

**Conifer Translational Genomics Network (CTGN – CAP)  
Quarterly Progress Report  
Year 1 / Quarter 4  
July 1 – September 30, 2008**

**General Progress / Administration**

The pace of activities on science-based objectives has significantly increased across the board. Components of the education and extension short-course, under development for years 2-4, were further refined and evaluated in an international workshop on genomics of adaptation in forest trees. A project brochure was completed, printed and distributed. Thirteen extension presentations were made by project personnel.

The first annual report was submitted in mid-July and subsequent requests for information were provided to CSREES personnel in August prior to approval of year two funding. Project Coordinator, Andrew Bower, resigned and took a position with the USFS July 1. It is anticipated that position will be filled, in parts, by two new hires, one of whom has already joined the team (Katie Tsang). Administrative duties are currently being handled by PD and co-PI's. An experienced, part-time PC is expected to join the team in January 09.

**Obj. 1: Validate previously discovered SNP by trait associations in Cooperative operational tree improvement populations.**

***North Carolina State University –***

Current Progress - A sample collection and shipping protocol was developed and sent to the Cooperative Tree Improvement Program members with a list of Loblolly pine genotypes to be sampled. Foliage sampling for DNA extraction and SNP markers is in full swing, and is about 25% complete. We identified 2555 loblolly pine selections that have progeny (full-sibs and/or half-sibs) or ramets in greenhouse and field trials with high-quality phenotypic data. When relatives of these selections are included, a total of 2798 selections have been identified for genotyping. Samples are being processed (put into vials with desiccants), sorted and labeled, and shipped to UC Davis in multiples of 96 samples.

Collection of wood cores (12mm) from replicated 9-year-old clonal trials of 170 clones commenced in September. Relative amounts of lignin and cellulose, the wood density, and other wood properties that are known to affect pulp yield and quality for papermaking will be determined and a subset of 20 different clones with a range of wood properties will be chosen. This subset of clones will be evaluated for more detailed analysis of processing efficiency for biofuel conversion, using different pulping processes and conversion methods (in collaboration with colleagues in the NCSU Wood and Paper Science Department). Foliage from the complete set of 170 clones will be collected for genotyping and the phenotypic data included in the CTGN database.

Phenotyping is now complete on the Genetics X Culture study (wood quality, growth, stem quality, rust incidence) which will provide additional breeding values for use in association studies with SNP genotypes on parents important to our breeding program.

Genetic analyses for sawtimber potential are complete for the Lower Gulf Elite Population, and the manuscript is in the final stages for submission. Again, these phenotypes provide breeding values for parents important to our breeding program that are suitable for use in association studies with SNP genotypes.

A high performance MacPro (dual quad-core Xeon 2.8 GHz CPUs, 32 Gb RAM) was purchased for analysis of large datasets, using funds from a different project (not CTGN funds). SimuPOP software was installed to carry out simulations for SNPs assisted selection in loblolly pine breeding. In addition, a Linux 64-bit version of ASReml software has been licensed to run simulated data to estimate SNPs effect on phenotype and gene substitution effect.

Jaime Zapata started as a PhD student in August and is funded by the CTGN. Jaime is an experienced forester with no previous experience in genomics, but he is keenly interested in learning how to apply new molecular marker technology and statistical analysis methods to tree improvement. His dissertation research will emphasize quantitative analysis of phenotypes and association with SNP marker data.

Funda Öğüt is also a new PhD student in Tree Improvement. Funda's research will focus on modeling marker assisted breeding. Funda came to NC State partly because of our research in MAS/MAB and our work with CTGN. Funda is not funded by CTGN, but her research will have relevance to the program.

Anticipated Activities Yr2Q1 - We anticipate finishing tissue collection of another ~1800 genotypes across loblolly pine tree breeding populations managed by NCSU.

Sampling of wood cores from replicated clonal trials of loblolly pine will be repeated on two other test sites. The task of collecting wood cores is expected to be completed in September/October 2008. We are modifying our current protocol for NIR spectroscopy of wood samples to allow high-throughput assessment of wood quality traits using the NIR method. Preparing wood cores for NIR scanning and data collection is expected to continue until February 2009.

