Report of the USDA National Clonal Germplasm Repository (NCGR), Davis, CA
To the W6 TAC
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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
Angela Medina, Biological Science Aid
Mary Parker, Secretary (Program Support Assistant)
Sergio De La Cruz, Pathways Technician
Anne Koehmstedt, Biological Science Technician (open)

UC Affiliates – Assistant Specialists

Judy Yang
Gloria (Patty) Diaz-Britz

Graduate Student

Dianne Velasco

Visiting Scientists

Currently:
Zhou Long, Xinjiang Agriculture University, micropropagation of *Prunus* (12 months)

Clearance received and will arrive in August, 2014:

Ewelina Jacygrad, Poland, pistachio rootstocks (3 years)
Thanh Pham Van, Viet Nam, micropropagation of *Prunus* (at least 12 months)
Carlos Trapero Ramírez, Spain, olives and other crops in the collection (5 months)
Rodrigo Infante, Chile, *Prunus* and kiwifruit (12 months)
Sung-min Jung, South Korea (1 month)
Youn Young Hur, South Korea (1 month)

Pending:
Placido Volo, Italy, micropropagation of olives (12-14 months)

Staff at the NALPGRU, Parlier

Curator position vacant, should be filled early 2015
Jerry Serimian, Biological Science Technician
Karen Wells, Biological Science Technician
Carmen Padilla, Pathways Technician
Veronica Padilla, Pathways Technician

Personnel Changes

Anne Koehmstedt's 4 year term position ended and is now vacant. Gabriela Romano (NALPGRU, Parlier), resigned.

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, however this year 5 lots each of 1000 walnut seeds were distributed as part of a NIFA-SCRI and 38 seed lots of Prunus species (*P. cerasifera, P. salicina, P. spinosa*) totaling 5700 seed for research at USDA Kerneysville, WV. The total number of orders has fluctuated since 2002 with the most during FY13, at 616 orders from a low in FY05 of 299 orders (Fig 1). So far during FY14, there are in excess of 750 orders.

Each item shipped is 3-5 cuttings/item (accession) (Fig. 2). 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.

For the first 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 22.6% of our grape dormant cutting distributions this current fiscal year, compared to 0% in the past. This is a silent upgrade to our distributions. With ancient clonal crops, viruses are often present in the propagules being distributed.

The NCGR is resuming distributing *Juglans* (walnut) germplasm. On advice from the *Juglans* CGC, beginning in 2010, walnut wood was not distributed because too little was understood about Thousand Cankers Disease. The size of scionwood that we distribute does not have sufficient caliper to be favored by the walnut twig beetle that spreads the fungus *Geosmithia morbida*. We have also worked with our phytosanitary inspectors and they are comfortable inspecting for twig beetle holes, therefore, will fill orders for *Juglans* in FY15.

Figure 1.

