Annual Report of the USDA National Clonal Germplasm Repository (NCGR), Davis, CA 2015

John E. Preece and Malli Aradhya
Research Leader & Geneticist, NCGR, USDA-ARS, Davis, CA 95616
Telephone: 530-752-6504 Fax 530-752-5974

INTRODUCTION

The National Clonal Germplasm Repository (NCGR) at Davis, receives, collects, preserves, evaluates, and distributes genetic resources of Mediterranean fruit and nut crops. These irreplaceable resources are maintained on a long-term basis to support domestic and international research efforts on germplasm enhancement, cultivar development, molecular biology, and other related research. The Repository operates in cooperation with the Plant Sciences, the Viticulture & Enology Departments, and Foundation Plant Services (FPS) at the University of California, Davis.

The National Arid Land Plant Genetic Resources Unit (NALPGRU) serves as an important germplasm regeneration center for other National Plant Germplasm System (NPGS) sites that have species and accessions that require long frost-free seasons or arid conditions for seed production or regeneration of vegetative propagules. A back up collection of *Corylus* is maintained for the National Clonal Germplasm Repository (NCGR), Corvallis, OR. In addition, the NALPGRU is the priority site for conservation of arid land plant species with potential as industrial crops. These genetic resources are acquired, conserved, characterized, and distributed to scientists worldwide. The NALPGRU is a worksite of the NCGR and is located at the USDA-ARS San Joaquin Valley Agricultural Sciences Center.

Permanent/Term Federal Staff at NCGR-Davis

John Preece, Research Leader
Malli Aradhya, Geneticist
Bernie Prins, Horticulturist (*Vitis*)
Carolyn DeBuse, Horticulturist (*Prunus*)
Jenny Smith, Biological Science Technician
Jeff Moersfelder, Greenhouse Manager
Howard Garrison, Field Manager
Salvador Rivas, Biological Science Technician
Mary Parker, Secretary (Program Support Assistant)
2 Biological Science Technicians (open)
Sergio De La Cruz, Pathways Technician
Kelly Liang, Pathways Technician
Da'mon Daniels, Pathways Technician
Debbie Dang, Pathways Technician

UC Affiliates – Assistant Specialists

Judy Yang

Graduate Student

Dianne Velasco

Visiting Scientists

Currently:
Ewelina Jacygrad, Poland, pistachio rootstocks (3 years)
Van Blume, Viet Nam, micropropagation of *Prunus* (at least 24 months)
Rodrigo Infante, Chile, *Prunus* and kiwifruit (12 months)
Sung-min Jung, South Korea (11 months)
Placido Volo, Italy, micropropagation of olives (9 months)

Staff at the NALPGRU, Parlier

Curator position vacant, Applications recently closed, interviews this summer
Jerry Serimian, Biological Science Technician
Karen Wells, Biological Science Technician
Carmen Padilla, Pathways Technician
Veronica Padilla, Pathways Technician

Personnel Changes

Two Biological Science Technician positions are open, Malli's lab manager and my tissue culture technician. It is expected that the Curator at the NALPGRU will be filled by the end of the FY.

Service

Distributions of NCGR germplasm are primarily winter collected, dormant cuttings or scionwood; although the NCGR also distributes leaves, summer cuttings, pollen, fruit and other plant parts as requested. Almost no seeds are distributed. Because dormant cuttings are primarily distributed, nearly all orders are shipped in late winter/early spring.

Each item shipped is 3-5 cuttings/item (accession) (Fig. 1). The number of items shipped has shown a steady increase since 2002, relating to increasing order size. There was an anomaly in FY 2012 because a group from UCDavis that had imaged tomato leaves, collected leaves from all of our *Vitis vinefera* vines, causing a spike in number of items shipped.

Most distributions (98.7%) are to domestic customers (Fig. 2). Of those domestic orders, 78.5% were to individuals, with the remainder shipped to federal and state agencies, colleges and universities, nonprofits, and commercial companies.

The 2015 orders are at an all-time high and have now tripled since 2011 (Fig 4). This overextended the resources of the NCGR and the State of California phytosanitary inspectors, resulting in the last orders being delivered in June, rather than March.

Therefore, the NCGR-Davis has adopted a new policy regarding to whom we will ship cuttings. Henceforth, we will ship to research and education entities when genetic diversity or genetic standards are a requirement. However, we will ship one large order each to not-for-profit horticultural groups, such as the California Rare Fruit Growers and the Hawaiian Tropical Fruit

Growers. That will shift the burden of the paperwork and sorting of orders to these groups and at the same time help them grow their membership. We expect that this will reduce our orders and shipping costs by up to 90%.

We are also truncating the time during which orders may be placed. This is to reduce redundancy and having shipping account numbers expire, this making ordering and processing more efficient. However, we will accept orders at any time from research and education entities.

The NCGR-Davis may be the first clonal repository to adopt this as a strict policy of only sending material to research and education entities; however, it brings the Repository in line with the National Seed Repositories. They were overwhelmed with orders several years ago. We based our "rejection" letter on those from the seed genebank in Geneva, NY and the NCGR-Corvallis.

We are also letting our customers know about other alternatives, such as the website called Plant Information Online that is operated by the University of Minnesota (http://plantinfo.umn.edu/default.asp). This website allows a person to search for a wide variety of cultivars, including many of our most popular. When the person clicks on a cultivar, it takes them to a list of nurseries who can provide plants.

