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California Sycamore Hybridization Study

Project #4109-01

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Executive Summary

Introduction

California sycamore (*Platanus racemosa*) is an iconic native tree species in California and northern Baja California. California sycamore trees are generally found along intermittent streams and floodplains subject to high intensity flooding, and are the dominant trees comprising sycamore alluvial woodland habitat. However, California sycamore is facing many threats as a species. One threat is a lack of regeneration, particularly from seed. Another factor affecting California sycamore is hybridization with a common non-native landscaping tree, London plane (*Platanus ×hispanica*). Hybridization with non-native trees can dilute native genetics, lead to reduced fitness through outbreeding depression, and may threaten the existence of California sycamores as a species. California sycamore × London plane hybrids are known to occur in Santa Clara Valley. However, the amount of hybridization is generally unknown because discerning native California sycamore trees from hybrids cannot be done through visual observation and requires genetic identification.

Research conducted by Johnson et al. along the Sacramento River in northern California found that California sycamore × London plane hybrids were common. The origin of the hybrids along the Sacramento River is unknown, but it has been hypothesized that many hybrids may have been inadvertently installed as part of habitat restoration projects. Previous research led by H. T. Harvey and University of California at Davis (UC Davis) targeted the genetic identification of sycamore leaf material from a variety of size classes and locations in Santa Clara County to determine the degree of hybridization occurring and to assess if California sycamores could be identified based on a threshold size (as a proxy for age) based on when London plane first became reproductive and could fertilize California sycamore seeds. This work determined that hybrid sycamores were present in wildland settings; however, the extent, sources, and potential sizes of hybrid individuals were not clear. This uncertainty was attributed to the small sample size of hybrids identified and the difficulty in achieving consistent replication of the genetic identification method used in the previous study. However, the hybrids identified in the previous study were small and some of the hybrids were potentially installed as part of a California Department of Transportation mitigation project.

Habitat restoration and mitigation projects throughout California have typically used nursery container stock grown from wild-collected California sycamore seed because collecting and propagating seed is relatively easy compared to propagating from cuttings. However, wild-collected seed may have been fertilized with London plane pollen and may be resulting in the inadvertent outplanting of hybrid trees, as mentioned above.

It has been hypothesized that there may be a distance between California sycamores and London plane trees at which London plane pollen dispersal, and thus hybrid fertilization of California sycamore seed, is low enough that the likelihood of hybridization is considerably reduced. Seed trees at this “safe” distance would thus be good candidates for collection of uncontaminated seed for propagation purposes. However, the distance at which this certainty may be achieved has never been tested. If this safe distance can be identified it will greatly facilitate selection of trees as seed sources for nursery propagation and outplanting for restoration and mitigation projects.

This study was designed with the following objectives:

1. Assess the extent and commonness of hybrid trees in Santa Clara County using genetic testing, by focusing on urban settings where hybridization is hypothesized to be more likely due to abundant London plane tree populations. Based the results of that testing, identify if there is a pollen dispersal distance threshold beyond which plane trees are unlikely to contaminate native trees.
2. Test seed from confirmed native trees identified as part of this study and/or from the previous genetics study, to determine if sources of uncontaminated seed are readily available and also identify the degree of hybridization in the seed of native trees relative to varying distances from London plane trees.

A detailed study design was developed, which included sampling leaves and collecting seed from a total of 96 sycamores at varying distances from dense urban centers where London plane trees are more likely to occur. The initial leaf samples were provided to UC Davis for genetic identification following the same process used for the previous work completed on sycamore samples from Santa Clara County. After multiple efforts it was determined that the process failed to extract enough high quality DNA from the collected leaves for genetic identification. The project then shifted to identifying and bringing on another plant genetics laboratory that had more extensive experience. The project team generated a relationship with Genomeadvisors Inc., which leads plant genetics analyses for the Invasive Spartina Project, and brought them into this study to replace UC Davis and California State University, Chico. Following a meeting with the UC Davis geneticists and Genomeadvisors Inc., the project moved forward with the primary goal of developing an accurate and reproducible DNA identification process.

Therefore, the majority of grant funding was dedicated to developing genetic identification methods. Secondarily, the frequency of hybridization between California sycamore and London plane was tested to assess how common seed produced by genetically identified California sycamore trees is and if the prevalence of hybridization decreased with distance from urban centers with London plane trees. This study also determined the genetic identity of unknown trees to bolster the database of known California sycamore and hybrid trees in Santa Clara County.