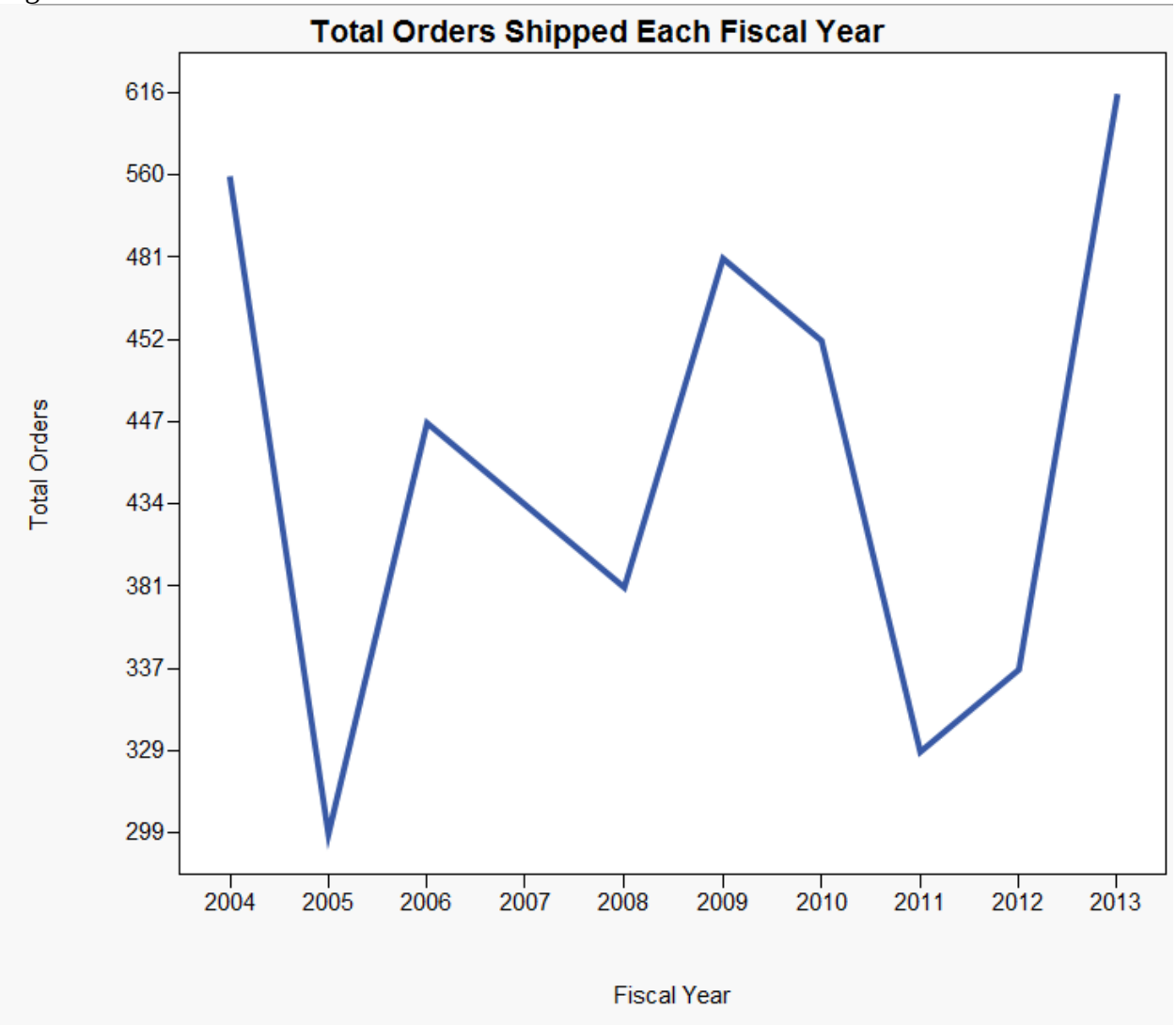
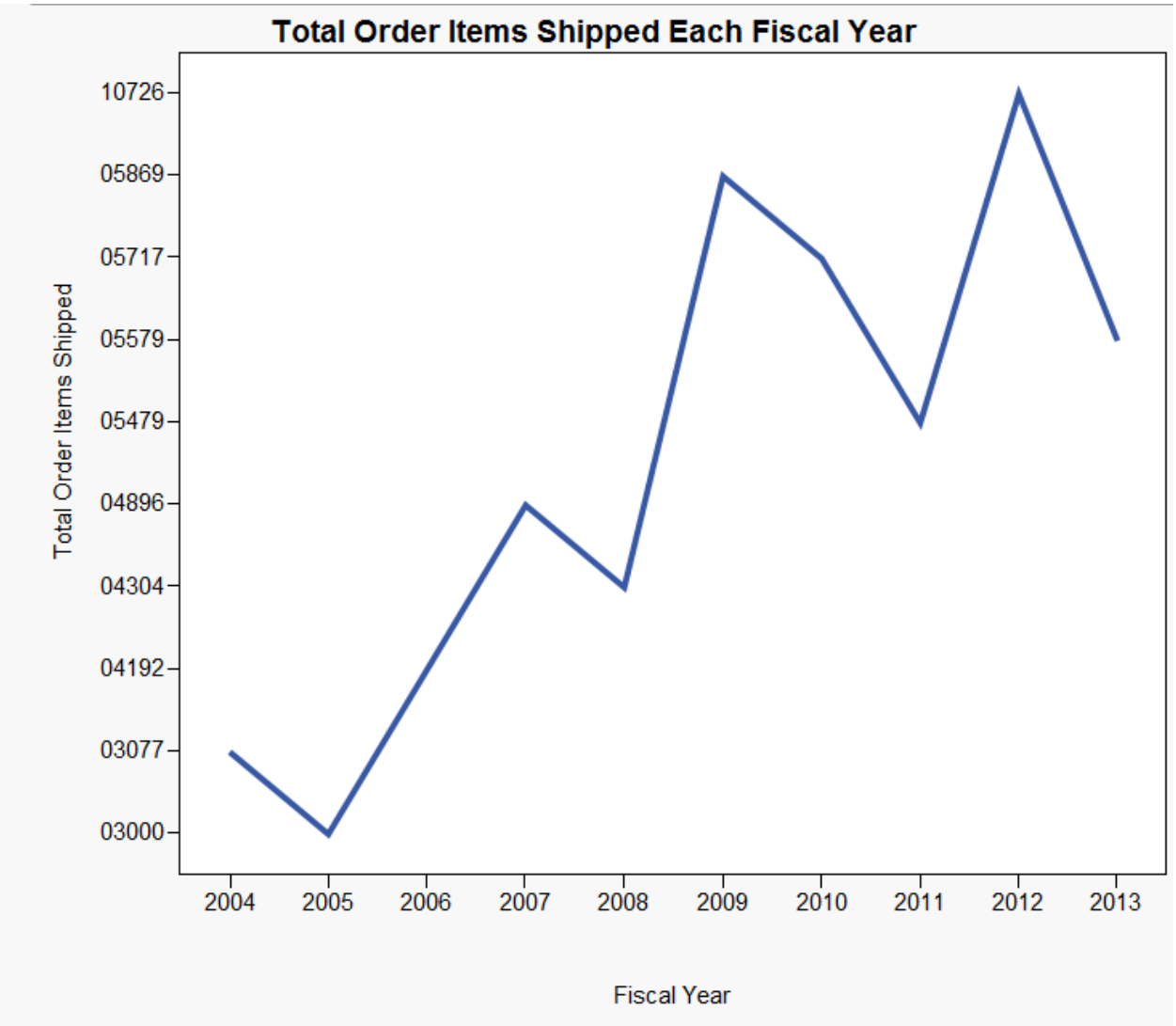