For the second time this year, *Vitis vinefera* accessions that were propagated from virus indexed plants from the UCDavis Foundation Plant Services (FPS), and grown under protection in a micromesh screen house were distributed. We have no plant pathologist on staff, so do not advertise these 200 accessions as virus indexed. This amounted to ???% of our grape dormant cutting distributions this current fiscal year, compared to 22.6% last year. This is a silent upgrade to our distributions. With ancient clonal crops, viruses are often present in the propagules being distributed.

Figure 1. Total orders shipped from 2004 – 2014. The blue line is the total number of orders shipped and the green line is the total number of items shipped in these orders. There are 3-5 cuttings/item shipped.

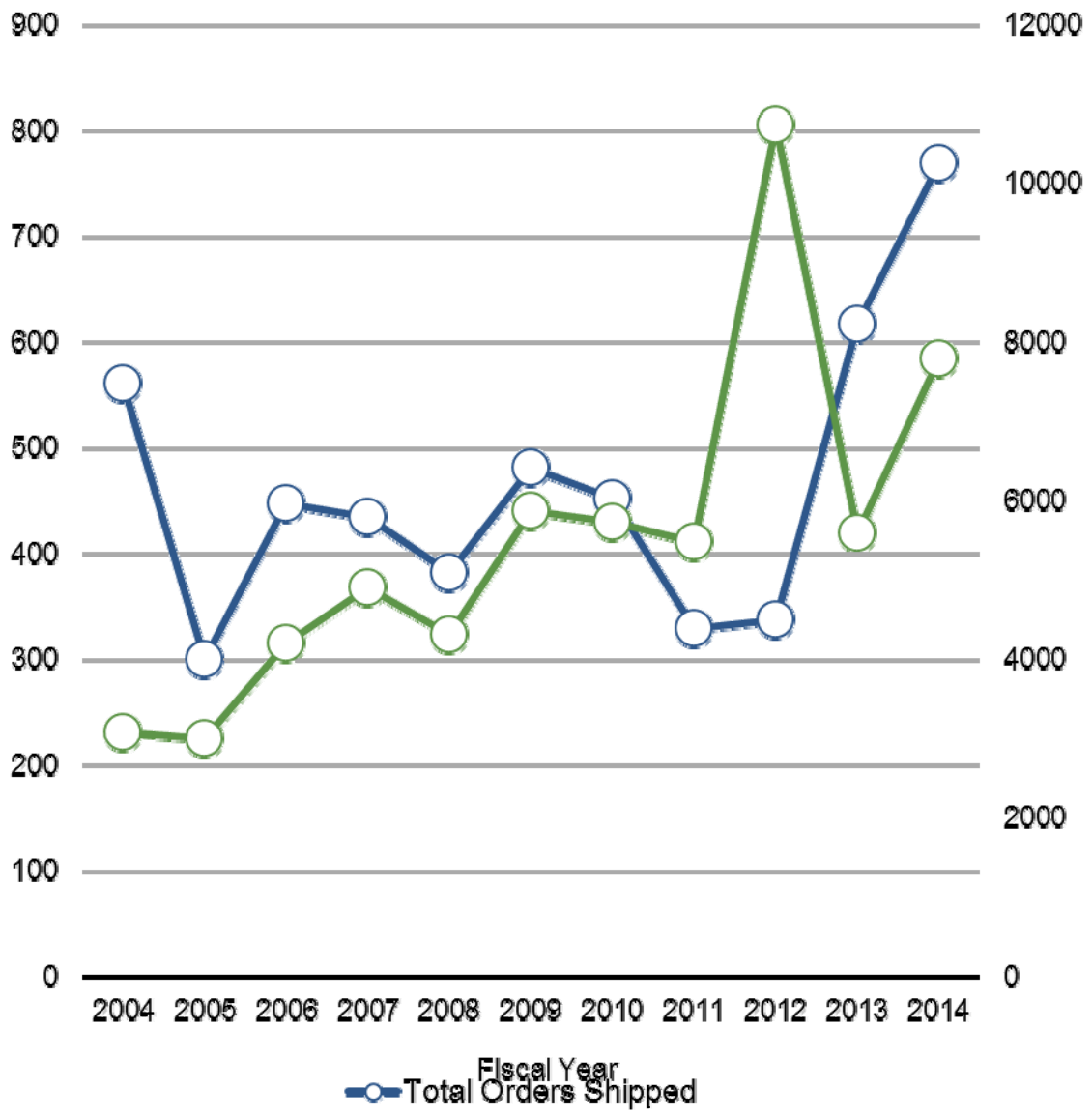


Figure 3. Domestic and international distributions during 2014.

