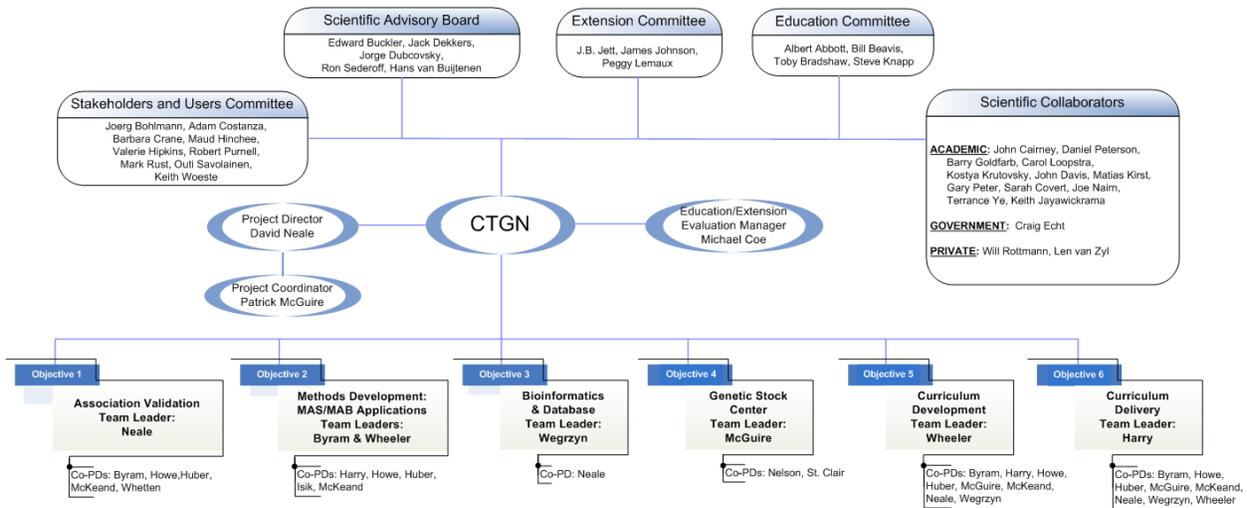


Response to Review of Integrated Conifer CAP (2009-01879)

Prepared by David Neale, June 26, 2009

USDA-CREEES Program Manager Dr. Ed Kaleikau requested the Conifer CAP (CTGN) PD David Neale to prepare a progress report (2007-2009) for the CTGN and respond to the major comments of the 2009 AFRI proposal review. This document attempts to fully meet that request. There have been only minor changes to the organizational structure since the project was first awarded and the six project objectives remain the same (see organizational structure and objectives below). In addition, reports from the Science Advisory Board and the combined Education and Extension Advisory Committees from the June 22-23, 2009 CTGN annual meeting are included at the end of this report (pages 15-19).



Objective 1.1 Genotype ~2500 trees from each of four Cooperative breeding programs (~10,000 trees in total) for 7600 candidate gene SNPs (UC Davis)

Progress

The goal of the CTGN is to validate marker-trait associations in four applied breeding programs that have previously been discovered in experimental populations under prior funding from NSF, USDA-IFAFS, and USDA-NRI. The populations genotyped and the number of SNPs are shown in Table 1.1.1. Furthermore, the CTGN validation populations and the number of SNPs to be genotyped are also shown in Table 1.1.1. We hope this clarifies issues raised by Reviewer #1 regarding past and present SNP genotyping.

Table 1.1.1. Experimental and validation populations relevant to the CTGN workplan.

Population/Funding source	Size/Species	SNPs	Reference
Experimental population			
Weyco/NSF	480/loblolly	58	González-Martínez et al. 2007
CCLONES/ADEPT-1	961/loblolly	46	González-Martínez et al. 2008
Weyco/ADEPT-2	480/loblolly	7600	Publication in progress
CCLONES/ADEPT-2	961/loblolly	7600	Publication in progress
NCSU/ADEPT-2	484/loblolly	7600	Publication in progress
FS-PNW/ADAPT	700/D-fir	384	Eckert et al. 2009
Validation population			
Loblolly NCSU	2200	7600	
Loblolly TAMU	1858	7600	
Loblolly x slash UF	524	7600	
Slash UF	1000	1536	
Douglas-fir OSU	2500	TBD	

TBD = to be determined.

References cited:

- Eckert A, AD Bower, JL Wegrzyn, B Pande, KD Jermstad, KV Krutovsky, JB St. Clair, and DB Neale. 2009. Association Genetics of Coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*, Pinaceae) I. Cold-hardiness Related Traits. *Genetics* doi:10.1534/genetics.109.102350
- González-Martínez SC, NC Wheeler, E Ersoz, CD Nelson, and DB Neale. 2007 Association Genetics in *Pinus taeda* L. I. Wood Property Traits. *Genetics* **175**:399-409. doi:10.1534/genetics.106.061127
- González-Martínez SC, D Huber, E Ersoz, JM Davis, and DB Neale. 2008. Association Genetics in *Pinus taeda* L. II. Carbon Isotope Discrimination. *Heredity* **101**:19-26.

The Neale wet lab facility at UC Davis is running a high-throughput DNA isolation protocol with assistance from custom LIMS sample tracking through a Plone-based web interface system. By means of this system, samples are accurately tracked from arrival through outsourcing for genotyping. The integration of all lab procedures into this LIMS has ensured identical handling of all tissues handled by the lab.