Figure 2.



The NALPGRU distributed 92 orders in 2013 and 73 so far during 2014. The numbers and accessions distributed are listed in Table 1. During 2014, the decommissioning of the *Atriplex*, *Bassia*, and *Proboscidea* collections was completed, therefore 483 *Atriplex* accessions distributed and thereby removed from the collection. Additionally, we stopped shipping *Bassia*, and *Proboscidea* in 2014.

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 and 2014.

Crop	# of accessions for 2013	# of accessions for 2014
Agave	7	4
Atriplex	91	483
Bassia	6	0
Cactus	148	97
Cucurbita	45	22
Ephedra	23	11
Hesperaloe	2	0
Hesperoyucca	2	1
Limnanthes	30	9
Parthenium	328	191
Physaria/Paysonia	70	22
Proboscidea	2	0
Salicornia	6	2
Simmondsia	28	33
Yucca	16	7

Acquisitions

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, plants of 14 new clonal apricot, plum or peach accessions were received from Armenia, Azerbaijan, China, Georgia, Spain, Taiwan, Thailand, Turkmenistan, or Ukraine. Because these are virus indexed trees, 2 copies each are being protected and grown in a micromesh screenhouse. In addition, 8 trees, representing 7 of these accessions have been planted in the field.

Bare root seedling *Prunus* was also received from APHIS and all 241 trees, representing 27 accessions from Georgia collected by Dr. Aradhya, species collected include *P. persica, P. dulcis, P. bucharica,* and a hybrid of *P. bucharica x spinosisium*. The seedlings were planted in the NCGR field nursery for genotyping and phenotyping to select the 5 seedling to put into the permanent collection.

In 2012, North American *Prunus* seed that had been collected in the eastern USA by Dario J Chavez, now University of Georgia, and Jose Chappero, University of Florida was received by the NCGR. These seeds were stratified and 36 seedling families of the following species are growing in the greenhouse: *P. hortulana, P. americana, P. rivularis, P. texana, P. angustifolia,*

P. umbellata, P. umbellata x geniculata, and *P. gracillis.* In addition, 3 *P. hortulana* seed lots were collected in Missouri by Jeffrey Carstens, USDA_ARS Plant introduction Station, Iowa, from which we have seedlings growing in the greenhouse.

