in loblolly, shortleaf, longleaf and slash pine by intensively sampling seed sources in the SSPSSS and using allozymes, cortical monoterpenes and DNA genetic markers to investigate variation patterns.
3. Investigate phylogenetic relationships among southern pines and related species using both morphological traits and DNA genetic markers.
4. Analyze dynamics for theoretical genetics models that describe density-dependent natural selection and for models of density-dependent interspecific competition.

5. Analyze range-wide genetic population structure in American chestnut using
   DNA genetic markers.
6. Investigate genetic population structure in natural populations of Fraser
   fir using biochemical markers.

Problem 1, Element (c) Research is needed on to elucidate the genomic
organization of forest species.

This research will attempt to integrate recombinational linkage mapping data
with physical mapping data to improve our understanding of forest tree genome
organization. Since 1990, the SIFG has focused on generating molecular marker
linkage maps in elite germplasm to facilitate traditional tree breeding and
improvement strategies. Linkage maps have been constructed for slash pine,
longleaf pine, eastern cottonwood, and chestnut. These maps are being used to
identify and subsequently select for genomic regions influencing specific
traits of interest such as growth rate, wood quality, and pathogen resistance
and stress tolerance within these species.

In spite of the fact that linkage maps can be used to identify and select for
specific traits of interest, there is much concern as to whether these
pedigree-specific maps will be transferable to other individuals within the
same species, or among more distantly related species and genera.

One method to integrate genetic maps across pedigrees and possibly genera is
through physical mapping. Physical mapping entails two main strategies,
mapping markers to contiguous overlays of large insert DNA libraries, and the
use of molecular cytogenetics techniques. Physical mapping of markers and
mapped sequences to intact chromosomes using molecular cytogenetics techniques
offers unique and compelling opportunities to address synteny questions and
the feasibility of integrating genetic maps into a consensus framework map for
species and genera. Identification of conserved marker-QTL linkages will
improve efficiency of traditional breeding and selection practices. Physical
mapping also will allow estimates of physical and genetic distance in
different parts of the genome, which is important in using many molecular
techniques in the large pine genome. Furthermore, information about the
physical map location and molecular cytology allow comparisons between the
genomes of different individuals and species. Highly conserved and divergent
chromosome structures and sequences can be identified, providing information
about the evolutionary relationships between them. Additional information
about genome conservation can be obtained using techniques such as genomic in
situ hybridization, which allows two or more genomes to be compared directly
at the molecular level. This technique also may be useful in backcross
breeding programs (such as for American chestnut) to distinguish which
individuals in the group containing the select marker have the highest
compliment of DNA from the recurrent species.

Accomplishments planned for the next 5 years include:

1. Integrate pedigree specific recombinational linkage maps using physical
   mapping.
2. Relate areas of physical to genetic distances of the genome.
3. Investigate phylogenetic relationships among southern pines and related
   species using both morphological traits and DNA genetic markers.
4. Demonstrate the utility of total genomic techniques in accelerating
   backcross breeding in forest trees.

5. Study conserved and divergent elements of the genome and the evolutionary relationships between species using comparative analysis.

Environmental consideration: The studies in this problem area are expected to have little or no potential for soil movement, water quality degradation, or impact on sensitive resource values and are therefore covered under FSH 1909.15, Chapter 30, "Categorical Exclusion from Documentation in an EIS or EA." Where environmental concerns may exist regarding particular studies, these will be evaluated within individual study plans or by Environmental Assessments or Environmental Impact Statements prepared with and approved by cooperating Region staffs.

Problem 2. Research is needed to elucidate the genetic processes involved in host-pathogen systems.

Problem 2, Element (a). Research is needed to elucidate the genetic processes involved in exotic, incipient or non-coevolved host-pathogen systems.