- 4179 loblolly samples entered in system to date
 - 3744 samples have been successfully extracted and submitted to Illumina Inc. for genotyping
 - Samples to be received will bring total number of loblolly samples to be genotyped up to 5700

- Genotyping data are available to Coop breeding programs through the Plone website.
- Isolated DNA samples are sent to either the Illumina Inc. facility in San Diego, CA or to the UC Davis Genome Center DNA Technologies Core Facility.

Future plans

Slash pine and Douglas fir samples will begin to be accepted and processed for DNA harvesting and then sent to the DNA Tech Core at UCD Genome Center for genotyping. The genotyping information will be made available through the Plone website.

Objective 1.2 *Validate SNP/Trait associations and estimate the effect of allelic substitutions in eastern loblolly pine breeding and testing populations (NCSU)*

Progress

Collection of foliage samples from trees in the NCSU loblolly pine breeding populations is nearing completion. By the end of June 2009, over 2500 foliage samples will have been collected and sent to UC Davis for DNA extraction and SNP genotyping.

The establishment of the Atlantic Coastal Elite (ACE) clonally replicated test population is proceeding well. It consists of progeny from parents that are being genotyped through the CTGN, and will be the first NCSU operational population to test the implementation of marker-trait associations identified in the CTGN.

Jaime Zapata, the PhD student supported by the CTGN at NC State, has successfully completed his first year of course work. He has also spent a significant amount of time working in both the lab and field and has become familiar with molecular-marker technologies.

Wood quality phenotypic analysis is proceeding. Of the original 170 clones analyzed using NIR spectroscopy, a subset of 18 clones was selected based on several traits, including lignin and cellulose content. For each selected clone, 3 ramets were harvested and are currently being analyzed by colleagues in the NC State University Wood and Paper Science Department. The objective of this experiment is to test for genetic variation in wood properties that affect the efficiency with which pine wood can be converted to ethanol by fermentation.

Future plans

The first field tests of clonally replicated elite loblolly pine families will be planted in Fall 2009 across six test sites in the Atlantic Coastal region. A second series of field tests of the same families will be planted in Fall 2010 at six additional sites. These clonally replicated field tests will serve as test populations for prediction of genetic merit based on the SNP associations detected in the breeding population.

Genotype data from the samples submitted for genotyping will be analyzed for association with phenotypic traits, with an emphasis on breeding values for height, volume, disease resistance, and stem form characters as the phenotypes of greatest interest to stakeholders such as the members of the NC State University Cooperative Tree Improvement Program.

Analysis of biofuel conversion efficiency on the selected clones should be complete within the next six months, and a calibration curve for near-infrared spectra to allow prediction of biofuel conversion efficiency from NIR spectral data will be constructed using the wet-lab data. This calibration curve will provide another phenotype for which SNP associations can be tested.

Objective 1.3 *Validate SNP/Trait associations and estimate the effect of allelic substitutions in western loblolly pine breeding and testing populations, and test the predictive power of markers in a forward selection population (TAMU)*

Progress

Germplasm has been collected for genotyping from 5 populations and phenotypic data tabulated for release to the public database. Collection required approximately 45 crew-member work days. Breeding population structure for all selections were established with classical co-ancestry matrices (Table 1.3.1).

Table 1.3.1. Summary characterization of established breeding populations.

Population	Description	Samples ¹	Phenotype data for release ²
E TX 1 st Gen	East Texas Breeding Population	560	County of origin, BV for height, diameter, volume, specific gravity, rust, stem straightness and forks
E TX 2 nd Gen	East Texas Breeding Population – offspring from E TX 1 st Gen	527	Parents, BV for height, diameter, volume, specific gravity, rust, stem straightness and forks
MS	Out group of selections from MS parents	192	Differential breakage patterns when exposed to hurricane force winds. Expect growth to also be available
Clonal line trial	Entries in two locations of replicated line trials related to the E TX populations	197	BLUP values for 4 year height, diameter, volume
CP Test	Control-pollinated progeny test of E TX 1 st Gen parents	574	Individual BLUP values for height, diameter, volume, specific gravity and stiffness (time of flight).
Total		1858	

¹ Not all submissions are unique.

² Not available for all selections.

Future plans

Additional measurements (stiffness (time of flight), wood specific gravity, and 5th year growth measurements) will be acquired from the clonal line trials. The phenotypic data will be uploaded to the project public database.

The markers, as they become available, will be used to characterize breeding population structure and evaluate alternatives for incorporating markers into progeny test analysis and for estimating impact for within family selection (using both the 2nd generation population and clonal line trials).

Objective 1.4 *Verify SNP/Trait associations in slash pine breeding and testing populations, and demonstrate response to selection of markers in a forward selection population (UF)*

Progress

- 2007-2008
 - Plant BC1 full-sib trial - Completed
 - Collect foliage from BC1 population for genotyping - Completed
 - Begin phenotyping of BC1 - Completed
 - Perform cross for mapping population - Completed

- 2008-2009
 - Collect and phenotype wood cores in the extant slash pine full-sib block plots (FSBP) – 500 of 1000 cores collected, not yet phenotyped
 - Collect foliage for genotyping in the FSBP – 500 of 1000 foliage samples collected
 - Analyze available phenotypes for the BC1 population – Completed, 2 papers should go to publication this fall, P. Munoz MS Thesis

Future plans

- 2009-2010
 - Continue phenotyping of the BC1 – After growing season
 - Plant mapping population – Grow in greenhouse and hot plant in spring 2010
 - Collect foliage from mapping population for genotyping
 - Generate genetic map for slash pine
 - Analyze phenotypes for the FSBP
 - Analyze new phenotypes for the BC1 population
 - Begin phenotyping mapping population
 - Continue phenotyping BC1, remove wood cores
- 2010-2011
 - Collect wood cores from mapping population
 - Continue phenotyping of BC1 and mapping population
 - Complete analysis and publish results

