PINEMAP Year 4 Progress Report 2
April 2015
Aim 3 (Genetics)

This is the final Aim progress report for year 4 (March 1, 2014-February 28, 2015). The information provided in these reports is used to track Aim-level outputs and outcomes over the course of the project and to fulfill NIFA reporting requirements via the annual continuation proposals and REEport progress report.

Please note that this is not a cumulative report. Rather, the purpose of this report is to gather information on progress since the previous progress report completed in September 2014.

To streamline this process, information reported in September 2014 is provided below, so you will simply need to review and update each section as necessary.

Please return the completed report to Grace no later than April 10

OUTCOMES/IMPACTS

Outcomes and ***Impacts*** are tangible results for stakeholders and society that the project has produced to advance on the societal challenge (e.g., ***changes in knowledge, actions, or conditions*** that result from project activities). Outcomes and impacts are similar, but impacts are typically longer-term; outcomes are used as a nearer-term proxy for impacts.

Describe how Aim-level activities, results, findings, techniques, or products contribute to project-level outcomes and impacts (e.g., changes in knowledge, actions, or conditions resulting from activities).

A narrative has been drafted below. Please modify or update as necessary.

Aim 3 activities will contribute to project-level outcomes and impacts by providing a genetic deployment tool necessary for stakeholders to make sound genetic deployment decisions under changing climatic conditions and by discovering genes and markers associated with important mitigation and adaptation traits that can be incorporated into future breeding programs. Aim 3 will work closely with Aims 1 and 2 to provide input and benefit from insights gleaned from the stand-level studies and modeling to make tree improvement more efficient. Simultaneously, knowledge of traits varying at the gene level should contribute to the ecophysiology studies.

OUTPUTS

Products

Products include published or in press peer-reviewed publications; other written materials such as white papers, research summaries, fact sheets, or popular press articles; audio or video products; etc.

The lists below summarize year 4 (March 1, 2014-February 28, 2015) products reported in the September 2014 Progress Report.

Please update as necessary and highlight in yellow any products added to the list for the April 2014 Progress Report.

Peer-reviewed publications

Koralewski, T.E., Brooks, J.E. and K.V. Krutovsky. 2014 Molecular evolution of drought tolerance and wood strength related candidate genes in loblolly pine (*Pinus taeda* L.). *Silvae Genetica* 63(1–2): 59-66

Koralewski, T.E., M. Mateos and K.V. Krutovsky. Conflicting genomic signals affect the phylogenetic inference in the southern pines from subsection *Australes* (genus *Pinus*, family Pinaceae). BMC Evolutionary Biology. In review.

Farjat, A.E., Isik, F., Reich, B.J., Whetten, R.W., McKeand, S.E. 2015. Modeling Climate Change Effects on the Height Growth of Loblolly Pine. Forest Science (in press, available online at http://www.ingentaconnect.com/content/saf/fs/pre-prints/content-forsci14075)

Theses/Dissertations

No new reported in Sept 2014 report (April-September 2014)

Other publications

No new reported in Sept 2014 report (April-September 2014)

Audio/video products

No new reported in Sept 2014 report (April-September 2014)

Events/Activities

Events/activities include presentations (oral and poster) given at meetings or conferences; workshops/trainings/courses conducted; and experiments/surveys/data collection conducted.

The table(s) below summarizes year 4 (March 1, 2014-February 28, 2015) events/activities reported in the September 2014 Progress Report.
Please update as necessary and highlight in yellow items added to the list for the April 2015 Progress Report.