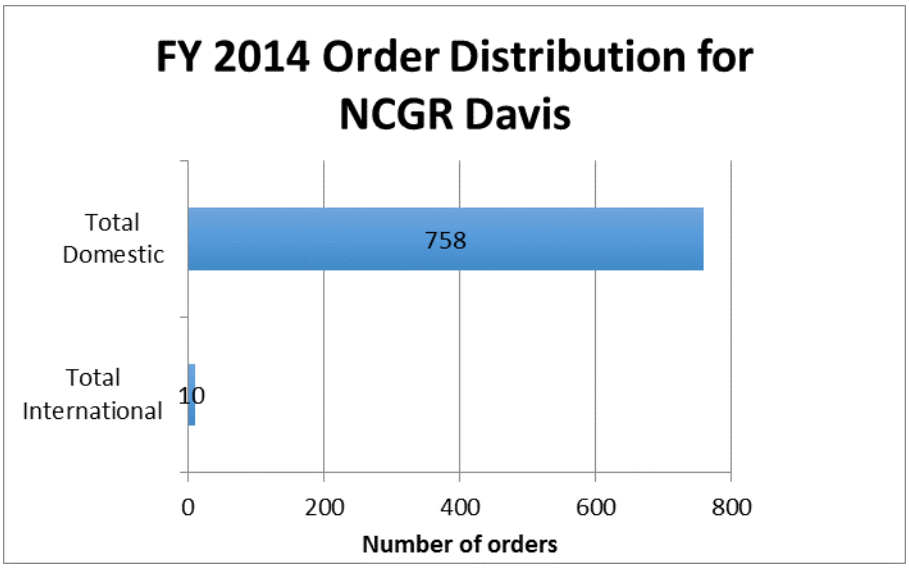
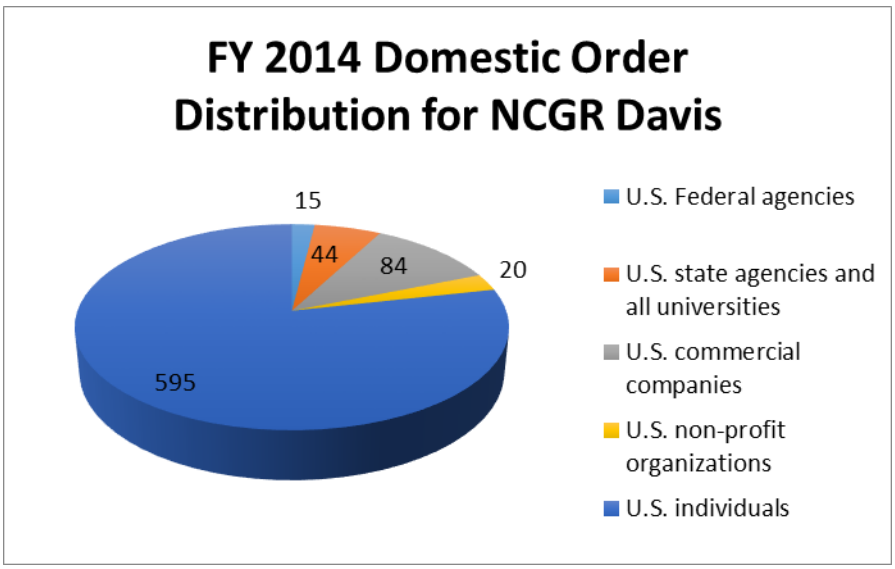


Figure 2. Distributions to domestic customers during 2014



The NALPGRU distributed 94 orders in 2014 and 42 so far during 2015. The numbers and accessions distributed are listed in Table 1.

The NCGR hosted 4 tasting events and The International Symposium on Energy and Protein Metabolism and Nutrition who had lunch at Wolfskill and a tour. Tours of the NCGR were provided to domestic and international individuals and groups.

Table 1. Number of accessions distributed by the NALPGRU by crop for 2013 - 2015.

Crop	# of accessions for 2013	# of accessions for 2014	# of accessions for 2015
Agave	7	15	12
Atriplex	91	501	10
Bassia	6	0	0
Opuntia	148	73	32
Cucurbita	45	38	5
Ephedra	23	0	1
Hesperaloe	2	4	0
Hesperoyucca	2	1	1
Limnanthes	30	15	76
Parthenium	328	207	114
Physaria/Paysonia	70	29	21
Proboscidea	2	0	0
Salicornia	6	4	5
Simmondsia	28	37	43
Yucca	16	16	4

Acquisitions

Plant exploration in the Republic Georgia 2014

Subtropical and temperate fruit and nut species germplasm with an emphasis on walnut (*Juglans regia*) and wild grape (*Vitis vinifera* ssp. *sylvestris*) were collected in the Republic of Georgia during 2014. A total of 153 accessions of wild and cultivated almond, wild *Prunus* spp. peach, apricot, and cherry, walnut (*J. regia*), and wine and table grapes were collected. All but *Prunus* spp. have arrived at the repository and are being propagated. The *Prunus* spp. germplasm accessions are in quarantine at the National Germplasm Quarantine Center, Beltsville and the grape accessions are in quarantine at the Foundation Plant Services, UC Davis.

New Acqusitions

From the Republic of Georgia, the NCGR received 91 seed accessions of *Juglans regia* and one of *Pterocarya* sp. collected in the wild from the country of Georgia. There was a total of 2200 seeds that underwent cold-moist stratification to meet their dormancy. At this writing, there are 774 plants (germinated seeds).

All international *Prunus* germplasm is received and quarantined by APHIS in Beltsville, MD. Cultivars and other clonal material receives thermotherapy and are micro shoot tip cultured (apical meristems and surrounding leaf primordia) and indexed for viruses, viroids, and other pathogens before being sent to the NCGR. This year, the NCGR received 232 new *Prunus*, representing 213 cultivars and 19 seedling families and a dozen taxa. Thirty-three of the accessions were from international sources and therefore, through APHIS; whereas the remainder were from northern California sources.