Objective 1.5 Validate SNP/trait associations in Douglas-fir (OSU)

Progress

New phenotypes. We measured wood stiffness, vegetative bud flush, or reproductive bud phenology on 830 clonally replicated parents in nine grafted seed orchards (Table 1.5.1). Clonal repeatabilities (i.e., estimates of broad-sense heritability) for some of the measured traits are presented in Table 1.5.1. Other traits have been measured but not yet analyzed (check marks in Table 1.5.1). We also measured fall cold hardiness on 141 full-sib families, with plans to measure fall cold hardiness on an additional 160 families in the fall of 2009. The measurement dates for the remaining traits are presented in Table 1.5.1.

Simulations. We developed a menu-driven Java program for simulating tree phenotypes and SNP haplotypes. This program will be used to (1) test alternative mating designs, field designs, and sampling strategies for QTL discovery and (2) evaluate alternative analytical approaches, including genomic selection. This program will also serve as a user-friendly educational tool for teaching students about association genetics. The program has five modules that simulate a QTL allele pool, genetic map, parents, progeny, and SNP haplotypes. Candidate gene assumptions that can be controlled by the user include the number of loci in the genome, number of loci genotyped, number of loci affecting the trait (QTL), and the probability that a genotyped locus is a QTL. The distributions of locus and alleles effects can be chosen from an exponential, gamma, or normal distribution, whereas QTL allele frequencies can be chosen from a Dirichlet distribution, or the user can specify that all alleles at a locus have the same frequency. The user can set the number of linkage groups or place QTL on specific linkage groups. Maps are created using the Haldane or Kosambi mapping function, and the distribution of loci can be chosen to have a uniform, normal, or Poisson distribution. A mixed-mating approach is used to simulate the first-generation parents. For this simulation, the user can control the proportion of selfed

progeny, parent-offspring matings, and matings among unrelated trees, half-sibs, and full-sibs. The user can specify the number of simulated parents and offspring per family using various mating designs (open-pollination, poly-mix, full-diallel, half-diallel, single-pair mating, or clonal replication). For open-pollination, the user can specify equal or unequal numbers of offspring per local male parent. Haplotypes of neutral SNPs are derived from haplotype libraries provided by the user. This allows the user to use real haplotypes or haplotypes simulated to match the characteristics of the species of interest. SNPs in the neutral haplotypes are replaced by quantitative trait nucleotides (QTNs) at the appropriate frequencies to form the QTL haplotypes. The user can specify the size of SNP discovery panel (to evaluate the effects of ascertainment bias), the maximum number of SNPs genotyped per locus, and the probability that the QTN are genotyped. In the future, we will add the ability to simulate missing SNP data and SNP genotyping errors.

Table 1.5.1. Progress and plans for obtaining new Douglas-fir phenotypes on parent trees and full-sib families.

Seed orchard or progeny test	No. of clones or families	Log stiffness	Tree stiffness	Increment cores	Fall cold hardiness	Vegetative bud flush	Flower bud phenology	
							Male	Female
Seed orchard								
BLM-I30	97	2010	2010	2010	–	0.93	2010	2010
BLM-I31	74	2010	2010	2010	–	0.88	2010	2010
BLM-P1	113	2010	2010	2010	–	0.94	2010	2010
BLM-P2	128	2010	2010	2010	–	0.87	2010	2010
Cowlitz	92	√	√	√	–	0.82	0.36	0.48
Gardiner	59	√	0.81	√	–	0.92	0.27	0.53
Hood Canal	82	0.40	0.16	–	–	–	–	–
Snoqualmie	133	√	2010	2010	–	0.90	0.71	0.76
Snow Peak	52	0.75	0.58	–	–	–	–	–
Progeny test								
Puget Sound	141	–	–	–	0.45-0.68	√	–	–
WA Cascades	160	–	–	–	2009	√	–	–

Dates 2009 and 2010 indicate when the data will be collected.

Other numbers are clonal or family heritabilities (repeatabilities).

Check marks (√) indicate that the trait has been measured. Dashes (–) indicate that traits will not be measured.

SNP discovery. We isolated RNA from five Douglas-fir tissues (stem, bud, shoot, seeds, and cambium) and submitted a pooled RNA sample to the Joint Genome Institute for 454 transcriptome sequencing (JGI co-PIs are Dean, Howe, Jermstad, Neale, and Rogers). We expect to receive data from as many as 1.2 million reads by late August or early September 2009.

Future plans

New phenotypes. During 2009-2010, we will continue to measure parental phenotypes in seed orchards and progeny phenotypes in progeny test plantations (see Table 1.5.1). In addition to the

materials shown in Table 1.5.1, we will seek out new seed orchard and progeny test materials to measure for wood stiffness and vegetative bud phenology, leveraging in-kind support provided by the members of the Pacific Northwest Tree Improvement Research Cooperative and the Northwest Tree Improvement Cooperative. Once data are acquired, phenotypic analyses are completed, and alternative sampling strategies have been evaluated with the simulation software, we will choose the 2500 Douglas-fir trees to genotype. The trees will be chosen based on the number of phenotypic traits available (new and existing), quality of the phenotypic data (e.g., heritabilities), and amount of information from relatives (i.e., extent of the measured pedigree).