We plan to continue to work on the simulation project to explore the parameters that are likely to be important for application of SNP technologies to breeding programs. We will supervise a PhD student to test the effects of varying the effective population size, mating design, trait heritability, and distributions of quantitative trait locus characteristics to identify the degree to which SNP genotyping can contribute significant value to applied breeding programs.

### ***University of Florida –***

Current Progress - Phenotyping of the BC1 population continues with monthly assessments of height and status and an additional trait, frequency of insect attack (tip moth 80% loblolly, 20% BC1 and 0 slash). The use of photographic techniques to obtain estimates of above ground components of biomass continues and excellent progress has been made during the pilot study. Needle samples from the BC1 population have been sent to UC Davis for DNA extraction and genotyping and this concludes sampling of the UF populations for the loblolly pine effort.

Work continues on improvement of a newer and more efficient version of the Bayesian SNP analysis program with SNP genotype imputation. In cooperation with the UF Stats Department, we are working closely with a programmer to implement changes to the code including intelligent priors for SNPs, a user specified design matrix and a user specified covariance matrix. These changes will be validated using simulated data. Comparisons are also being made between results from Linear Mixed Models and the Bayesian program.

Expected Progress YR2Q1 - The BC1 and slash and loblolly progenitors in the High Springs trial will be phenotyped after growth cessation by MS student, Patricio Munoz, for above ground carbon partitioning, needle length, number of branches and pest resistance. An analysis of first season growth versus the morphological traits will seek to assign cause using path coefficients. If SNP genotypes are available on the BC1, Patricio will perform association tests against all the phenotypes.

Collection of wood cores for phenotyping of slash pine families will begin in this quarter.

Further progress is expected in comparing analytical techniques for SNP analysis of pedigreed pine populations. Analysis of simulated data should discern among analytical techniques and competing statistical models within techniques.

### ***Oregon State University –***

Current Progress - Wood quality data and foliage was collected from the Cascade Timber Consulting Mason Seed Orchard in collaboration with Bill Marshall (Cascade Timber Consulting). This orchard had 218 trees from 52 clones that were recently harvested. Foliage was for DNA isolation and SNP genotyping. Wood stiffness was measured on standing trees using the FAKOPP TreeSonic, and then remeasured on logs using the Fibre-gen HM200.

Similarly, wood quality data from the Roseburg Resources Lebanon Seed Orchard was collected, in collaboration with Sara Lipow (Roseburg Resources). Wood stiffness was estimated from acoustic velocity measured using the FAKOPP TreeSonic (547 standing trees from 59 clones). In the next quarter, we will collect increment cores and foliage, and then measure acoustic velocity on the felled logs using the HM200.

We continued developing the Tree Genome Simulator. Current functions include simulation of (1) various probability distributions; (2) QTL alleles and quantitative trait genotypes for the parental breeding population; (3) SNP haplotypes for QTL alleles (based on characteristics of user supplied haplotype samples); (4) genetic linkage maps; (5) open-pollinated genotypes and phenotypes of progeny in field tests; and (6) genotypes and phenotypes of ramets in clonal field tests. These functions operate using a graphic user interface (GUI).

In a related activity, Douglas-fir tissues were collected for EST sequencing via the DOE Joint Genome Institute Community Sequencing Program. Tissues were sent to CTGN Team Leader Jeff Dean at UGA. Tissues, which were collected from a single clonally-replicated genotype, included (1) terminal and lateral buds; (2) cambium; (3)

developing seeds from developing cones; (4) current year shoots, including needles, stem, and buds; and (5) current year stems (i.e., shoot excluding needles).

### ***Texas A&M University –***

#### ***UC Davis –***

Effort was focused on lab facility set-up and evaluation for large-scale, high-throughput DNA isolation of needle and megagametophyte tissue samples. All analytical tools were physically moved into a lab at a different locale better suited for this high-throughput DNA isolation protocol and for creation of a Stock Center to house needles and megagametophyte tissue samples and DNA that will be available for future distribution.

Many pieces of labware and equipment were either received or serviced according to the needs of the isolation and quantification protocol as determined by Andy Bower. Training for the liquid handling robot (epMotion 5075) was assigned to Katie Tsang (new laboratory personnel).