Seedling families were planted in the NCGR field nursery for genotyping and phenotyping to select the 5 seedling to put into the permanent collection.

In addition, during FY14, the NCGR has received 1 accession of *Ficus* as a cutting, 6 seed accessions of Georgian *Punica* (pomegranate), 1 *Actinidia* (kiwifruit) accession, and 5 *Juglans* seed accessions. The NALPGRU has 25 new accessions in FY14.

Collection maintenance and propagation

The NCGR is actively repropagating *Prunus* and other crops that are in peril or in need or renewal. During the past year, we budded 796 accessions, resulting in 3064 grafted rootstocks. Most of the rootstocks were clonal and donated to the NCGR by Duarte Nursery. Included in this was the repropagation of the entire apricot collection. The rootstock was older than desirable and grafting success was less than ideal, so repropagation of this crop will continue into 2016.

The University of California, Davis pulled out its peach cultivar block, which contained many valuable genotypes that were not in the collection, so we propagated 64 accessions, representing 8 species. In addition, because of the California drought, a private plant collector contacted us to propagate out of his collection before it died from no water. From him, we propagated 135 accessions representing 7 *Prunus*.

This year, focus is on repropagating weak or dying *Prunus* and 54 accessions have been budded as of this week. Also 55 new accessions (*P. salicina, P. persica* and *P. dulcis* hybrids) have been propagated which were donated by *Prunus* breeders from UC Davis, Dr. Tom Gradziel, and from USDA, Dr. David Ramming, and Dr. Craig Ledbetter. Again, Duarte Nursery donated *Prunus* rootstock.

The fig collection was partially repropagated and 174 trees have been planted at Wolfskill. Another 400 cuttings are being rooted. The additional trees will be planted where the old olives (repropagated 10 years ago) and figs are located. They will be removed and the land prepped for the new trees that may be fall or spring planted, depending on their size.

To produce quality scionwood, the walnut collection was hedged this year. Sierra Gold Nursery is grafting about 200 accessions for a cost/tree. This is the beginning of repropagating the entire *Juglans* collection. Land to plant the new trees is not currently available, however options are being explored, including negotiations the UCDavis for additional land, or moving a portion of the collection to the NALPGRU in Parlier since there is land available at the SJVASC.

Evaluation and Research

Grants focused on the collections at the NCGR-Davis: NIFA-SCRI, $2.9 million (Walnut Rootstock Development, 2014-2016); CDFA-SCRI, $129,000 (Olive Knot Evaluation, 2013-2014); California Fig Institute, $2,000 (Fig Cultivar Trial, 2015); USDA-ARS-NPGS, $14,000, (micropropagation *J. cathayensis* 2015-2016); California Pistachio Board, $291,000 (genotyping *P. atlantica* x *P. integerrima* UCB1 rootstock, 2014-2017) and $46,000 (developing molecular markers for Pistachio cultivars); and California Olive Committee, $22,500 (dwarfing rootstocks for olive, 2015), CDFA-SCRI, $221,000 (dried persimmon evaluations of many accessions, 2014-2016), California Almond Board, $300,000 (breeding disease-resistant almond rootstock, 2015-2017).

Non-genomics Research activities

Almond

Genes from crop wild relatives can more easily be incorporated into rootstocks than scions because nut quality is not a concern. As long as there is graft compatibility and the proper rootstock attributes, interspecific F_1 hybrids are used frequently for fruit and nut crops, such as commonly used peach x almond hybrids for almond production.

In an study designed to "mine" the collection for beneficial phenotypes, crosses were made with almond crop wild relatives (CWR) resulting in 190 hybrid genotypes from a set of peach x wild almond species and plum crosses. The goal is to develop disease resistant or tolerant rootstocks. The focus is on testing for susceptibility to *Agrobacterium tumefaciens* (crown gall) and *Phytophthora* root rot followed by genotyping using the genotyping-by-sequencing (GBS) approach. These form the basic genetic resources for association analysis to identify markers linked to disease resistance.

These interspecific hybrids continue to be grown and propagated by cuttings and micropropagation to produce plants for further replicated disease testing. More hybrids are being embryo rescued and micropropagated at a local commercial micropropagation laboratory to produce plants for replicated disease testing.

Olive

A field trial was planted in May, 2014 looking at 4 NCGR accessions for their potential as dwarfing rootstocks. Dwarfing rootstocks are needed for high density planting. We are testing *O. oblonga, O. cuspidata, and O. olea* 'Nitskaya' and 'Dwarf D.' 'Manzanillo' will be used as

the scion and there will be two controls, ‘Manzanillo’ on its own roots and ‘Manzanillo’ grafted to itself. ‘Sevillano’ is planted in border rows and serves as the pollinizer.

The entire olive collection has been and is being rooted as stem cuttings. These are being provided to Dan Kluepfel, ARS plant pathologist for inoculation with *Pseudomonas syringae* pv. *savastanoi* to access hypersensitivity and level of tolerance or resistance in the collection. There are wide differences in resistance among accessions, with some showing good promise.