Presentations

Author(s)/Presenter(s)	Title	Type	Date	Venue/Location
Byram, T.	Valuing the family tree: Open-pollinated vs. control-pollinated families	Presentation (Meeting)	April 25-26, 2014(Meeting)	2014 Four State Forestry Conference, Texarkana, TX
Casola, C.	How forest genomics at Texas A&M can impact the breeding program	Presentation (Meeting)	May 6-7, 2014	WGFTIP Contact Representatives Meeting, Jasper, TX
Casola, C.	Using genomics to study pine tree adaptation and phenotypic variation	Presentation (Meeting)	March 25-26, 2014	WGFTIP Executive Committee Meeting, Shreveport, LA
Farjat, A., F. Isik, S. McKeand, and R. Whetten	Modeling Growth Performance of Loblolly Pine Families Under Differing Climate Condition	Poster Presentation	May 14-16, 2014	PINEMAP Annual Meeting, Athens, GA

Author(s)/Presenter(s)	Title	Type	Date	Venue/Location
Gonzalez-Ibeas D., Martinez-Garcia P.J., Famula R., Loopstra C., Puryear J., Neale D., Wegrzyn J.	Survey of the sugar pine (Pinus lambertiana) transcriptome by deep sequencing.	Poster Presentation	January 2015	Plant and Animal Genome, San Diego, CA
Koralewski, T.E., H.-H. Wang, and T.D. Byram	In search of optimal strategies for delineating assisted migration guidelines as applied to loblolly pine families from the Western Gulf region of the USA	Poster Presentation	May 14-16, 2014	PINEMAP Annual Meeting, Athens, GA
Lu, M., K. Krutovsky, C.D. Nelson, T. Byram, and C. Loopstra	Phenotyping environmental adaptation and stress mitigation traits in the clonal loblolly pine association mapping population ADEPT2	Poster Presentation	May 14-16, 2014	PINEMAP Annual Meeting, Athens, GA
Lu,M., K.Krutovsky,C.D. Nelson, T. Byram, T. Koralewsky, and C. Loopstra	Genetics of Adaptation and Stress Mitigation Traits in the Clonal Loblolly Pine (Pinus taeda L.) Association Mapping Population	Poster Presentation	May 14-16,2014	PINEMAP Annual Meeting, Athens, GA
Lu M, Krutovsky K, Nelson CD, Koralewski T, Byram T, Loopstra C.	Association Research and Exome Capture in a natural population of Pinus taeda L.	Poster Presentation	December 2015	Texas A&M University, MEPS/Horticulture Fall Poster Symposium
Nelson CD, Abbott AG	Alternative approaches to tree breeding for no-analog physical and fiscal environments	Presentation (Meeting)	August 25 – 29, 2014	IUFRO Forest Tree Breeding Conference, Prague, Czech Republic
Paul R., Kuruganti S., Stevens K., Gonzalez-Ibeas D., Martinez-Garcia P.J., Liechty J., Vasquez-Gross H., Grau E., Loopstra C., Zimin A., Holtz-Morris A., Koriabine M., Yorke J.A., Crepeau M., Puiu D., Salzberg S., deJong P..J, Mockaitis K., Main D., Langley C.H., Neale D., Wegrzyn J.	Genome annotation and repeat sequence characterization in sugar pine (Pinus lambertiana)	Poster Presentation	January 10 – 15, 2015	Plant and Animal Genome, San Diego, CA
Taylor, E.	Impact of faster growth on a thinning decision support tool	Presentation (Meeting)	May 6-7, 2014	WGFTIP Contact Representatives Meeting, Jasper TX
Townsend, L. and R. Whetten	Identifying Genetic Variation in Loblolly Pine	Poster Presentation	May 14-16, 2014	PINEMAP Annual Meeting, Athens, GA
Vogel, J	Can silviculture and genetic deployment affect the sensitivity of managed pine stands to disturbance?	Presentation (Meeting)	May 6,-7, 2014	WGFTIP Contact Representatives Meeting, Jasper TX