The isolation protocol using Invitrogen's Chargeswitch gDNA Plant extraction kit was performed manually to optimize DNA extraction results and has now been translated into an automated protocol to be carried out by the Eppendorf robot. Ideally, three 96-well plates can be run through the automated procedure in one day resulting in DNA isolation of 288 pine needle samples.

We have developed, and continue to optimize, a protocol for the shipping and receiving of pine tissue for storage and/or DNA extraction.

### **Obj. 2: Develop and economically evaluate new methods incorporating marker-assisted selection into conifer tree breeding programs.**

Information from TAMU will be included in the Yr2q1 progress report.

### **Obj. 3: Develop databases (TreeGenes) and web-based tools to facilitate all aspects of the CTGN project.**

#### ***UC Davis-***

Significant time and effort has been expended on developing project personnel. Elly Chen was hired as a programmer (grade I) to help with website and database management. Ben Figueroa was hired as a programmer (grade 3) to work as an interface developer and database administrator. Alex Voong (programmer, grade 1) left for another position at the University. Recruitment is underway for an additional programmer (grade 1) to assist with software pipelines and website management. Plone and website development for the CTGN project is complete. Files including reports, project status, and presentations continue to be updated on this platform. Public view documents are updated on the public website.

The extensive resequencing database and interface resource has been termed ***DiversiTree***. This interface provides full user access to genotypic and phenotypic data.

Database design and interface development are also underway for the genetic stock center (to serve Obj. 4). The database will support barcode scanned stocks as a method for maintaining inventory count as it is processed. Development will be tied into existing forest tree genetic resources including the TreeGenes database and the DiversiTree Re-sequencing database and interface.

**Obj. 4: Develop an international genetic stock center for conifers.**

***USFS- Southern Institute of Forest Genetics***

Rootstock inventories for 2009 grafting have been enhanced to include several sizes and ages of material (Table 1). Age 1 and 2 rootstocks in tubes or pots at this stage will provide a useful distribution of tree calipers for optimizing scion-rootstock size matching.

Table 1. Rootstock inventory (September 23, 2008) for loblolly pine grafting at the Harrison Experimental Forest, Saucier, Mississippi.

<b>Age (growing seasons)</b>	<b>Container Type</b>	<b>Number</b>
1	Tube	580
1	Pot	1100
2	Pot	400
<b>Total</b>		<b>2080</b>

***USFS – Pacific Northwest Forest Exp. Station***

*Douglas-fir* – The Douglas-fir graft-compatible root stock seedlings are growing in the greenhouse at Oregon State University. They will be moved out of the greenhouse this fall to shut them down and harden them off. The seedlings will need to be grown for another growing season before they are large enough for grafting. This fall we will do an inventory and probably sow a few more seeds in the winter to have extra seedlings for grafting should some of the first grafts fail.

***Obj. 5: Develop an education plan for undergraduate and graduate curriculum in genomics-based breeding for forest trees.***

Components of the education and extension short course, under construction for delivery in years 2-4, were further developed and evaluated in abbreviated formats in presentations given in two different international venues (see below). An early draft of a flyer announcing the upcoming short course was created. A final version is now being constructed. With the help of UC Davis staff, a website was created for the workshop at <http://www.pinegenome.org/ctgn/workshop.html>. Registration details and other information pertaining to the workshop will be posted at this website as further information becomes available.

In July, co-PIs Howe and Harry hosted a day-long session for a summer course entitled "Sustainability of Natural Resource Management in the Pacific Northwest" organized by Rick Meilan, of Purdue University. Other course co-sponsors include NCSU, and the Swedish University of Agricultural Sciences. Besides Meilan, instructors included 5 other faculty, including Prof. Mats Olsson from SLU. Course attendees included 14 students

from the US and 6 from the EU. Howe and Harry's task was to present an overview of how biotechnology, specifically genetics and genomics tools, can contribute to sustainable forest practices. Presentation materials included excerpts from materials being assembled for the CTGN workshop, supplemented by other materials. The visit included an afternoon field session hosted by Mike Albrecht and Sara Lipow at their Roseburg Resources facility.