Pistachio

P. atlantica and *P. integerrima* are wild relatives of the edible *P. vera* that are in the collection, and their F_1 hybrid, named ‘UCB1’ is a commonly used rootstock by the California pistachio industry. Working collaboratively with the UCDavis, Foundation Plant Services, 1000 UCB1 seedlings were planted on a research plot at Russell Ranch. A doctoral student from Poland, Ewelina Jacygrad phenotyped them at the end of the first (late 2013) and second (Jan. 2015) growing seasons, as well as summer suckering and pruning data. The California Pistachio Board funded a 3 year project where the phenotyping will continue and, working with the UCDavis Genomics Center, a genetic map is being constructed and markers being investigated for phenotypic traits.

Walnut

The walnut rootstock development NIFA SCRI is focused on the wild *Juglans* germplasm in the NCGR collection, specifically *J. microcarpa, J. ailantifolia, J. major, J. hindsii, and J. cathayensis.* The most promising seedlings for crown gall and Phytophthora resistance/tolerance are from *J. microcarpa* and for lesion nematode, *J. cathayensis*. Both open pollinated and seeds produced by controlled crosses were produced from the collection. This year, the most promising mother trees were crossed with *J. regia* to produce interspecific ‘Paradox’ seedlings for cloning and testing for disease resistance.

J. cathayensis has been difficult to clonally propagate on its own roots. Cuttings generally do not root and micropropagation is difficult and slow. Research is underway to improve micropropagation of this important source of genes for resistance to lesion nematode.

Cold storage of in vitro cultures and other in vitro studies

A plum/almond hybrid has established well in vitro and is being used to study the effects of refrigerated storage on health and survival of the microshoot cultures. The overall goal is to begin backing up the collections using in vitro and cold storage methods.

Studies are also underway on micropropagation of olive. Media and plant growth regulators are being optimized and different genotypes are being micropropagated. Some of the genotypes are the same as being used in the olive dwarfing rootstock study.

Genomics Research Activities

WALNUT (Juglans regia)

Biogeography and location of glacial refugia of walnut (*Juglans regia*)

Study of climatic oscillations during the Quaternary glacial period that shaped the distribution and genetic structure of extant trees offers insights into the evolutionary forces driving species distribution, diversification, and survival. Therefore, understanding the genetic consequences of past climatic changes is critical for the genetic conservation, management, and sustainable utilization of tree genetic resources and to address the imminent threats of climate change. Modern distribution of walnut within the section Juglans of the family Juglandaceae probably represent post glacial expansion, colonization, and cultivation comprising the diversity resulting from complex interactions of evolutionary forces, human selection and domestication. Palynological data indicated that walnut populations were extirpated from Eastern Europe to southwestern Turkey at the end of the Last Glacial Maximum. Nevertheless, small isolated populations probably survived in glacial refugia in the western Mediterranean, Black sea (Euxinian vegetation) and the Caspian (Hyrcanian vegetation) regions as far east as the Balkans and up north into the Baltic and Carpathian regions and westward in southern Italy and in the southern Iberian peninsula. Post-glacial expansions from different refugia into higher latitudes probably occurred during the Holocene.

We used the pattern of distribution of genetic diversity and species niche modeling approaches to investigate the historical biogeography and to establish the location of the Pleistocene refugia, where walnut survived during the last glaciations. Our analysis of genetic structure and differentiation of a diverse walnut germplasm collection based on genetic polymorphism at 19 microsatellite loci revealed six major regional groups corresponding to the regions of historical walnut distribution in the world (Fig. 4). The pattern of distribution of genetic diversity within and among different regions suggests that the walnut from the southwest Asia, mainly from northern Afghanistan and North-West Frontier regions of Pakistan and northwestern Himalayas up to the warm sheltered coves of the southwestern Pamir harbors the greatest genetic diversity probably suggesting that the region protected walnut in small refugia during the Late Cenozoic glaciations. Presence of a large number of low frequency alleles unique to this population suggest that they are of recent origin and maintained by a dynamic mutation-selection balance in a highly heterogeneous environment with a great potential to generate genetic diversity. Expansion and contraction of walnut populations during interglacial periods probably resulted in further isolation of subpopulations within among regional groups as evidenced by significant deficiency of heterozygotes and inbreeding coefficients for all groups across loci contributing for moderate differentiation within groups. There is evidence in the principal coordinate analysis for gradual post glacial dispersal of walnut from the southwest Asian and the Caucasus regions to central Asia and Eastern Europe. The patterns of distribution of genetic variability within and among groups also suggest that the SW Asia, the highly variable region probably harbored the most glacial refugia contributed for further slow spread of walnut to neighboring regions of central Asia and Europe. This migration probably was supplemented by the human introductions during the Roman period. Mild genetic differentiation as indicated by the CA and PCA supports circulation of genetic variation within and among regional populations as a result of combined effect of gene flow and human mediated dispersal events. However, the Bayesian CA exhibited clear differentiation among groups with a small percentage