Author(s)/Presenter(s)	Title	Type	Date	Venue/Location
Wegrzyn J., Stevens K., Paul R., Gonzalez-Ibeas D., Martinez-Garcia P.J., Liechty J., Vasquez-Gross H., Kuruganti S., Grau E., Loopstra C., Zimin A., Yorke J.A., Crepeau M., Puiu D., Holt C., Yandell M., Salzberg S., deJong P..J, Mockaitis K., Main D., Langley C.H., Neale D.	Sugar pine annotation	Oral Presentation	January 10 – 15, 2015	Plant and Animal Genome, San Diego, CA
Whetten, R.W.	Testing gene expression patterns as predictors of pine family performance	Poster Presentation	May 14-15, 2014	PINEMAP Annual Meeting, Athens, GA
Whetten, R.W, Lu, M., Krutovsky, K., Palle, S.R., Seeve, C.M., Loopstra, C.A.	Predictive Accuracy of Models Based on Multiple Genomic Attributes in Loblolly Pine (*Pinus taeda* L.)	Poster Presentation.	January 10 – 15, 2015	Plant and Animal Genome, San Diego, CA
Zhang, J., S.A. Gezan, and G.F. Peter	Integrating Climate and Genetic Effects of Loblolly Pine by Universal Response Functions	Poster Presentation	May 14-16, 2014	PINEMAP Annual Meeting, Athens, GA

Trainings, workshops, and courses

No new reported in Sept 2014 report (April-September 2014)

PROGRESS NARRATIVE

Please provide a *brief* summary of progress on each deliverable/task/input/output listed below. In many cases, a one sentence summary may suffice. If there is no progress update on an item, leave blank. Please highlight additions in yellow.

- Do not include any figures or tables, but please do include quantifiable measurements, if available (i.e., # of plots measured, # of samples, # of runs, # of people reached, etc.)

Genotyping

Task: Collect more phenotypic data on ADEPT2 (8/31/15)

Mengmeng Lu and PINEMAP intern Nathalie Reiley spent three weeks collecting tissue samples and data from the ADEPT2 clonal trial planted near Saucier MS in May and June, 2014. The phenotypic data available now include: specific leaf area, branch angle, crown width, total height, growth height, diameter, and number of fusiform rust galls. Measurements of carbon isotope discrimination and stomata density are underway on foliage samples; the data will be used to test hypotheses about the relationship of water use efficiency and growth under field conditions. These phenotypic measurements will be used in conjunction with genotypes obtained from the same cloned individuals to test for associations between genetic and phenotypic variation.

The Virginia Tech group is using an electrolyte leakage test to measure cold hardiness in the ADEPT2 population at both Gainesville, FL, and Saucier, MS. Lateral branch cuttings were collected in January 2015, and current year needles subsequently sectioned and frozen at three test temperatures. Conductivity of the resulting solution was measured as a proxy for freeze-induced cell death. Preliminary results from these assays show extensive genetic variation in freeze tolerance both within- and among-population, and a relationship between provenance latitude of origin and freeze damage (greater damage in the southern provenances comared with the north). These data will be employed to seek genotype-phenotype relationships using data generated by the Florida PINEMAP group (led by Gary Peter). As freeze tolerance (or lack thereof) is a primary determinant of the suitability of loblolly pine for a given site, these data will provide insights into possible expansion of the planting range of the species under climate change, and allow for the selection of both families and allelic combinations that are favorable for a given site under future predicted climatic regimes.

Dana Nelson and the USFS group at Saucier collected additional tissue samples and phenotypic measurements from the ADEPT2 clonal planting at the Harrison Experimental Forest. The tissue samples were collected to provide DNA for other researchers interested in analysis of this experimental population.

Task: Genotyping experiments underway at VT, NCSU, TAMU, and UF (8/31/15)

The Virginia Tech group used genotyping-by-sequencing to genotype the Plantation Selection Seed Source Study (PSSSS) trial located in Appomatox County, Virginia. Genotyping of this study is of interest due to its location at the northern edge of the current loblolly pine range, and will complement co-PI Whetten's sampling/genotyping of the same families planted in Georgia. DNA was extracted from 960 individuals, which were chosen to maximize overlap with families present at sites being genotyped by the NC State group. Libraries were prepared using a double-digest GBS approach with MspI (common cutter) and PstI (rare cutter). Samples were multiplexed with in-line barcodes, and are currently in the queue for 10 lanes on an Illumina HiSeq instrument at the Virginia Bioinformatics Institute.