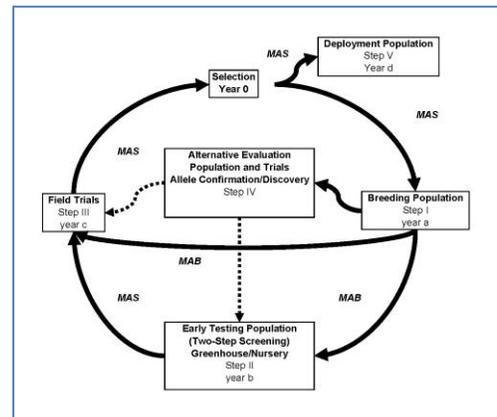
Simulations. We will use the simulation software to test alternative sampling strategies for association tests. In particular, we will evaluate alternative numbers of parents and progeny to genotype. These sampling strategies will be evaluated using analyses that focus on combined analyses of within-family linkage and population-level linkage disequilibrium. Once optimal distributions of parents and progeny are determined, we will choose the trees to genotype based on the criteria listed above.

SNP discovery. Over the next few months, we will investigate the feasibility of using CTGN funds to sequence RNA from multiple tissues, developmental stages, environmental conditions, and genotypes using Solexa (Illumina) sequencing. We will use the 454 sequence as a reference and the shorter Solexa reads for SNP detection and digital gene expression using the RNA-seq approach. The Solexa sequence will be used for SNP discovery and SNP chips will be designed. SNP genotyping and association analyses will occur in the last half-year of the project.

Objective 2.0 Develop and economically evaluate new methods incorporating marker-assisted selection into conifer tree breeding programs

Progress

Continued progress on Objective 2 has been made on two fronts: conceptualization and graphical description of the logistical nature of MAS implementation in the tree breeding cycle, and the creation of an analytical tool for conducting stochastic simulation of tree breeding alternatives using MAS, the objective of which is to provide economic insights on probability of success (financial return) for an array of tree breeding scenarios. Features of both components were presented in the “Genomics of Tree Breeding and Forest Ecosystems, Module 8” short-course recently conducted in Davis, CA (see figure, right).



Since the inception of the current grant, an expanded view of Objective 2 goals has developed. Efforts promoted by co-PDs to develop analytical methods for incorporating SNP association data in routine selection indices or BLUP-derived BV estimates (see Obj. 1.2 – NCSU Activities) have been embraced as the third leg of MAS implementation in forest tree breeding (Obj. 2). An entire module (#9: Linear mixed models and molecular markers) was incorporated in the short-course as a result of this work. Progress in all three areas is sufficient to warrant preparation of peer-reviewed manuscripts.

At TAMU a beta version of simulation software for ranking alternative breeding programs was completed.

Future plans

Before the end of grant year 2 (10/1/09), outlines of three peer-reviewed papers on MAS implementation in forest tree breeding will be completed and circulated among PDs working on this objective. Drafts of all three papers will be completed by the end of the first quarter of year 3 funding (1/1/2010), and papers will be submitted to a single journal, as a series, by the end of the second quarter (3/1/2010). Tentative topical descriptions and authorship of the three papers are noted as follows:

- Marker-informed tree breeding strategies (N. Wheeler and T. Byram)
- Inclusion of markers in BLUP-derived BV estimates for advanced generation selection in forest trees (F. Isik and R. Whetten)
- Stochastic simulation of the economic risk of marker-informed breeding in forest trees (T. Byram and N. Wheeler)

The possibility exists for an additional paper to be added to this series based on simulation work being conducted at Oregon State University.

At TAMU a release version of the simulation software for ranking alternative breeding programs will be completed and a paper will be published demonstrating its use.

Objective 3.0 *Develop databases (TreeGenes) and web-based tools (Dendrome) to facilitate all aspects of the CTGN*

Progress

Development of Plone Project Website

- Plone system implemented for CTGN project content
- Content related to education and extension activities updated
- Content related to presentations and data updated regularly

DNA Inventory System for Integration into the TreeGenes database

- System designed for the collection of tree metadata and phenotypic data from participating co-ops
- Manual assignment of phenotypes including detailed descriptions and standardizations
- Controlled logins created to allow participants to continually upload data to the system relating to specific tree identifiers
- Functions to establish links between sample barcodes and tree identifiers used by the originating database is complete.
- Full integration of this data in the TreeGenes database

Development of Custom LIMS system for Integration into DNA Inventory

- LIMS system to guide activities of the wet lab including sample preparation and DNA isolation
- Full tracking based on barcode identifier through to plate population
- Allows for tracking of concentrations as well as inter and intra-plate controls
- Full integration with equipment used for the process
- Interface allows for data on all plates and samples utilized to be reviewed at any time
- Full integration of this data into the TreeGenes Database

Future plans

Development of Genotyping Interface for Integration into DNA Inventory

- Automated receipt and processing of genotypic data

- Export of required data points from Illumina including: score data, raw fluorescent values, and actual genotype values
- QC functions and full integration of this data into the genotype module of the TreeGenes database.

Full Integration of all data in TreeGenes into the DiversiTree Interface

- DiversiTree interface to include phenotypic data for each tree identifier that can easily be queried by public users.
- Interface to include genotypic data for each SNP successfully processed by Illumina. This will be able to be queried by public users.
- Ability to perform large-scale queries as an enhanced feature

Interface development for the Stock Center

- Provide full interface and database for ordering, tracking, and shipping of samples
- Integration of the Stock center with existing resources (DiversiTree and TreeGenes database) so that information available relating to specific samples can be obtained by users

Objective 4.0 *Develop an international genetic stock center for conifers*

Progress

A public genetic stock center for conifer species.