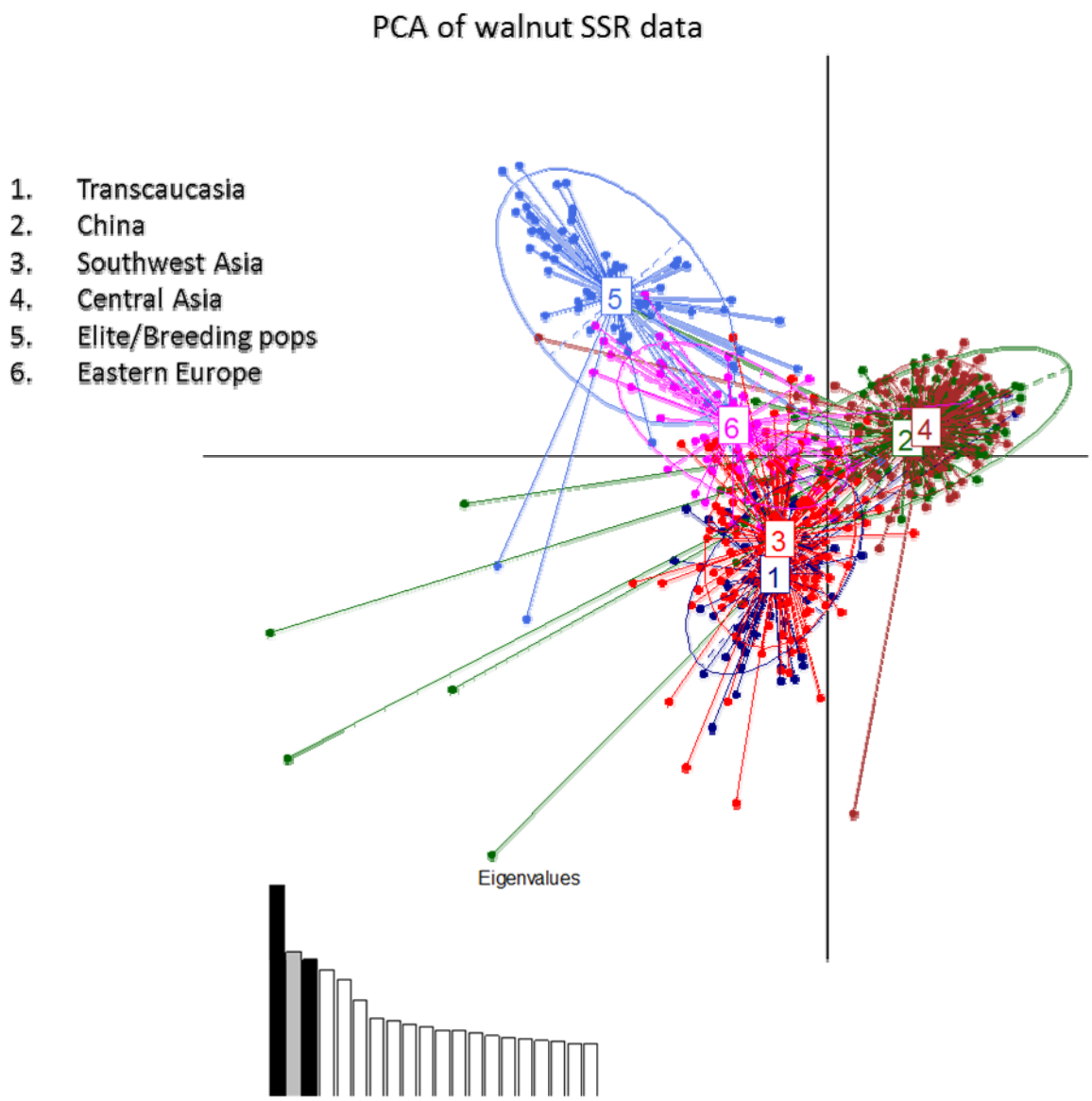


Figure 4. Genetic differentiation in cultivated walnut (Juglans regia)

of genotypes showing genetic admixture probably due to shared ancestral polymorphisms or recent dispersal mediated by human migration along the silk routes and gene flow between bordering populations. We are modeling the current distribution of walnut based on 19 bioclimatic variables with the hope that this model when implemented with LGM climatic variables will predict the location(s) of walnut refugia.

A walnut physical map reveals a whole-genome duplication, polyploidy-dysploidy cycles, and slow rates of genome evolution.

A bacterial artificial chromosome (BAC)-based physical map with embedded gene-derived BAC-end sequences (BES) can facilitate studies of genome structure and evolution, genome comparisons, and the development of a reference-quality genome sequence. In this study, we report the construction of a comparative physical map for walnut and analyze with it walnut genome structure and synteny with other Rosid genomes. We constructed a genetic map containing 1,524 single-nucleotide polymorphism markers. The linkage groups averaged 65.4 cM, and recombination rates were greatly reduced in the centromeric regions. We then fingerprinted 124,890 BAC clones, assembled contigs, anchored them on the genetic map, and constructed a minimal tiling path across them. We ordered 15,203 exonic BES along BAC contigs and quantified synteny of the physical maps with the grape, poplar, apple, cucumber, and Medicago truncatula pseudomolecules. We uncovered a whole-genome duplication (WGD) preceding radiation of Juglandaceae in Fagales and were able to allocate the 16 walnut chromosomes into 8 homoeologous pairs. We estimated the nucleotide substitution rate in the walnut lineage as 2.3×10^{-9} substitutions $year^{-1}$, which was 6.5 times slower than the rate reported for Arabidopsis but similar to that reported for poplar. We added one more WGD to the growing number dating to the Cretaceous-Tertiary boundary. We showed the rates of nucleotide substitution, synteny loss, the rate of dysploid chromosome number reduction during the polyploidy-dysploidy cycles to be slower in long-lived woody perennials than in short-lived herbs.