Methods

Genomeadvisors Inc., a private plant genetics lab, followed a modern approach to develop a genetic identification method to identify California sycamores, London planes, and hybrids. Specifically, 10 genetic microsatellite markers were selected for use in genetic identification, and a workflow was developed for the genetic identification. The process included ordering labelled microsatellite markers (with fluorescent tags) and further refining the method to allow for multiplex polymerase chain reaction (multiplex PCR) in which multiple primers can amplify DNA sequences with the microsatellite markers simultaneously. Leaf collections that were previously identified by UC Davis were used to evaluate the genetic identification method and the results were verified using genemapper software. The computational program STRUCTURE subsequently assigned samples to the reference California sycamore or London plane populations, or categorized them as hybrids. Following successful verification of the testing method, individual trees and saplings propagated from seed (described below) were genetically identified using this approach.

Eight populations of putative California sycamore trees that were accessible and varied in distance from urban centers were selected throughout Santa Clara County, as described in the Sycamore Hybrid Genetics Study Design, previously produced by H. T. Harvey & Associates. The selected populations were located near Coyote Creek Golf Course, Henry Coe State Park–Hunting Hollow, Henry Coe State Park–North Fork Pacheco Creek, Little Alamitos Creek, Llagas Creek, Pacheco Creek, Uvas Canyon County Park, and Uvas Road. A thorough search for seed was conducted in each of the identified populations; however, seed was uncommon across all of the study populations. Seed was collected on December 18–20, from single trees at Coyote Creek Golf Course, Henry Coe State Park–North Fork Pacheco Creek, Little Alamitos Creek, and Pacheco Creek, and from two trees at Llagas Creek. Seed was collected from trees that were previously genetically identified by UC Davis and trees that were genetically identified as part of this study. The collected seeds were propagated at the Grassroots Ecology Nursery in the foothills of Palo Alto, California, and leaves from 80 seedlings (3–12 seedlings per seed tree) were collected on August 29, 2019. Leaf collection from wild trees occurred between August 14, 2019, and September 1, 2019, at Coyote Creek Golf Course, Henry Coe State Park–Hunting Hollow, Henry Coe State Park–North Fork Pacheco Creek, Little Alamitos Creek, Llagas Creek, Pacheco Creek, Uvas Canyon County Park, and Uvas Road. During leaf collection, the location of each tree sampled was mapped using a global positioning system (GPS) and the diameter at breast height (DBH) of each tree was measured using a DBH tape. Eighty leaf collections were made across the 8 populations. All sycamore leaf collections, including the leaves of seedlings propagated from the collected seed, were mailed to Genomeadvisors Inc. for genetic identification.

As stated in the study design and in order to not exceed the available finding, 96 samples were genetically identified in this study. Forty-eight of the samples were previously identified by UC Davis. These samples were used as reference standards for the rest of the study and allowed the genetic identification of the remaining unknown 52 samples. Because the number of unknown samples that could be genetically identified was reduced due the necessity of establishing the new genetic identification methods and reference samples, the seedling and leaf collections were ranked in priority for genetic identification. Identifying seedlings grown from seed collected across varying distances from urban centers in Santa Clara County was the highest priority, and at least three seedlings from each seed tree were identified to capture potential variation of fertilization within the same tree. If the seed was collected from a tree of unknown identity, seedlings were not genetically identified until it was confirmed that the seed tree was a California sycamore. Therefore, the second priority for genetic identification was identifying a subset of seed trees to determine whether their seedlings also merited genetic identification. The testing of young saplings that were presumably recruited from seed in wildland settings was given third priority, and identifying larger trees from which seed was not collected was the lowest priority. In accordance with these priorities, genetic identification was conducted for 39 seedlings grown from collected seed, 5 seed trees, 7 saplings, and 1 larger non-seed tree. This resulted in only identifying seed collected from 5 populations and leaves collected from 6 populations. All seedling and leaf collections that were not genetically identified are being stored at Genomeadvisors Inc. for future identification pending additional funding.

Results

Genetic Identification

Genomeadvisors Inc. successfully established an efficient and replicable method for identifying California sycamore, London plane, and hybrids. Genotyping of leaf collections from trees that were previously identified by UC Davis had a success rate of 92%; only 1 reference sample could not be genetically identified. The failed genetic identification was determined to be due to the sample's low quality genetic material and not attributable to the genetic identification methods since all microsatellite markers failed for this sample. Thus, the genetic identification methods was considered successful.

Seed Collection Identification

Of the 39 seedlings that were genetically identified, 29 were California sycamores, 7 were hybrids, and 3 could not be identified due to low quality genetic material. The hybrid seed was collected from Llagas Creek, Pacheco Creek, and Henry Coe State Park–North Fork Pacheco Creek. These locations span the complete range of distances from urban centers within Santa Clara County used in this study.