The stock center will be headquartered at UC Davis (PD D.B. Neale) and field archive locations will be at the Southern Institute of Forest Genetics, Southern Research Station, USDA Forest Service in Saucier, MS (co-PD D. Nelson) and at the Pacific Northwest Research Station, USDA Forest Service in Corvallis, OR (co-PD B. St. Clair). The two Forest Service sites will develop and maintain clonal archives of all genetic mapping and association mapping populations for loblolly pine and Douglas-fir. In total, this resource is nearly 4000 clones, of which much was developed by co-PD N. Wheeler while at Weyerhaeuser Company. Weyerhaeuser has agreed to release these materials to the stock center for public archival, curation, and distribution.

Douglas-Fir Genetic Stock Center

Seed for graft-compatible Douglas-fir rootstock was sown in spring 2008 and 2009. A total of 1,500 seedlings are currently being grown in containers in Corvallis, Oregon. The 1-year-old seedlings were transplanted into larger pots in spring 2009.

Loblolly Pine Genetic Stock Center

Base and QTL Three-generation populations

Reference loblolly pine mapping populations Base and QTL are now archived with four ramets per clone at the Harrison Experimental Forest clone bank in Mississippi. Two reps of two ramets per clone were planted in either 2007 or 2008. Replacements were grafted in 2007 and 2008 and fill-in planted in May 2008.

Additional ramets (ranging from 1 to >10) from each clone were planted in 2007 and 2008 at the Erambert Seed Orchard, also in south Mississippi, but about 40 miles north of the Harrison.

NCSU Association population

The NCSU Association population archive was initiated in Spring 2009. 1202 rooted cuttings representing 413 unrelated clones were obtained from CTGN collaborators at NC State University.

UC Davis Molecular Genetic Stocks Center

There are significant molecular biology resources and regents for conifers that have been generated and collected by Neale and reside in -80°C freezers: cDNA libraries and clones, BAC libraries and clones, mapping and association population DNAs, PCR primer sets, etc. Much of this resource has been collected by Neale and resides at UC Davis.

Future plans

Douglas-Fir Genetic Stock Center

The second sowing will be transplanted into larger pots in late summer 2009. Scion from 600 clones of the linkage mapping/QTL three-generation pedigree will be collected from trees at the Institute of Forest Genetics in Placerville, California, in January 2010 and grafted onto the rootstock. Some rootstock will be held over for grafting replacements the following year. The grafts will be maintained in the greenhouse during 2010 and planted at a site 20 miles north of Corvallis, Oregon, in winter 2011. Records for the clonal archive will be kept in the TreeGenes database.

Loblolly Pine Genetic Stock Center

Base and QTL Three-generation populations

Additional fill-in planting at the Harrison Experimental Forest is planned for summer 2009, once soil moisture is high enough. A final batch of ramets from each Erambert Seed Orchard clone will also be planted in summer 2009, again once soil moisture is adequate. This will complete the Base and QTL genetic archive.

A full inventory and mapping for the clone bank archive and overflow archive areas will be completed. The two areas will be maintained and tissue samples will be provided or coordinated upon request.

NCSU Association population

The rooted cuttings from CTGN collaborators at NC State University will be planted at the Erambert Seed Orchard to serve as a genetic archive. We will target 3 ramets per clone from as many clones as are viable and still available in 2009/2010. This number should approach 500. A secondary archive of at least 3 ramets per clone will be developed in the future by grafting onto seedling rootstocks using the rooted cuttings as scion stocks. The grafts will be planted at a second location on the Erambert Seed Orchard. The two areas will be maintained and tissue samples will be provided/coordinated upon request.

Other species

A priority list of populations to archive will be developed by the CTGN investigators and archiving of the highest priority population will be initiated.

UC Davis Molecular Genetic Stocks Center

The database and web resource components covering all three components of the CTGN Genetic Stock Center will be developed by the Dendrome and TreeGenes Database staff, at UC Davis.

This web resource will completely integrate the biological and genetic data and accession information for the living clonal and preserved molecular components of the collection. The site will offer access for requesting and distribution of the resources to other research units in the US and internationally.

Objective 5.0 *Develop an education plan for undergraduate and graduate curriculum development in genomics-based breeding in forest trees*

Progress

1. Preparation and delivery of an intensive five-day short-course on Genomics in Tree Breeding and Forest Ecosystems was the primary, but not only, focus of the Education PDs over the last nine months. The short-course, held June 15-19, 2009, on the UC Davis campus, was attended by 24 participants, representing seven countries. Participants included nine graduate students/post-doctoral fellows and 15 professionals, nine of whom had Ph.Ds. There were seven tree breeders in the group.

Primary instruction was provided by David Harry and Nick Wheeler, with support from co-PDs F. Isik, R. Whetten, T. Byram, and J. Wegrzyn. Additional assistance came from A. Eckert and V. Hipkins. The short-course consisted of ten modules presented in Powerpoint format (54 – 110 slides in length). Six of the modules featured exercises that extended concepts presented in modules to manipulation of data sets in relevant genomics software applications.

Course attendees completed evaluation questionnaires before and after the short-course, to gauge overall affect of the course on their general knowledge level, and they completed an evaluation document on quality of presentations. The latter was quickly summarized and presented to project advisory board members at the annual meeting held the following week (June 22-23).

2. A second, major, accomplishment of the Education objective was the creation and delivery of a special topics course “Association Genetics and Breeding” at Oregon State University (3 quarter credits, winter quarter, 2009). Largely organized and taught by PD D. Harry, the course featured guest lectures from PDs within the Conifer, Wheat, Barley and Solonaceae CAP projects. The course was attended by 10 graduate students representing Forestry (4), Crop and Soil Science (3), Horticulture (1), and Animal Science (2). Course structure included 13 lectures (80 minutes), 3 class periods featuring data and software exercises, and 3 class periods for discussion topics.