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

To help the NCGR repropagate *Prunus* that is in peril or in need or renewal, in 2013, 1,850 Nemaguard peach rootstock trees, 50 Myro 29C rootstock trees, and 25 Citation interspecific peach & plum rootstock trees that had been micropropagated by Duarte Nursery were donated. Their value was $5775.00. This allowed for the repropagation of the peach collection, which included 286 clonal *P. persica* accessions, 217 *P. persica* seedling accessions and 46 related accessions. The new, budded trees were planted at Wolfskill this year. Additionally, other *Prunus* accessions that were weak or dying, were budded (13 species: 84 accessions).

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 repropagated and has been partly planted at Wolfskill. Ground in being prepared for the planting. This will also require removal of the old olive trees. They were repropagated nearly 10 years ago. The figs are being planted differently this time, with the Capri (male) figs with the wasps located closer to the north end of the Repository at Wolfskill and the cultivars toward the south end. This can better allow for evaluations and determining which accessions require caprification (pollination) and which do not (common figs).

Sierra Gold Nursery is grafting 23 of the walnut accessions onto clonal RX1 Paradox rootstock. This includes 12 *Juglans californica,* a species that is not growing well in the collection. Renewal pruning will be done to force higher quality scionwood from the weak walnuts.

The current focus is on renewal pruning for the collection at Wolfskill. Additional, temporary labor has been hired and a row of Paradox walnut rootstock (not accessions) that has been shading valuable accessions has been removed.

The NCGR is moving into using pressure bomb readings to schedule irrigations. This will save valuable water and the trees will grow better. We are working with Dr. Bruce Lampinen, UCDavis state almond and walnut specialist who is providing guidance on monitoring the collection.

Evaluation and Research

Grants focused on the collections at the NCGR-Davis: NIFA-SCRI, $1,1 million (Walnut Rootstock Development, 2013-2014); CDFA-SCRI, $129,000 (Olive Knot Evaluation, 2013-

2014); California Fig Institute, $3,500 (Fig Cultivar Trial, 2014); USDA-ARS-NPGS, $24,000, (tropical walnut evaluation, micropropagation 2013 – 2015); California Pistachio Board, $291,000 (genotyping *P. atlantica* x *P. integerrima* UCB1 rootstock); and California Olive Committee, $22,500 (dwarfing rootstocks for olive, 2014).

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. Among the initial 20 accessions tested, there are wide differences in hypersensitivity, judging by size of the olive knots.

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 growing season (late 2013). The California Pistachio Board recently funded a 3 year project where the phenotyping will continue and, working with the UCDavis Genomics Center, Ewelina is coming back to do genotyping by sequencing as a post-doc.

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 are being produced from the collection.

From the 2013 harvest from 56 accessions, 22,506 seeds were placed into stratification 9/11 or 9/17/2013. From 4/22-4/25, 10,554 seeds were sown and from 5/19-5/20, 4,964 seedlings were transplanted. Other seeds continue to germinate and will for another year or more.

Some of the mother trees produce abundant suckers for layering, and others produce none. Last year, Wes Hackett was successful with layering of two *J. microcarpa* in the field and others in the greenhouse. This year on the mother trees that did not sucker, a girdle was made half way around the circumference and Promalin (BA + GA_4-GA_7) was applied in 50% white latex paint to half of the wound and the other half was painted with just the 50% paint. Suckers on two of the wounded trees have been layered.

The tropical walnuts will not survive in the field in Winters, CA and have been maintained in pots. They were not moved to a tropical collection in the NPGS because the walnut breeding program is in Davis, CA. Work is underway to introduce the tropical walnuts into micropropagation as a method to propagate clones and to back up this valuable germplasm.

Genomics Research Activities

Almond

Genome resequencing of a dozen genotypes of wild *Prunus* spp. that have been used in the hybrid rootstock generation (see above, *P. dulcis, P. argentea, P. kansuensis, P. davidiana, P. bucharica, P. kuramica, P. arabica, P. persica,* and *P. fenzliana*) are being currently performed at the Beijing Genome Institute. The sequence data equivalent of 30x the average size of *Prunus* genome of the species received are being aligned with the reference peach genome sequences to discover SNPs.

Grape (*Vitis* spp.)