The NC State group has begun an experiment to test the relative value of estimating genetic covariance separately for genetic variants that may affect gene function versus genetic variants that affect gene regulation. As a proxy for detecting variants that affect gene regulation, gene expression levels are to be measured in RNA samples isolated from whole three-month-old seedlings (above- and below-ground tissues combined), with the expectation that the genetic covariance among individual trees of gene regulation patterns at this age will be similar to that of trees at age six, when progeny tests are measured. The 42 parent trees used in this experiment are from the Lower Gulf Elite Population, a collaborative experiment among the Cooperative Forest Genetics Research Program at UF, the Cooperative Tree Improvement Program at NC State, and the Western Gulf Forest Tree Improvement Program of the Texas Forest Service. Field trials of progeny from a partial diallel mating design have been established and measured, so phenotypic data are in hand for use in genetic analyses to test the relative accuracy of statistical models using genetic covariance matrices based on pedigree, on genetic variants in gene coding regions, on covariance of gene expression patterns, or some combination of these. A second set of 40 parent trees with progeny test results in hand is also available, so results from the initial first experiment can be verified in a second population.

The group at Texas A&M has done exome capture on 384 ADEPT2 clones using approximately two million Roche Nimblegen baits covering 38,000 genes. The results from the first 20 trees were very promising, with approximately 3 million SNPs detected in regions sequenced at least five times in all 20 trees. DNA sequencing is complete or underway for all 384 clones, but the data have not yet been completely analyzed.

The group at Texas A&M is waiting for sequencing results from the first test of Nimblegen hybrid capture reagents, to confirm the utility of that method before moving forward with more genotyping of the ADEPT2 clonal population, and the group at Virginia Tech is waiting for sequencing results from GBS libraries prepared from the Appomattox County, Virginia test site of the PSSSS test series. NC State is preparing to grow seedlings from two sets of about 40 parents that were used in large partial-diallel mating designs to create about 250 full-sib families. The phenotypic data from those progeny tests are in hand, and the seedlings will be used for RNA-seq experiments to test the hypothesis that gene expression levels can be used to model genetic variation in family mean performance.

Task: Genotyping complete (sequencing done, data analysis on going) (5/31/15)
No progress reported in Sept 2014 report (April-September 2014)

Deliverable: Upload ADEPT2 phenotypes into TerraC (Date?)
No progress reported in Sept 2014 report (April-September 2014)

Task: Discover genetic variant data (SNPs in VCF files) (8/31/15)
No progress reported in Sept 2014 report (April-September 2014)

Deliverable: Upload genetic variant data into TreeGenes (8/31/15)
No progress reported in Sept 2014 report (April-September 2014)

Deliverable: Tree breeding tools available to breeding programs (2/28/16)
No progress reported in Sept 2014 report (April-September 2014)

Deliverable: Better adapted germplasm (2/28/16)
No progress reported in Sept 2014 report (April-September 2014)

Seed deployment tool for DSS

Deliverable: Version 1 Seed deployment tool (11/30/14)
North Carolina State University: Additional analysis of variation among families within seed sources, using maximum and minimum annual temperature and total precipitation as climate variables, shows that 40% to 60% of the variation in family performance relative to locally-adapted checklot families can be explained by three climate factors. The predictive power of these models will be tested by cross-validation to evaluate the stability of the models, and additional climate factors will be tested to assess which models have the best predictive power. If this method is successful with the PSSSS data, it can be extended to include other progeny test data to evaluate the approach across a more diverse set of germplasm from the current breeding population.