Workshop syllabus

Course Outline

- **Day 1. Introduction and Basic Principles**
 - *Module 1. Basic Principles in Population and Quantitative Genetics*
 - *Module 2. Introduction to Conventional Tree Breeding*
- **Day 2. Genetic Polymorphisms and Analyses**
 - *Module 3. Genetic Markers*
 - *Module 4. Molecular Population Genetics*
- **Day 3. Complex Trait Dissection**
 - *Module 5. Genetic Maps and QTL mapping*
 - *Module 6. Association Genetics*
- **Day 4. Marker Assisted Breeding**
 - *Module 7. Marker informed breeding in T1: Discovery/Proof of Concept*
 - *Module 8. Marker informed breeding in T1: Implementation Strategies & Economics*
- **Day 5. Gene Resource Management and Forest Ecosystem Applications**
 - *Module 9. Linear Models and Molecular Markers*
 - *Module 10. Genomic Applications in Genetic Resource Management*

www.pinegenome.org/cton





Attendees and teachers of the 2009 short-course, “Genomics of tree breeding and forest ecosystems”, held in Davis, June 15-19, 2009.

Additional activities in support of CTGN goals (TAMU)

1. PD T. Byram prepared and presented 1.5 hr session on economic simulation at the CTGN short-course. UC Davis, June 15-19, 2009.
2. Used four hours of our annual Contact Representative’s Meeting as a training session on Tree Breeding and Molecular Markers. Presentation was done by Dave Harry on behalf of the CTGN

Future plans

Course materials developed for the Oregon State University course and the summer short-course will be made available for incorporation in curricula at other institutions within our grant and subsequently to institutions external to our grant. Currently, the course is expected to be delivered at UC Davis and North Carolina State University during the 2009/2010 academic year. The CTGN staff will expedite delivery and modification of the materials, as required. The short-course will be offered, likely in modified format, to include more exercises and less theory.

At TAMU, there will be continuing efforts to train and inform members of the WGFTIP with presentations from the CTGN group at the annual Contact Representative’s Meeting.

Objective 6.0 *Develop and deploy an extension curriculum for continuing education in genomics-based breeding for practicing tree breeders and forest tree gene resource managers*

Progress

In the first 1.75 years of the project, Co-PDs have delivered over 40 outreach presentations, including invited talks, class lectures, posters, talks to professional societies, conference talks, and so forth (summarized by venue, # of participants, titles, etc in previous progress reports). We have developed and widely circulated a CTGN CAP brochure.

In May, 2009, Extension PDs prepared and delivered PowerPoint presentations to all three of our southern pine cooperative membership meetings. The Douglas-fir community was addressed with a similar talk in June. The objective of these meetings was to further develop our CTGN message. In the past we have provided a very brief overview of markers, marker informed breeding, and genomics. This year greater detail was provided. Presentations varied in length between 2 and 5 hours.

A powerful extension tool has been the interaction among cooperative directors in which each shares particular knowledge or expertise with one another. Several PDs (directors) are working on simulation tools related to association genetics (see Objective 1.0) and this information is constantly being shared. NCSU PD F. Isik delivered two workshops in December 2008 on use of BLUP tools that incorporated potential use of SNP markers in BV estimation. The workshops were well attended, including members of our coop staffs and regular membership.

The recently completed short-course (noted in Education section of this report) served a significant extension function. At least 7 attendees were directly or indirectly associated with tree breeding programs. The short-course provided an opportunity for continuing education and development of skills. At least two prominent breeders indicated the short-course gave them great confidence that marker informed breeding will play a role in their programs and they were ready to get started. This clearly validates our extension logic model that seeks to change attitudes and behaviors.

Additional activities in support of CTGN goals (NCSU):

- On May 19-20, the Program held its 53rd Advisory Meeting in Raleigh, NC. Over 40 guests from ~20 companies and state agencies were present. Several presentations were given involving CTGN, including one from PD Dave Harry on the basic fundamentals of indirect selection, markers and their various applications in tree improvement, as well as a brief introduction to the concept of association genetics.
- Dr. Fikret Isik organized the 2009 North American Quantitative Forest Genetics Workshop, held 31 May 2009 in conjunction with the Southern Forest Tree Improvement Conference at Virginia Tech in Blacksburg VA. The theme of the workshop was “Use of genetic markers in forest tree breeding”. Dr. Ross Whetten presented a talk entitled “Simulation of genomic selection in loblolly pine breeding populations”, co-authored by Dr. Isik, to describe results of studies exploring how SNP genotype data can be used in the loblolly pine breeding program at NCSU.

Additional activities in support of CTGN goals (TAMU):

1. Acquired ASREML software and training for the tree improvement staff
2. Presentation: Byram, T.D. and N.C. Wheeler. 2008. The promise and unresolved challenges of marker assisted breeding in southern pine tree breeding programs. Presented at the IUFRO-CTIA Joint Conference, Quebec City, Canada, August 25-28, 2008.

Future plans

In addition to the short course, we are considering a workshop intended to provide working solutions to use of SNP associations in BV estimation. This would be targeted to a very small audience of coop directors, their staff, and select members of the coops who are familiar with quantitative genetics.