The SIFG is currently investigating levels of resistance in American chestnut x Chinese chestnut hybrids and their progeny to the chestnut blight fungus. Molecular markers are being incorporated into this program and are being used to identify regions of the chestnut genome conferring this resistance. For the American chestnut-blight fungus pathosystem, the goals are to identify and introgress these genomic regions from various accessions of Chinese chestnut into American chestnut through backcrossing. The ultimate goal is to pyramid regions until acceptable levels of resistance are obtained. Experience and knowledge gained from the above research will be used to better understand and help manage for other economically or ecologically important incipient forest tree diseases such as Dutch elm disease, pitch canker, and dogwood anthracnose.

Pitch canker was discovered in the Appalachian region in the mid-1940s. No great attention was paid to this disease for nearly three decades until a widespread epidemic began in the mid-1970s. This led to the discovery of new symptoms, new hosts, much epidemiological information, and even a new identity for the infecting fungus. The genetics of this host-pathogen association, however, has not been well studied. This disease offers forest geneticists and pathologists a unique opportunity to compare an incipient pathosystem too more established, coevolved systems, such as the fusiform rust disease on southern pines.

Accomplishments planned for the next 5 years include:

1. Identify additional molecular markers specific to American chestnut as well as Chinese chestnut.
2. Map these and existing DNA genetic markers in backcross lines developed from two different sources of resistance.
3. Fine-scale map around putative chestnut blight fungus resistance loci.
4. Utilize DNA genetic marker data to select resistant American chestnut hybrid progeny and rogue undesirables from experimental plantings.
5. Utilize these markers to assess levels and partitioning of genetic variation in wild populations of American chestnut to help better estimate the number of recurrent parents needed at each breeding location, as well as to determine how many locations are needed across the natural range of the host species.

6. Determine the nature of genetic resistance in slash pine to the pitch canker fungus and identify molecular markers for putative host resistance genes.

Problem 2, Element (b). Research is needed to elucidate endemic or coevolved host-pathogen systems.

Fusiform rust is the major disease of loblolly and slash pine in the southeastern United States. Despite 40 years of phenotypic selection and breeding in both species against the fusiform rust fungus, the genetic basis of this host-pathogen interaction is not clearly understood. Recent efforts to characterize the genetic interactions involved in the development of this disease have led to the hypothesis that the interaction may conform to a complementary genetic system. In such a genetic system, specific reaction genes in the host and specific pathogenicity genes in the fungus evolved together to determine whether a compatible or incompatible reaction occurs (i.e., infection or no infection, respectively). Past research has reported both increased infection percentages associated with serial inoculation of slash pine families and higher infection and spermatia sporulation on seedlings from sources nearest the origin of inocula. These findings are consistent with the hypothesis of coevolution and reciprocal adaptation of complementary genes in the host and pathogen. The formation of a spermatium is an indication of a successful infection (the compatible interaction of host and pathogen) and is certainly a prerequisite for pathogen fecundity. Increased cultivation of exclusive pine genotypes selects from the pathogen population those genotypes with complementarity; the selected pathotypes increase in frequency because they are the only forms that can infect the improved varieties of pine.

Because the expression of resistance is a function of the genetics of the host, the genetics of the pathogen, the environment, and their interaction, it is necessary to control these factors under experimental conditions in order to understand the system. Research in this element involves developing and maintaining individual clonal isolates of the fungus with varying levels of pathogenicity, and in challenging individual clones of pines, developed both as rooted cuttings in vivo and as somatic emblings through tissue culture in vitro, with these fungal isolates in an artificial inoculation system. The long-term goal is to develop efficient systems for genotyping host-resistance loci and pathogen cultures at their complementary avirulence loci. This information will give breeders a way to monitor pathogen avirulence-allele frequencies in potential planting areas, and then to select the appropriate families for planting. It also will serve as a model for investigating the genetics of other complex, coevolved host-pathogen systems.