Leaf Collection Identification

Of the 13 genetically identified leaf collections (including saplings, seed trees, and the larger non-seed tree), 6 were California sycamores, 4 were hybrids, and 3 could not be identified due to low quality genetic material. Hybrids were located at Little Alamitos Creek, Lower Llagas, and Henry Coe State Park–North Fork Pacheco Creek, spanning the distance from urban centers in Santa Clara County. The hybrids were observed to have DBHs between less than 1 inch and 20 inches. However, it should be noted that no trees analyzed as part of this study had a DBH larger than 25 inches.

Of the 5 seed trees that were genetically identified, 3 were California sycamores and 2 were hybrids. The hybrid seed trees were from Little Alamitos Creek and Llagas Creek, both of which are near urban centers. Of the 7 saplings that were genetically identified, 3 were California sycamores, 1 was a hybrid, and 3 could not be identified due to low quality genetic material. The hybrid sapling was collected from Henry Coe State Park–North Fork Pacheco Creek which is the farthest population from urban centers in our study, although there may be London plane trees planted at private rural residences within 2 miles of the sapling location. The larger non-seed tree that was analyzed was identified as a hybrid and was collected from Llagas Creek which is near an urban center. One of the hybrid seed trees was also near this non-seed tree hybrid at Llagas Creek.

Key Findings

- An efficient and replicable method for identifying California sycamore, London plane, and hybrids was established.
- Hybrid seed was collected from genetically identified California sycamore trees from locations that cover the range of distances from urban centers, where London plane trees are common. Thus, it can be inferred that London plane pollen likely travels and successfully fertilizes California sycamore seeds over long distances and that there may not be a distance from urban centers that is safe for California seed collection without the use of genetic identification.
- A hybrid sapling was identified in a location where no habitat restoration or active planting has ever occurred. Thus, hybrids may be present in other locations where natural regeneration from seed is occurring.

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Section 1. Introduction

California sycamore (*Platanus racemosa*) is an iconic native tree species found in California and northern Baja California. California sycamore trees are generally found along intermittent streams and floodplains subject to high intensity flooding, and are the dominant trees comprising sycamore alluvial woodland habitat. Sycamore alluvial woodland is a rare and valuable habitat type defined as an open to moderately closed canopy, winter-deciduous, broad-leafed riparian woodland dominated by well-spaced California sycamores (Holland 1986). However, California sycamore, and thus sycamore alluvial woodland habitat is facing many threats.

One threat to California sycamore is a lack of regeneration, particularly from seed. California sycamore trees are often observed recruiting from basal sprouts or buried limbs, but this asexual reproduction lacks the benefit of increasing genetic diversity from recombining DNA that occurs with regeneration from seed. Genetic diversity supports the species' ability to adapt to changing environmental conditions. The reduction in regeneration from seed is likely caused by many factors including hydrologic modifications of creeks that have led to other altered abiotic and biotic conditions (San Francisco Estuary Institute-Aquatic Science Center 2018). For example, many rivers are dammed and have regulated water releases that decrease the number of high intensity floods. This means that there may be a lack of freshly deposited alluvium that is believed to be necessary for the recruitment of California sycamores. Additionally, with regulated and often perennial water flows, other riparian vegetation has become dominant which may increase competition with California sycamore seedlings and reduce their establishment success.

Another factor affecting California sycamores is hybridization with a common non-native landscaping tree, London plane (*Platanus ×hispanica*) (Johnson et al. 2016). Hybridization with non-native trees can dilute native genetics, lead to reduced fitness through outbreeding depression, and may threaten the existence of California sycamores as a species (Anttila et al. 1998; Johnson et al. 2016). Additionally, while sycamore hybrids are not currently recognized by the California Invasive Plant Council as invasive (Cal-IPC 2020), hybridization between native and non-native species is a common evolutionary pathway that can lead to invasiveness of non-native species (Schierenbeck and Ellstrand 2009). Thus, knowing the extent to which hybrid California sycamore × London plane trees exist, and to what degree hybridization is currently occurring is of great benefit to conserving and restoring California sycamore populations and sycamore alluvial woodland habitat. However, the amount of hybridization is generally unknown because discerning native California sycamore trees from hybrids cannot be done through visual observation and requires genetic identification.