Emphasis this coming year will be on broadening our exposure to a wider audience. We will do this through direct encounters (*short-course, class room lectures, seminars and Co-op meeting talks (co-op members, co-op directors, and staff)*), consultative interactions (*meetings with co-op members to advise and assist them with genomic applications (i.e., fingerprinting orchard parents, locating vendors, assisting with contracts, etc)*), On-line Resources (*putting shortcourse materials on-line so that other institutions can use them to develop their own courses, eXtension commitments*), and peer-reviewed papers. Our target is to make at least 15 contacts per year.

Additional planned activities in support of CTGN goals (TAMU):

At TAMU, there will be continuing efforts to train and inform members of the WGFTIP with presentations from the CTGN group at the annual Contact Representative's Meeting.

Scientific Advisory Board Report

Jack Dekkers and Chuck Langley

Substantial progress has been made on each of the research objectives. Coordination on aspects of the project (sample collection, genotyping, data base development) are good, but further planning and collaboration will be needed to ensure the project will deliver on its goals of validating SNPs and providing stakeholders with tools for MAS/MAB.

Objective 1. Validate SNP associations discovered in prior grants in operational tree improvement populations.

Data base system for entry, storage, and retrieval of phenotype and genotype data has been developed and is in place.

Recommendation: to facilitate future use of the data base and joint analysis of populations, attention needs to be given to:

- *Further standardization of phenotypes*
- *Entry of raw phenotypic data*
- *Pedigrees*

A comprehensive system for receipt of field samples, DNA extraction, tracking and genotyping has been developed and is in place.

Genotyping platforms are in place and operational

- 1536 Golden Gate panel
- 7,600 iSelect panel

Recommendation: Incorporate plans for population-specific gene calling – using raw bead data and Illumina Genome Studio software.

Recommendation: Develop plans to facilitate future work with further scaling-up to larger panels and for subsequent re-genotyping using larger panels.

Individual collaborator projects for validation and discovery have made good progress on developing populations and collecting phenotypes and samples for genotyping. The different validation populations and data sets are at different stages of development. Genotyping has started for some populations. The complete path that will be taken in each population to achieve the goal of validating associations detected in earlier studies is not clear.

It is unclear to what extent the emphasis in each population/project is on using LD versus within-family linkage – this has large impact on the individuals that should be genotyped and on how the results should be implemented in breeding.

Recommendation: Evaluate the impact of alternate measures of phenotype (raw phenotypes/BLUPs) in association analyses.

Recommendation: *Develop a detailed time-line for each population for sample submission, genotyping, and analysis.*

Recommendation: *Use genotyping strategies (individuals to genotype) that would enable subsequent use of genotypes for future projects (e.g. high-density – low-density genotyping strategies, as in NAMS design).*

Recommendation: *Use genotyping data to further evaluate the extent of LD in breeding populations, how LD has changed during the cycles, and the consistency of LD across populations. Look for evidence of geographic differentiation in SNPs associated with specific traits.*

Recommendation: *Develop detailed strategies and plans for data analysis, including separate analysis of each population, joint analysis of multiple populations, who will develop and conduct which analyses on which populations, etc. Consider sharing/exchange of populations and software. For associations analyses, consider Genomic Selection-type of analyses of all SNPs jointly.*

Recommendation: *Consider evaluation of SNP associations with more detailed expression, morphological and physiological phenotypes.*

Objective 2. MAS/MAB applications

Several simulation programs have been developed to assess various partially complementary and partially overlapping aspects of the use of markers for association analysis, QTL mapping, and the application of markers in MAS/MAB. Different simulation software may be needed depending on the targeted application/audience.

Recommendation: *Coordinate development and use of simulation programs across groups and integrate software where possible. Also integrate software with methods of analysis.*

Recommendation: *Use simulation software to identify optimal population structures and SNP densities required for LD and/or linkage analysis – use LD that exists in operational tree breeding populations.*

Recommendation: *Use simulation software to identify importance of LD vs linkage in MAS/MAB in operational tree breeding populations.*

Recommendation: *Focus on development of genetic evaluation software that will allow stakeholders to incorporate marker information in BV estimation.*

Software (Simetar) has been developed as a tool for education and to allow rapid evaluation of alternate breeding programs. It is not clear to what extent these incorporate multiple-trait breeding goals (with economic values), multiple-trait selection indexes, or use of selection index methods to incorporate marker information and to model BLUP EBV and to evaluate impacts on

inbreeding. Incorporating these concepts would also capitalize on ‘teaching opportunities’ to further promote use these basic breeding methods.

Recommendation: Expand Simetar to incorporate additional concepts and aspects of comprehensive multi-trait breeding programs.

Objective 3 - Bioinformatics

Through Treegenes and Dendromes, a comprehensive web-based system has been developed with genomic resources for conifers, which serves an important role for the US and worldwide conifer genetics and genomics community.

Recommendation: It will be important for the conifer genetics and genomics community to provide feedback on utility and suggestions for improvement of the resource.

Recommendation: A stable source of longer-term funding to ensure maintenance and further development of the resource must be found.

Objective 4 – Conifer Genetic Stock Center -

Living and DNA-based resources have been established to preserve genetic material from previous research projects. It is not clear how these resources will be used, other than that the genetic material will be maintained through these efforts.

Recommendation: Need efforts to advertise existence of these resources to the research and stakeholder communities, including opportunities to collect and evaluate additional phenotypes.

Recommendation: Expand efforts to other species

Recommendations: Given the long generation intervals and the rapidly changing technologies (higher-density SNP panels – complete sequencing), determine what populations would be most useful to establish now for future R&D

General consideration: the group should further discuss the future and impact of tree breeding in the US in the context of the changing global industry and market shifts and emerging science, and evaluate the implications for research.