Genetic diversity, structure, and patterns of differentiation in the genus *Vitis*

Vitis (Vitaceae) is a taxonomically complicated genus with ca. 60 taxa divided into two subgenera, *Vitis* and *Muscadinia*. We used population genetic approaches to gain insights into the genetic diversity, patterns of evolutionary differentiation and to decipher the taxonomic status of some of the controversial taxa within the genus *Vitis*. The distance- and model-based analyses were used to examine the phylogenetic structure within the genus *Vitis* using simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers. The results closely matched the current classification, but some discrepancies in the identity of taxa at the specific and subspecific levels were still evident. The East Asian the North American *Vitis* exhibited strong divergence and each group showed further differentiation into several subgroups with North American subgroups roughly matching the described series. The model based cluster analysis indicated 14 clusters as optimum to explain the genetic structure within the genus *Vitis* with most clusters containing a moderate frequency of admixed genotypes suggesting interspecific gene flow within the subgenus *Vitis*. Hierarchical partitioning of molecular variation indicated that a significant amount of the total variation (~74% and ~69% for SSRs and AFLPs, respectively) is accounted for by intraspecific variation as compared to the levels due to genetic differentiation among species within series (~17% and ~20% for SSRs and AFLPs, respectively) and among series within the genus *Vitis* (~9% and ~10% for SSRs and AFLPs, respectively). Overall, *Vitis* possesses mild genetic structure characterized by reticulation and incomplete lineage sorting of ancestral polymorphisms.

Wild grape (*Vitis vinifera* ssp. *sylvestris*) genetic characterization

We have analyzed 350 wild grape seedlings from 10 accessions collected in Azerbaijan using a standard set of eight markers to identify diverse subset to be planted in field. In another collaborative project involving NCGR, Viticulture-Enology Department, UC Davis, and Instituto Nacional de Investigacion y Technologis Agraria y Ailmentaria, Spain, we are further examining the domestication of cultivated grape and identification of domestication alleles in wild grape on a comprehensive sampling of wild and cultivated grape species. In this project, currently we have assembled a dataset comprising 20 microsatellite loci for cultivated (*V. vinifera* ssp. *vinifera*) and wild grape (*V. v*. ssp. *sylvestris*) with a wide representation of geographic and climatic diversity. The aim of the project is to decipher the biogeographic and domestication history of cultivated grape and genetic and evolutionary consequences of domestication in terms of genetic diversity of cultivated grape.

Walnut (*Juglans* spp.)

Characterizing the walnut genome through analyses of BAC end sequences

To gain insight into the structure and evolution of the walnut genome, we constructed two bacterial artificial chromosome (BAC) libraries, containing a total of 129,024 clones, from in vitro-grown shoots of *J. regia* cv. Chandler using the HindIII and MboI cloning sites. A total of 48,218 highquality BAC end sequences (BESs) were generated, with an accumulated sequence length of 31.2 Mb, representing approximately 5.1% of the walnut genome. Analysis of repeat DNA content in BESs revealed that approximately 15.42% of the genome consists of known repetitive DNA, while walnut-unique repetitive DNA identified in this study constitutes 13.5% of the genome. Among the walnut unique repetitive DNA, Julia SINE and JrTRIM elements

represent the first identified walnut short interspersed element (SINE) and terminal-repeat retrotransposon in miniature (TRIM) element, respectively; both types of elements are abundant in the genome. As in other species, these SINEs and TRIM elements could be exploited for developing repeat DNA-based molecular markers in walnut. Simple sequence repeats (SSR) from BESs were analyzed and found to be more abundant in BESs than in expressed sequence tags. The density of SSR in the walnut genome analyzed was also slightly higher than that in poplar and papaya. Sequence analysis of BESs indicated that approximately 11.5% of the walnut genome represents a coding sequence. This study is an initial characterization of the walnut genome and provides the largest genomic resource currently available; as such, it will be a valuable tool in studies aimed at genetically improving walnut.