GRAPE (*Vitis* spp.)

Genetic diversity and differentiation within and between cultivated (*Vitis vinifere* ssp. *sativa*) and wild (*V. v.* ssp. *sylvestris*) grapes

In a collaborative project involving the NCGR, Davis, Viticulture-Enology Department, UC Davis, and Instituto Nacional de Investigacion y Technologis Agraria y Ailmentaria, Spain, we are examining the genetic consequences of domestication of cultivated grape and identification of domestication alleles in wild grape on a comprehensive sampling of wild and cultivated grape species. This project included 1525 accessions of cultivated and wild grape accessions representing 12 geographic sources (five cultivated and seven wild grape populations) across the native distribution of these species using 21 microsatellite loci. A manuscript is under preparation which describes the genetic diversity, and patterns of distribution of genetic diversity within and between the wild and cultivated grape and identify domestication alleles. There was considerable variation for the average number of alleles/locus among cultivated grape populations ranging from 12.7 for Georgia to 8.1 for western European. Western European cultivated grape generally recorded lower number of alleles except for Italian cultivated grape

which recoded 10.3 alleles/locus. The cultivated grape from Mediterranean and the Caucasus regions were more variable than western European grape. Overall, wild grape from Georgia were the most variable with an average of 13.25 alleles/locus as compared to wild grapes from the other Caucasus countries, Azerbaijan, Armenia. Surprisingly, wild grape from Italy and Croatia were also moderately variable. We suspect that some of the wild grape from Georgia, Croatia, and Italy may be hybrids containing alleles from the cultivated grape. Most populations exhibited inbreeding except for the ones from Spain and western European cultivated grape groups. There was significant differentiation among the 12 groups included in the study. The principal components analysis of the genotypic data indicated that the wild grape from Azerbaijan and Italy have diverged from the rest of the groups, which were somewhat less differentiated from each other (Fig. 5). Generally cultivated groups have diverged from the wild grape groups to various degrees reflecting on the domestication history and directional selection as a result of long history of selection for wine and table grapes in different grape growing regions of the world.