REPORT
Education and Extension Committees
Conifer Translational Genomics Network
Coordinated Agricultural Project
University of California, Davis
June 22-23, 2009

Discussion Participants: Jim Johnson, Tom Blush, Paul Gepts, Michael Coe, Peggy Lemaux

Education

Primary educational efforts of the CTGN over the past year included the recently held shortcourse, entitled Genomics in Tree Breeding and Forest Ecosystems, as well as a quarter-long course at Oregon State University. The shortcourse, originally intended mainly for graduate students, attracted 23 participants but few were actually grad students (4 graduate students, 5 post-doctoral fellows, and 14 industry representatives). Based on the extensive survey done by the Conifer CAP professional evaluator, the course was quite successful, with generally favorable evaluations.

Recommendations:

- Suggestions for future iterations of the shortcourse include focusing more on applications and less on theory.
- The wide range of experience and educational backgrounds of the participants in the first shortcourse made the course challenging. The instructors did a very commendable job on the first time through the curriculum as a short course. Depending on the makeup of future shortcourses, perhaps the course could be organized to accommodate this diversity by, for example, using remedial readings or providing instruction before the class begins.
- Other constituencies could be surveyed to determine if there is interest in putting together a combined genomics shortcourse. One approach might be to coordinate with other CAPS to see if a general genomics/plant breeding section of the shortcourse could be followed by separate sections that are crop-specific. Perhaps this could be discussed with the other CAPs to see if such an approach would be marketable and create a new pool of graduate students and other participants. The modular organization of the course lends itself to this type of approach.
- To increase graduate student interest in forestry careers, it was suggest that a couple of short You-Tube movies be created that feature some interesting aspects of the profession, as well as interviews of young people who have chosen this career. Offer students at participating institutions a small “prize” for the winning video – a very successful venture by the American Society of Plant Biologists
(<http://www.chlorofilms.org/index.php?module=Pages&func=display&pageid=6>).

Extension

Extension activities during this past year consisted mainly of presentations to the tree improvement cooperatives, about 50 presentations in total. The CTGN sees direct encounters, consultative interactions, on-line resources, and peer-reviewed papers as outreach products for next year. Plans are also to continue with presentations to co-op directors and members. The Advisory Board appreciates that the materials in these presentations were at a level more appropriate to the interests and educational levels of this clientele. Historically some

technologies have been “oversold” to the forestry community. CTGN is clearly making an effort to help the community understand the MAS technology but not to overemphasize its potential. CTGN is very proactive in conducting evaluations of both the education and extension activities. Michael Coe, Cedar Lake Research and Consulting Group, LLC, from Portland, OR is leading these evaluation efforts.

Recommendations:

- The focus of these presentations should be more on possible applications (see below) than on understanding the intricacies of the scientific approaches. Some industry participants are technicians, some with only a high school education, making it difficult for them to take full advantage of the information offered.
- Success of extension depends on adoption of new technologies and changes in attitudes and practices. But, at present the science is still developing and there are no concrete examples in forestry; however, other crops do have successes with MAS and using these is another means of communicating its potential utility.
- For some target groups, there may also be an infrastructure problem, since some do not have the laboratories and equipment to adopt the technology. So the focus should be on changing attitudes toward the utility of the technology so that they will be receptive when applications of the technology are ready to be adopted.
- Activities of co-op directors should focus on continuing to expose their members to the information covered in CTGN presentations. Is this information being included in yearly reports, newsletters, web sites, listserves, etc.?
- CTGN might consider follow-up sessions with the co-op directors to determine if CTGN can provide additional information or help in encouraging continued educational activities for their members. Having directors comfortable with this information will be helpful in advance of having the products of the technology available.
- Annual reports of the co-ops should be linked to the CTGN website and vice versa in order to provide more continuity in informational exchange.
- A quarterly newsletter from the CTGN might keep target audiences connected to the project by providing backgrounds of CTGN participants, by describing successes of MAS in other crops, and by summarizing in lay language papers on topics of interest.
- The AFRI approach to extension relies heavily on eXtension and has become the mandated primary extension tool for the new CAPs; “older” CAPs are also strongly encouraged to adopt this approach. SolCAP has been given funding to coordinate eXtension activities of the CAPs and to prepare them for inclusion in the eXtension website. CTGN should contact SolCAP to determine how to coordinate posting of both education and extension resources on eXtension.
- Development of one or more fact sheets on marker assisted selection and breeding as a profession might be included in the U.S. Forest Service/UC-Davis:”Why We Care About Genetics” series. These could also be distributed following training sessions.

General Concerns

- The committee has some concerns about how CTGN will effectively carry on the education and extension program after David Harry leaves. Dave communicates very effectively with a broad educational range of clientele, brings much knowledge, enthusiasm, and commitment to the project, and thus will be very difficult to replace.

Communicate effectively to different target audiences at the appropriate level is not easy; care should be taken to identify an individual with a proven track record in this regard.

- The committee also suggests that a replacement be sought for Extension Advisory Board member, J.B. Jett, who has retired.
- Advisory Boards can provide useful feedback on direction and approach, but it is most useful to Conifer CAP if individuals are actively involved and attend meetings. Perhaps some members of the Advisory Boards, who are not able to actively participate, might be replaced with others who are more committed to assuming this responsibility.

Summary

Overall, the Education and Extension Advisory Boards feels that the CTGN has done a very good job in the education and extension arenas. CTGN benefits from having small and very well-defined education and extension target audiences and individuals committed to effectively delivering information to these groups.