Genome-wide SNP discovery in walnut

A genome-wide set of single nucleotide polymorphisms (SNPs) is a valuable resource in genetic research and breeding and is usually developed by re-sequencing a genome. If a genome sequence is not available, an alternative strategy must be used. We previously reported the development of a pipeline (AGSNP) for genome-wide SNP discovery in coding sequences and other single-copy DNA without a complete genome sequence in self-pollinating (autogamous) plants. Here we updated this pipeline for SNP discovery in outcrossing (allogamous) species and demonstrated its efficacy in SNP discovery in walnut (*Juglans regia* L.).
The first step in the original implementation of the AGSNP pipeline was the construction of a reference sequence and the identification of single-copy sequences in it. To identify single-copy sequences, multiple genome equivalents of short SOLiD reads of another individual were mapped to shallow genome coverage of long Sanger or Roche 454 reads making up the reference sequence. The relative depth of SOLiD reads was used to filter out repeated sequences from single-copy sequences in the reference sequence. The second step was a search for SNPs between SOLiD reads and the reference sequence. Polymorphism within the mapped SOLiD reads would have precluded SNP discovery; hence both individuals had to be homozygous. The AGSNP pipeline was updated here for using SOLiD or other type of short reads of a heterozygous individual for these two principal steps. A total of 32.6X walnut genome equivalents of SOLiD reads of vegetatively propagated walnut scion cultivar ‘Chandler’ were mapped to 48,661 ‘Chandler’ bacterial artificial chromosome (BAC) end sequences (BESs) produced by Sanger sequencing during the construction of a walnut physical map. A total of 22,799 putative SNPs were initially identified. A total of 6,000 Infinium II type SNPs evenly distributed along the walnut physical map were selected for the construction of an Infinium BeadChip, which was used to genotype a walnut mapping population having ‘Chandler’ as one of the parents. Genotyping results were used to adjust the filtering parameters of the updated AGSNP pipeline. With the adjusted filtering criteria, 69.6% of SNPs discovered with the updated pipeline were real and could be mapped on the walnut genetic map. A total of 13,439 SNPs were discovered by BES re-sequencing. BESs harboring SNPs were in 677 FPC contigs covering 98% of the physical map of the walnut genome.

The updated AGSNP pipeline is a versatile SNP discovery tool for a high-throughput, genome-wide SNP discovery in both autogamous and allogamous species. With this pipeline, a large set of SNPs were identified in a single walnut cultivar.

Walnut genetic linkage map and QTL analysis of economic traits

Four hundred twenty-eight F_1 individuals from the mapping population ('Chandler' x 'Idaho') have been genotyped using the SNP chip developed previously. Only 45% of the markers were segregating normally in the test-cross (1:1) and F_2 (3:1) fashion without any distortions, among them only 2215 markers (37% of total markers assayed) were successfully mapped following regression mapping approach with Kosambi's mapping function into 16 linkage groups. The length of linkage groups ranged from 39 cM to 99 cM with total length of 1108 cM and number of markers ranged from 40 to 202. The largest gap in the map is about 15cM in linkage group 3 with 40 markers.

Although a number of economic (quantitative) traits segregating in the cross were also mapped using three different methods: (1) Kruskal-Wallis (K-W) analysis; (2) interval mapping (IM); and (3) multiple-QTL models (MQM), most of them accounted for low LOD scores and showed diffused patterns of LOD score distribution, sometime across the entire linkage group suggesting a complex genetic architecture and probably low heritability. However, detection of markers was possible through inclusion of cofactors in a series MQM scans to identify and localize markers with major effects. However, analysis of important traits such as lateral vs. terminal bearing habit, harvest date, some of the nut traits showed agreement between MQM and K-W analyses indicating the validity and utility of markers. Based on the QTL analyses, we have tagged several markers linked to lateral vs. terminal bearing, leafing, flowering, and harvesting dates. These markers are currently being validated in diverse genetic backgrounds. These results are in agreement with the association analysis performed on the NCGR germplasm data reinforcing the value of markers selected through QTL analysis.

Genetic diversity, structure and differentiation in cultivated walnut (*Juglans regia* L.)

We used 763 genotypes comprising 317 diverse accessions representing the modern range of distribution of walnut (*J. regia*) maintained at the National Clonal Germplasm Repository, USDA-ARS, Davis, California and a number of elite germplasm, breeding lines, and advanced selections from the California walnut breeding program to assess genetic structure and differentiation in walnut.

Nineteen microsatellite loci, WGA001, WGA004, WGA009, WGA069, WGA089, WGA106, WGA118, WGA178, WGA202, WGA223, WGA225, WGA237, WGA318, WGA321, WGA331, WGA332, WGA338, WGA349, and WGA384 were assayed using standard PCR methods. The walnut germplasm examined exhibited considerable polymorphism with observed number of alleles ranging from 8 for WGA089, WGA237, and WGA384 to 20 for WGA 202 with an overall mean of 12 alleles/locus (Table 1). The observed and expected levels of heterozygosity indicated significant deficiency of heterozygotes for all loci as compared to Hardy-Weinberg expected levels. The observed heterozygosity ranged from 0.327 for WGA225 to 0.650 for WGA009 with an overall mean of 0.501 and the fixation index, which indicates non-random assortment of alleles due to significant population sub-structuring, ranged from 0.148 for WGA009 to 0.354 for WGA331 with an overall mean of 0.285.