California sycamore × London plane hybrids were found to be common along the Sacramento River in northern California (Johnson et al. 2016). The origin of the hybrids along the Sacramento River is unknown, but it has been hypothesized that many hybrids may have been inadvertently installed as part of habitat restoration projects. Previous research led by H. T. Harvey and University of California at Davis (UC Davis) targeted the identification of leaf material from a variety of locations and size classes of sycamores to determine the degree of hybridization occurring in Santa Clara County and to assess if California sycamores could be identified based on a threshold size (as a proxy for age) based on when London plane first became reproductive and could fertilize California sycamore in Santa Clara County (H. T. Harvey & Associates et al. 2019). This work determined that hybrid sycamores were present in wildland settings. However, the extent, sources, and potential sizes of hybrid individuals were not clear. This uncertainty was attributed to the small sample size of identified hybrids and the difficulty in achieving consistent replication of the genetic identification method used in the previous study. However, the hybrids identified in the previous study were small and some of the hybrids were potentially installed as part of a California Department of Transportation mitigation project (H. T. Harvey & Associates et al. 2019).

Habitat restoration and mitigation projects throughout California have typically used nursery container stock grown from wild-collected California sycamore seed. However, wild-collected seed may have been fertilized with London plane pollen and result in the inadvertent outplanting of hybrid trees, as mentioned above. Propagating sycamores from cuttings of known California sycamore trees is a feasible solution for some projects, but generally has a lower success rate and does not come with the added benefit of recombining DNA and increasing genetic diversity that comes with propagating by seed. Thus, using wild-collected seed would be preferable to cuttings if genetic identification of nursery stock could easily be identified.

It has been hypothesized that there may be a distance that California sycamores exist from London plane trees at which London plane pollen dispersal is low enough that the likelihood of hybridization becomes considerably reduced and it may be considered safe to collect seed for propagation and outplanting. However, the distance at which this certainty may be achieved has never been tested. If it can be determined that hybrid seed is either absent or extremely limited from California sycamores at a particular distance from urban centers (i.e., where London planes are common), these trees could potentially be used as seed sources for nursery propagation and outplanting for restoration and mitigation projects.

The primary goal of this study was to develop an efficient and accurate genetic identification method that can be easily replicated and made available for ongoing California sycamore genetics research. Therefore, the majority of grant funding was dedicated to developing genetic identification methods. A secondary goal was to assess the frequency of hybridization between California sycamore and London plane by testing how common seed produced by genetically identified California sycamore trees is and if the prevalence of hybridization decreased with distance from urban centers with London plane trees. This study also determined the genetic status of unknown trees to bolster the database of known California sycamore and hybrid trees in Santa Clara County.

Section 2. Methods

2.1 Genetic Identification

Genomeadvisors Inc., a private plant genetics laboratory, followed a modern approach to develop a genetic identification method to identify California sycamores, London planes, and hybrids. Specifically, 10 genetic microsatellite markers (i.e., DNA loci) were selected for use in genetic identification, and a workflow was developed for genetic identification. The 10 microsatellite markers were the same as those used in a similar study based along the Sacramento River in northern California (Johnson et al. 2016). The 10 loci were plms29, plms53, plms71, plms92, plms109, plms113, plms122, plms130, plms136, and plms176. Once the PCR signal for each loci was strong enough for sequencing, one PCR product from each of the 10 loci was selected and sequenced using the Sanger method. Sanger sequencing included using both forward and reverse primers to confirm the identity of the microsatellite repeat. A microsatellite DNA repeat sequence was observed for all loci except the reverse and forward PCR product of plms_71. However, the genotyping for plms_71 was still successful during sequencing. The process was then further developed by ordering labelled microsatellite markers (with fluorescent tags) and further refining the method to allow for multiplex polymerase chain reaction (multiplex PCR) in which multiple primers can amplify DNA sequences with the microsatellite markers simultaneously. The microsatellite markers were then tested using genemapper software. After successful identification of the microsatellite markers following multiplex PCR, the computational program STRUCTURE version 2.3.4 (Pritchard et al. 2000) was used to assign the samples to reference California sycamore or London plane populations, or categorized them as hybrids. The program implements a model-based clustering method for inferring population structure using genotype data consisting of unlinked markers. The program assumes a model in which there are a specified number of populations, or the number of populations may be unknown. Additionally, the program assumes that the loci are at Hardy-Weinberg equilibrium and linkage equilibrium within populations. Each population was characterized by a set of allele frequencies at each locus. Sample individuals were probabilistically assigned to populations, or jointly to two or more populations if their genotypes indicate that they are hybrids. The program assigned identities to each sample based on the microsatellite data. This process was run for a 10,000 replication burn-in period and was then run for 100,000 replications. If the samples were identified as California sycamore or London plane in 90% or more of the replications, they were assigned to that population. If the sample had been identified as both California sycamore and London plane between 10% and 90% of the replications, the individual was classified as a hybrid.