Texas A&M University has used a multinomial logit regression approach to develop a Categorical Universal Response Function (CURF) to delineate potential deployment zones for loblolly pine (*Pinus taeda* L.) using 15 year measurements from the Western Gulf Forest Tree Improvement Program (WGFTIP) Geographic Seed Source Study (GSSS). The CURF uses performance categories for the response variable, and the model assigns a probability score of a given seed source’s response falling into each of these categories. Minimum temperature of the coldest month, summer precipitation, and variation measures of these two metrics for both the seed source provenance and the test location were used as independent variables. Planted tree volume, accounting for both survival and growth (a proxy for timber yield) was used as the response variable. Even with this limited number of environmental input variables, model performance was good, with the AUC score ranging from 0.785 to 0.808, depending on the parameters included and the evaluation criteria. The models developed with the CURF approach can be readily implemented in a Decision Support System as they 1) suggest sets of adapted families from which foresters can choose based on local knowledge, 2) can be easily expanded to include other variables, and 3) can be applied to outputs from projected climate scenarios to extrapolate into the future.

Deliverable: Final seed deployment tool (2/28/15)
The AIM 3 Group will be working with the Deployment Decision Support Group to produce an interactive web application to produce 'heat-maps' showing historic and projected values for climate variables identified as important in our analysis

Version 1 of genetic deployment tool with provenance information (Goal Date?)
No progress reported in Sept 2014 report (April-September 2014)

Comment [2]:

Version 2 of genetic deployment tool with progeny information (Goal Date?)
No progress reported in Sept 2014 report (April-September 2014)

Determine appropriate genome reduction methods for genotyping by sequencing (Goal Date?)
North Carolina State University: The relatively low yield of marker loci from the double-digest GBS method should still be useful for construction of kinship matrices based on Identity-By-Descent using reference-genome-based haplotypes, and these matrices will be incorporated into genetic analysis of the PSSSS data to assess the added value obtained from more precise measures of relationships. We are working in collaboration with Trudy Mackay's research group in the Genetics Program at NC State to re-assess the potential value of RNA-seq measurements of gene expression as predictors of phenotypic variation, based on unpublished results they have recently obtained in Drosophila lines derived from wild populations. Independent analysis of public data on gene expression levels and phenotypic variation in the mouse Collaborative Cross has confirmed the observation that genetic variation in levels of gene expression can be used to train a statistical model with some power to predict phenotypic variation, so a pilot experiment to determine the scale and power of an RNA-seq experiment on the PSSSS population is being planned.

Texas A&M University: Genotyping using Nimblegen SeqCap EZ System with the exon annotation based probes is underway. 20 samples have been sequenced in two lanes using Hiseq-2500 as a test. 158million and 170million reads were produced, respectively. The reads are currently being analyzed.

New markers for genotypes in ADEPT2, CCLONES, and PSSSS populations (Goal Date?)
Virginia Tech is using GBS to genotype ~1000 progeny of the PSSSS trial at Appomattox, VA, which will complement ongoing work at NC State that involves genotyping the same families at more southern sites. This will allow for testing of predictions based on genome-wide data across two different points in the climate space in which loblolly pine is planted.

Phenotypes for adaptive traits in ADEPT2 & CCLONES (Goal Date?)
In collaboration with Dana Nelson (USFS), Virginia Tech researchers are measuring cold hardiness for the ADEPT2 population at Saucier, MS. We will begin with a preliminary test in late fall and assuming genetic variation in this trait exists, we will proceed to a full replicated test in winter.

Phenotypes growth and responsiveness to nutrition in ADEPT2 & CCLONES (Goal Date?)
No progress reported in Sept 2014 report (April-September 2014)

Phenotype wood density and lignin content of ADEPT2) (Goal Date?)
No progress reported in Sept 2014 report (April-September 2014)

Discover alleles associated with water relations (existing phenotypes, new markers) Goal Date?)
No progress reported in Sept 2014 report (April-September 2014)

Discover alleles associated with nitrogen responsiveness (existing phenotypes, new markers) (Goal Date?)
No progress reported in Sept 2014 report (April-September 2014)

Discover & validate alleles and genes associated with growth and adaptive traits in 3 populations (new markers) (Goal Date?)
No progress reported in Sept 2014 report (April-September 2014)

Molecular tools for accelerating tree improvement and deployment (Goal Date?)
No progress reported in Sept 2014 report (April-September 2014)

Develop new region-wide cross population for southwide field test (Goal Date?)
No progress reported in Sept 2014 report (April-September 2014)

BROAD IMPACTS

Provide a short narrative describing broad impacts (i.e., far-reaching and possibly unanticipated outcomes resulting from Aim work). Specifically, please highlight leveraged funds and/or partnerships with other projects/external collaborations.