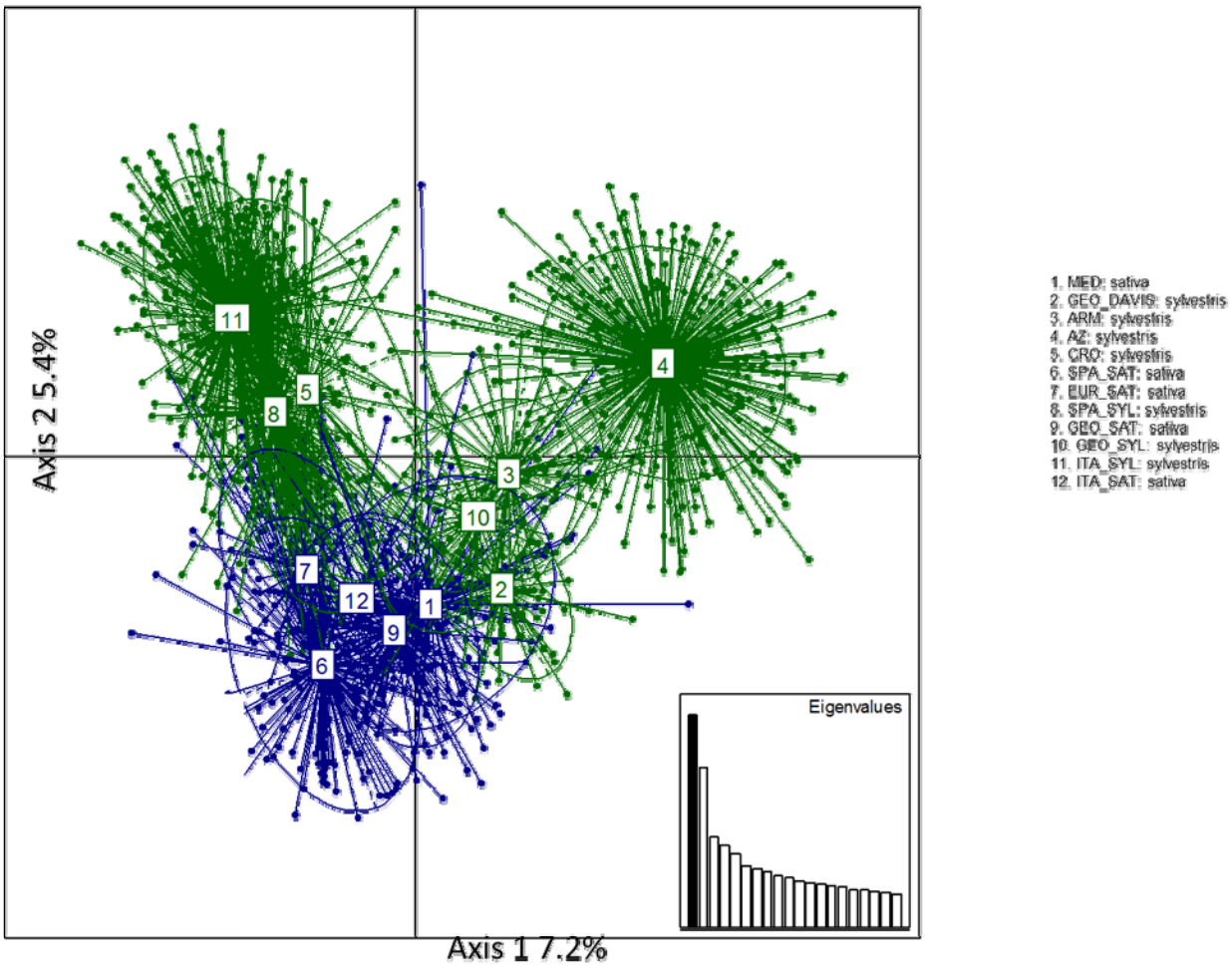


Figure 5. Genetic divergence within and between cultivated (Blue: Vitis vinifera ssp. sativa) and wild (Green: *V. v.* ssp. *sylvestris*) grape.

STONEFRUITS (*Prunus* spp.)

Apricot germplasm evaluation - As a part of ISTC Project K-1920 on the Estimation of apricot and pear genetic diversity and germplasm preservation in Kazakhstan, ~148 genotypes representing 6 populations of apricots from three distinct regions, Koturbulak, Urukty, and Ketman were genotyped for 12 microsatellite loci. The data has been subjected to distance based cluster and principal components analyses to visualize the genetic structure and differentiation. The results should permit identification of diverse apricot populations for long-term in situ conservation. The results are being written up into a publication.

This data set has now been enlarged to include 21 microsatellite loci with two main objectives: (1) to analyze genetic diversity and differentiation in the collection and (2) to select diverse seedling individuals in various seed accessions received over time at the repository. These objectives contribute to the genetic characterization and effective management of collections.

Caspian and the Central Asian Almond germplasm evaluation - A recently collected almond germplasm (~395 genotypes) from Georgia and Kyrgyzstan were assayed using 15 microsatellte loci. The data will be analyzed to assess the genetic diversity, population structure, and differentiation within and between the two countries located on either side of the Caspian Sea. The final goal is to select diverse genotypes within and among progenies for inclusion in the main almond collection block.

Cherry evaluation - As a training project in molecular marker technology, a visiting Chinese Scientist from the Xinjiang Agriculture and Forestry University, Xinjiang analyzed 152 cherry genotypes including some Chinese sour cherry DNA samples for 12 microsatellte loci. The visiting scientist had hands on training in PCR amplification, capillary electrophoresis and fragment analysis techniques. Further statistical treatment of data and interpretation of results were performed.

Development of genomic tools for almond rootstock improvement - *Prunus* germplasm utilization

There are three main objectives: (1) Develop molecular markers linked to disease-pest resistance for use in almond rootstock breeding programs, (2) Discover single nucleotide polymorphisms (SNPs) using genotyping-by-sequencing strategy, and (3) Genotype commercial and experimental rootstocks and newly produced genetically diverse interspecific hybrids. Development of improved rootstocks with durable resistance to soil borne diseases is a priority to the California almond industry. Currently used rootstocks such as 'Nemaguard' and other peach x almond hybrids are susceptible to one or more of these diseases affecting almond production in California. With restrictions on use of fumigants and to minimize environmental impact, the industry reliance on rootstocks with field resistance to soil borne pests and diseases is increasing. Host plant resistance is the most durable and sustainable form of protection against soil borne pests and pathogens.

In this project an attempt has been made to produce novel interspecific hybrids involving peach, wild almond species and diploid plums that are potential donors of resistance to soil borne diseases and in some cases drought tolerance. A two step approach was used: (1) produce and evaluate diverse interspecific hybrids for resistance to soil borne diseases; and (2) develop and identify SNP markers linked to resistance to develop effective juvenile selection strategies to rapidly develop improved rootstocks.

During 2014, ~ 1900 clonal plants from a set of 34 diverse interspecific hybrids involving *P. persica, P. argentia, P. tangutica, P. dulcis, P. bucharica, P. kuramica, P. davidiana,* and *P. kensuensis* have been produced for disease evaluation scheduled for Spring 2015. Meanwhile, genotyping-by-sequencing of 190 diverse, currently used, under field testing, and experimental hybrids has yielded ~221 million reads of which 18 million unique reads were aligned to the published peach genome sequence and, after filtering, 164,742 SNPs were assembled. Further SNPs with low LD and low representation across hybrids were filtered out to arrive at 7444 (7k) SNPs for the association analysis.

The association analyses performed following the mixed linear model implemented in TASSEL and PLINK software packages indicated association of several SNPs with crown gall (CG) with R^2 values ranging from 0.09 to 0.11, which is considered significant for complex traits such as disease resistance with low heritability. While these analyses are still preliminary, at this time, we have identified significant association of markers with CG, but the analyses failed to come up with any markers for *Phytophthora*, root knot or lesion nematode infestations. We suspect that lack of consistent and adequate disease testing data has resulted in failure to detect markers associated with disease resistance. We are further filtering the data set using a number of filtering criteria to eliminate leaky data among the SNP loci identified in the GBS analysis for further analyses.

Publications in 2014 and 2015 (NCGR staff bolded)

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