The second international venue was through PD Neale's organization of an international workshop held in Italy in August (**Population, Quantitative and Comparative Genomics of Adaptation in Forest Trees' Centro di Ecologia Alpina, Monte Bondone, Trento, Italy, August 3-5, 2008**). The conference website (below) includes links to the various presentations (<http://conferences.cealp.eu/conferenceTimeTable.py?confId=4>). In addition to Neale, speakers included CTGN team members Wheeler, Eckert, and Wegryn.

Education and extension co-PIs began planning for a course to be offered at Oregon State University in the winter quarter, 2009. The course is being built around the materials now under development for the planned project short-courses. By presenting these materials to a broader audience of graduate and advanced undergraduate students, the co-PIs will evaluate and revise the materials in advance of the first offering of the shortcourse, planned for June, 2009.

*Synergistic activity.* In mid July, co-PIs Wheeler and Harry hosted high school teacher (and Kenan fellow) Harold Mackin on a three-day visit to Oregon State University. While technically a part of the ADEPT2 project, the visit included consultations with plant and animal breeders affiliated with the OSU campus, including the National Clonal Germplasm Repository (<http://www.ars-grin.gov/cor/>).

Expected Progress YR2Q1 - The upcoming quarter will be a busy time for the education team. We will be nailing down registration and other administrative processes for the upcoming shortcourse. As previously mentioned, a finalized flyer for the shortcourse will also be completed. Harry and Wheeler will meet with Howe in Corvallis during early October to plan out activities needed to complete these tasks. In October, Harry will present a lecture on population and evolutionary genetics to an introductory forest biology course at OSU.

***Obj. 6: Develop extension plans for continuing education in genomics-based breeding for practicing tree breeders and forest tree gene resource managers, and develop education and extension evaluation protocols for all activities.***

The fourth quarter presented the CTGN team with the first opportunity to integrate feedback from our education (Obj 5) and extension (Obj 6) activities. Feedback is being gathered in several ways. First, our advisory committees provided oral and written comments from our first annual meeting in Raleigh. In July, education and extension co-PIs reviewed and discussed these comments and have begun modifying content and activities as appropriate.

Also in July, project evaluator Michael Coe summarized input from questionnaires distributed to PNW coop members in late June. Feedback was generally positive, particularly in helping staff gain additional background information on marker applications and new genotyping technologies. Presentation materials and approaches are being tweaked to accommodate the feedback provided. Coe's summary of the feedback from the PNW coop meeting is appended at the end of this document.

One noteworthy observation concerns our ability to use presentation materials for multiple audiences. We were concerned that materials designed primarily for the planned workshops might not be appropriate as extension materials. While we obviously must remain mindful of our audience, participant feedback offered assurance that our overall approach and content is on target.

Finally, in addition to feedback on developed materials, Coe is continuing to collect baseline data for coop staff, members, and participants. These data are being assembled using a combination of surveys and interviews. Collection of baseline data began at our annual meeting in Raleigh, and has since continued with other Coop staff who did not attend. This process is being done using a combination of surveys and phone interviews. Collection of baseline information will be completed by the end of 2008.

Co-PI Harry served on the NRI's Animal Genome panel specifically to bolster expertise for integrated proposals. This was an opportunity to gain added insights as to how marker-breeding extension and education activities are being organized and executed in a distinct but closely related field.

Presentations with an education and/or extension component completed during the fourth quarter included the following:

<b>Activity</b>	<b>Presenter/ Author</b>	<b>Title</b>	<b>Date &amp; Location</b>	<b>Venue &amp; Audience</b>
Oral Presentation	Glenn Howe David Harry	Forest Genetics and Genomic Tools: Contributing to Sustainable Natural Resource Management in Pacific Northwest Forests	July 1, 2008, Corvallis, OR	Purdue-NCSU-SLU joint Summer Shortcourse "Sustainability of Natural Resource Management in the Pacific Northwest." OSU Visit. 20 US and EU students and 6 faculty.
Oral Presentation	Fikret Isik	Population Improvement in the Era of Varietal Forestry (emphasized CTGN component of the research)	July 22-25, 2008, Charleston, SC	IEG-40 Meeting Scientists and Forest Industry, International conference, 100+ attendees
Oral Presentation	Fikret Isik	Genetic Improvement of Wood Quality Traits in <i>Pinus taeda</i> (emphasized CTGN component of the research)	August 25-28, 2008. Quebec City, Canada	IUFRO-CTIA 2008 Joint Meeting. Adaptation, Breeding and Conservation in the Era of Forest Tree Genomics and Environmental Change Scientists and Forest Industry, International conference, 300+ attendees
Oral Presentation	Steve McKeand	Loblolly Pine: A Clear Choice for Biofuels Feedstock Production in the South (emphasized CTGN component of the research)	August 18, 2008 Bloomington, MN	Short Rotation Crops International Conference, 100+ attendees
Oral Presentation	Glenn Howe	Presenting the CTGN Project	September 17 Schroeder Seed Orchard, Oregon	Stimson Lumber Company Representatives
Oral Presentations	Nicholas Wheeler  David Neale  Andrew Eckert  Jill Wegrzyn  Andrew Eckert	Introduction to population and quantitative genetics and genetic markers Introduction to genetic and QTL mapping and association genetics Population structure and demography Resequencing and PineSap software Estimating diversity and divergence. DNASam software	August 3-5, Centro di Ecologia Alpina - Fondazione E. Mach, Trento, Italy	Beginning and advanced graduate students in forestry programs across Europe and the United States
Oral Presentation (Keynote)	David Neale	Genomic Discovery for Application in Tree Breeding and Forest	August 25 , Quebec, Canada	IUFRO / CTIA Symposium Adaptation, Breeding and Conservation in the Era of

address)		Health Management		Forest Tree Genomics and Environmental Change Attended by several hundred tree breeders, students, scientists.
Oral Presentation	Tom Byram and Nick Wheeler	The Promise and Unresolved Challenges of MAB in Southern Pine Tree Breeding Programs	August 25 , Quebec, Canada	IUFRO / CTIA Symposium Adaptation, Breeding and Conservation in the Era of Forest Tree Genomics and Environmental Change Attended by several hundred tree breeders, students, scientists.
Oral Presentation	David Neale	Forest Tree Translational Genomics	August 26-28, 2008	National Plant Genome Initiative Workshop University of California Irvine, CA
Oral Presentation	David Neale	???	September 30 – October 3, 2008	IPlant Collaborative-sponsored "Grand Challenge Workshop: Mechanistic Basis of Plant Adaptation" Biosphere 2. Oracle, Arizona.
Brochure	Extension and Education Team	Conifer Translational Genomics Network – CAP	Distributed in late Summer	To: CSREES, all committee members, team members.

#### Expected Progress YR2Q1 -

The NSCU Cooperative Tree Improvement Program is organizing two workshops on genetic data analysis using ASReml (<http://cnr.ncsu.edu/tip/news.htm>). The use of SNP marker data in genetic analyses will be a focus of the workshops. The first workshop will be held on October 6-10, 2008 focusing on advanced level phenotypic and genetic data analysis. The second workshop is being developed by Fikret Isik for beginners on December 8-9, 2008. We expect tree breeders, graduate students, and CTGN participants will attend the workshop to improve their skills to analyze complex data.

Nick Wheeler will attend the Stakeholders and Outreach Committee meeting of Arborea II, a large genomics project centered out of Laval University, Quebec, Canada. As a member of the Stakeholders committee, he will evaluate project activities as well as present a talk on the extension and education components of the CTGN project.

David Neale will give two talks in October:  
Ecological Genetics Symposium. Purdue University, West Lafayette, IN. October 5-8, 2008

A World of Uncertainty: Thresholds and Dilemmas in Ecology. Discovery Lecture Series. Purdue University, West Lafayette, IN. October 7, 2008.

Attachment: Feedback evaluation, as summarized by evaluator Michael Coe is attached here. This feedback is from the annual meeting of the PNWTIRC from June, 2008. To open the attachment, click on the icon below.



Feedback\_  
PNWTIRC-NWTIC.