The NCSU Cooperative Tree Improvement Program is also participating in another NIFA Coordinated Agricultural Project, the Integrated Biomass Supply System (IBSS) project funded at University of Tennessee-Knoxville under the bioenergy component of the CAP program. The IBSS research conducted by NCSU CTIP is focused on chemical and physical properties of loblolly pine biomass, with a strong focus on wood. These properties are relevant to PINEMAP objectives because they affect the market for southern pine forest products, including solid wood products, pulp and paper as well as potential bioenergy markets that may develop.

Close ties are maintained between PINEMAP researchers and the PineRefSeq CAP, also funded by the bioenergy component of the CAP program, to fully exploit the value of the draft loblolly pine genome sequence for the breeding and genetic analysis objectives of the IBSS and PINEMAP research efforts. Texas A&M University applied the results of the draft loblolly pine reference genome assembly and annotation from PineRefSeq team for designing the capture probes used in the Nimblegen hybrid-capture sequencing experiment now being analyzed. The SNPs discovered from the captured exome sequences will be a supplement for the current pool of molecular markers which could be used in screening for better-adapted genotypes.

The application of the multinomial logit regression approach to the development of environmental models to guide assisted migration appears to have broader application to other areas such as the ecophysiological modeling of invasive plant movement.

TRAINING

A CUMULATIVE list of all Aim 3 undergraduate and graduate students, postdocs, and technical/research personnel trained under this project and descriptions of their research focus and/or role in the project is provided below. Additions/ changes from the Sept 2014 progress report are highlighted in blue.
Please update as necessary and highlight in yellow any updates made for the April 2014 Progress Report.

Last name	First name	Position	University	Role
Bawa	Rajesh	Ph.D. Student	VT	Research focus: Conducting Phenotyping of adaptive traits (i.e., cold hardiness and bud phenology) in the loblolly pine progeny trials and working with universities across region to conduct genome-wide association mapping between these traits and the re-sequencing data.
Farjat	Alfredo	Ph.D. Student	NCSU	Research focus: Carrying out joint analysis of progeny test data and climate data for the Cooperative Tree Improvement Program.
Koralewski	Tomasz	Postdoc	TAMU	Building a location database for the progeny tests and the parents represented in those progeny tests that can be linked to climatic data, and performing URF analysis to optimize seedling deployment.
Lu	Mengmeng	Ph.D. Student	TAMU	Research focus: Evaluating efficiency of the Agilent SureSelect target gene enrichment system for Illumina GAII and HiSeq high-throughput sequencing instruments, based on capture probes designed using the Agilent eArray tool and the most recent and comprehensive unigene set consisting of 35,550 sequences representing individual genes expressed in loblolly pine.
Kohlway	Will	Undergraduate Research Assistant	NCSU	Working on pilot scale experiments to test genotyping-by-sequencing as a cost-effective means of genotyping the experimental populations. (Deleted in Sept 2014 report, but this is cumulative list)
Rusche	Ben	Undergraduate Research Assistant	NCSU	Working on pilot scale experiments to test genotyping-by-sequencing as a cost-effective means of genotyping the experimental populations. (Deleted in Sept 2014 report, but this is cumulative list)
Townsend	Laura	MS	NCSU	Research focus: Genetic variation in loblolly pine for adaptability to climate variables.
Zhang	Jianxing	Ph.D. Student	UF	Research focus: conducting analyses linking progeny test performance with climatic data and developing a database for the URF analysis to optimize seedling deployment.