Research also will involve studies on physiological interrelationships of the host and pathogen in organized tissues of the tree as well as in tissue cultures developed in vitro from specific tissues of the host. Since cultures developed in vitro from slash pine collective, seedling tissues are known to elaborate rust fungus-biocidal substances at effective levels, the identity and biosynthetic control of these substances will be determined. Further, any differential synthesis by unique cultures from specific organized host tissues will be appraised. In vitro cultures developed from specific pine tissues will be examined both for their differential reaction to clonal isolates of the rust fungus, and their synthesis of any chemical elicitors of defense reactions. Gametophyte seed tissue will be appraised for the presence of rust fungus reaction genes, for immediate subsequent clonal somatic embryogenesis from embryonic tissues within that genotype. This will permit rapid clonal

multiplication of host genotypes that are either resistant or susceptible to various clonal isolates of the fusiform rust fungus.

Accomplishments planned for the next 5 years include:

1. Determine the extent of genetic variation in the fusiform rust fungus
    population using DNA genetic markers by sampling across the entire range
    of the rust fungus population.
2. Continue identification of complementary gene pairs in the fusiform rust
    pathosystem on slash pine.
3. Map identified avirulence genes in the pathogen to assess genetic
    diversity and monitor gene frequency shifts in the pathogen population.
4. Develop a panel of differential host genotypes to verify gene frequency
    shifts in the pathogen population.
5. Develop regional panels of differential rust fungus genotypes to assess
    the resistance of the host material being deployed in that region.
6. Employ immature pine gametophyte tissues for recognition of rust fungus-
    resistance genes, thus permitting subsequent clonal somatic embryogenesis
    using embryo tissues of same host genotype.
7. Identify and determine the biosynthetic regulation of fusiform rust
    fungus-biocidal compound(s) elaborated constitutively by tissue cultures
    of those southern pines otherwise attacked in vivo to the rust fungus.
8. Appraise reaction to in vitro inoculation using single spore-derived
    axenic fusiform rust fungus cultures on differential morphotypes of pine
    tissue cultures generated from single pine genotypes.
9. Identify and determine mechanism(s) of action of pathogen and host-
    elaborated chemical elicitors of defense reactions to fusiform rust fungus
    in southern pines.

Environmental consideration: The studies in this problem area are expected to
have little or no potential for soil movement, water quality degradation, or
impact on sensitive resource values and are therefore covered under FSH
1909.15, Chapter 30, "Categorical Exclusion from Documentation in an EIS or
EA." Where environmental concerns may exist regarding particular studies,
these will be evaluated within individual study plans or by Environmental
Assessments or Environmental Impact Statements prepared with and approved by
cooperating Region staffs.

11. Cooperations

The SIFG has a long and productive history of collaborative research with
universities, industries, government agencies and others. Currently, the SIFG
cooperates on research with 22 colleges and universities in the U.S. and two
in Europe, one genetics institute in Europe, the three southeastern forest
industry tree improvement cooperatives, four private foundations, and with six
Research Work Units in three Research Stations and Region 5's National Forest
Gel Electrophoresis Laboratory, and Region 8.

12. Staffing

The scientific staff consists of seven permanent full-time scientists,
including the Project Leader and one to two full-time scientists on term,
post-doctoral appointments. Scientists from other organizations visiting the
SIFG to do research in any one year spend on average three weeks, in total
contributing generally one additional scientist-year. There are currently
eight full-time permanent technicians, one administrative assistant and one
secretary, and four to six part-time intermittent student biological and
forestry aids. Four seniors are supported on the Senior Community Service
Employment Program, by the U.S. Department of Education.

Scientist Years for Each Year of the RWUD

Problem Area	1999	2000	2001	2002	2003
1	5.7	5.5	6.0	6.0	6.0
2	2.3	2.5	3.0	3.0	3.0
Total	8.0	8.0	9.0	9.0	9.0

Conduct of this research will require eight to nine scientists per year, with an average annual cost of $250,000 per year for laboratory research and $200,000 per year for non-laboratory research (appropriated funding).

Budget for Each Year of the RWUD (x $1,000)

Problem Area	1999	2000	2001	2002	2003
1	1,280	1,070	1,300	1,300	1,300
2	474	684	750	750	750
Total	1,754	1,754	2,050	2,050	2,050