Multivariate genetic structure of walnut germplasm collection was elucidated using a neighbor-joining cluster analysis using a genetic distance matrix assembled based on Nei and Li distance and five major groups closely matching with the geographic affiliation of different walnut accession were identified. The Eastern European accessions from the Balkans, Carpathians, Russian, western European mainly French and the Albanian accessions showed close genetic affinity with the Elite germplasm and breeding lines from the California walnut breeding program. The Chinese and the Central Asian germplasm mainly from Kyrgyzstan formed two unique but closely allied groups. The South and West Asian germplasm from Afghanistan and neighboring Tajikistan, India, Nepal and Pakistan formed a loose conglomeration with at least six subgroups exhibiting subtle differentiation among them. Transcaucasian germpalsm from Azerbaijan and Georgia formed an exclusive group closely associated with the South and West Asian group.

Walnut germplasm examined presents considerable amount of genetic variation and the pattern of distribution of genetic variation indicate that nearly 87% of the total variation is common to all the geographic and genetic groups. The remaining 13% of the variation accounts for significant genetic differentiation. The results are suggestive of the South and West Asian broad regions probably represent the center of diversity and domestication of walnut.

Stonefruits (*Prunus* spp.)

We have over time assembled 16 loci microsatellite data for all *Prunus* spp. However, there are gaps in the datasets and for some species, we have up to 20 loci information. Overview of the data indicate that Plums form the center of the genus Prunus in terms of taxonomic and genetic diversity.

Peach (*P. persica*)

As a part of germplasm management, we have genotyped several progenies of *Prunus persica* and its wild relatives using 6 polymorphic microsatellite loci to identify genetically diverse subset of five individuals to be planted in a new peach block. We compared multilocus genotypes with the field observations on these seedling progenies to maximize both genotypic and phenotypic variation in the selected subset. Further, the entire Peach collection was genotyped for 15 microsatellite loci as a part of germplasm rejuvenation and management. We are in the process of adding five additional loci to make up to a total of 20 loci. The genotypic data will be used to analyze the amount and pattern of genetic diversity within the collection. We also performed similar analysis of almond (*P. dulcis*) progenies currently planted in nursery block for preliminary field observations of nut traits and other horticultural traits.

Apricot (*P. armeniaca*)

The apricot collection was sampled for leaf samples to extract DNA for genotyping using a similar set of microsatellite markers as that of peach. Again, this is part of routine germplasm rejuvenation and management at the repository, while contributing for the analysis of genetic diversity in the collection.

Other Species

Enrichment of the persimmon (*Diospyros* spp.) collection

As a part of enrichment of persimmon collection, we have undertaken genotyping of a wild persimmon (*Diospyros virginiana*) collection maintained by a private nurseryman in Indiana to identify genetically diverse trees. About 130 individual tree samples have been genotyped using microsatellite markers and compared with our in-house collections, which have been already characterized using 14 microsatellite loci. Genetically and phenotypically diverse accessions identified from this study will be added to NCGR collection to enrich the secondary gene pool of Persimmon.

Olives (*Olea europea*)

In collaboration with the visiting scientist from Spain, Dr. Pilar Rallo, we have genotyped 96 accessions of NCGR olive collection using a set of tetra- and hexa-nucleotide SSR loci.

Pomegranate (*Punica granatum*)

Two hundred thirty five accession of pomegranates have been genotyped for 15 microsatellite loci and data is being assembled for analysis of genetic diversity and population structure.

Jojoba (Simmondsia chinensis)

Jojoba is a dioecious plant grown for its oil rich seeds of industrial value. Early sex determination permits the NALPGRU to plant two male and two female plants of each accession, avoiding having the accession be represented by only one sex. Therefore, 120 *Simmondsia chinensis* genotypes were fingerprinted using two CAPs markers reported to be linked to sex locus. The marker loci were PCR amplified and restricted with restriction enzymes, HindIII and HinfI and the products run on a 2% Agarose gel to examine the banding patterns associated with the sex of plants. The assay was successful in determining the sex of Jojoba plants and is being used to guide our new plantings.

Publications in 2013 and 2014 (NCGR staff bolded)

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