2.2 Sample Collections

Eight populations of sycamore trees were identified throughout Santa Clara County. These locations were selected based on where California sycamore trees existed, were accessible and that varied in distance from urban centers, as described in the Sycamore Hybrid Genetics Study Design (H. T. Harvey & Associates 2018). These locations complement previous work where sycamores have been identified by increasing the sample size in some locations and adding additional locations. The selected populations were located near Coyote

Creek Golf Course, Henry Coe State Park–Hunting Hollow, Henry Coe State Park–North Fork Pacheco Creek, Little Alamitos Creek, Llagas Creek, Pacheco Creek, Uvas Canyon County Park, and Uvas Road (Figure 1).

2.2.1 Seed Collections

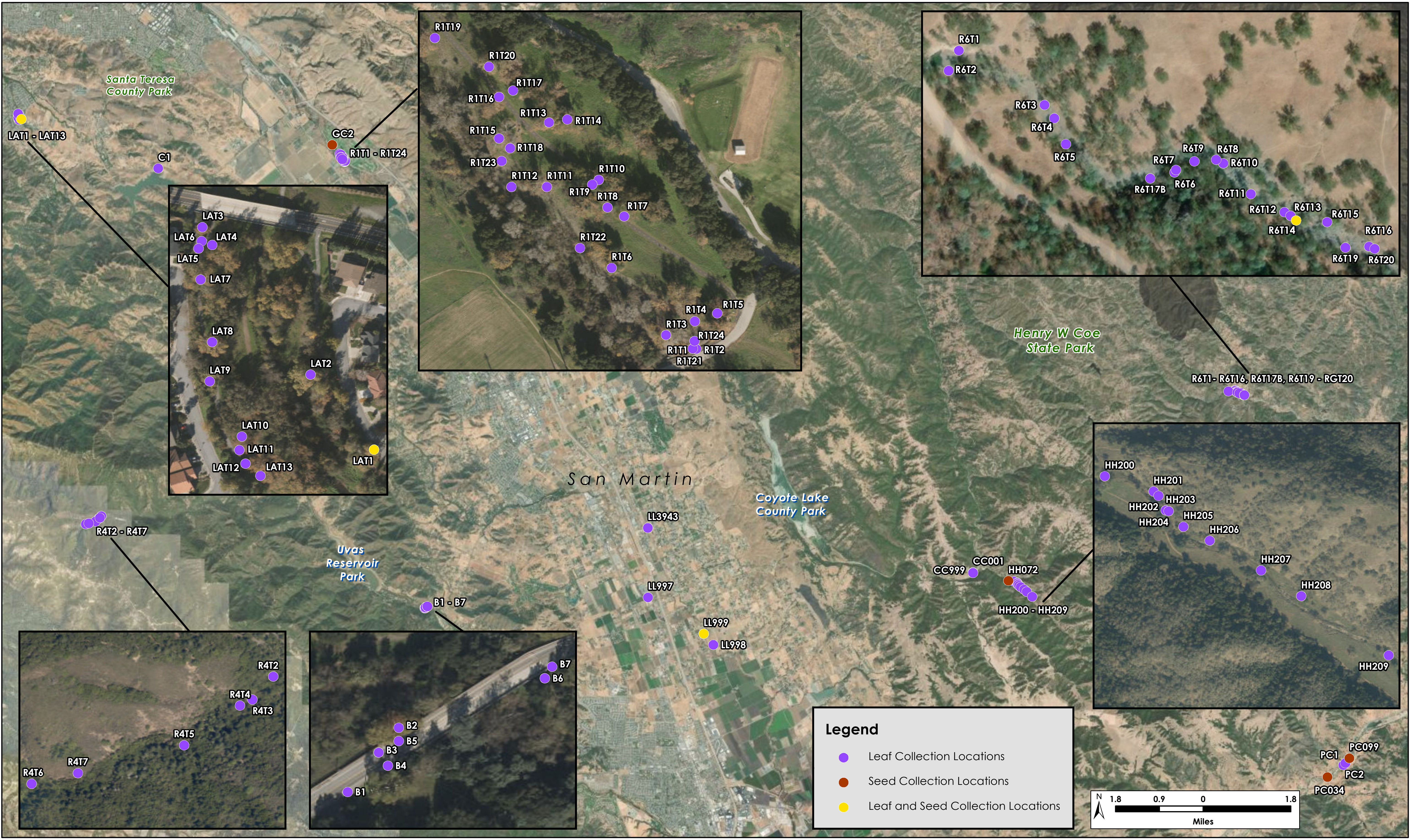
A thorough search for seed was conducted in each of the identified populations described above. Seed was uncommon across all of the study populations; only 6 seed trees were observed. Seed was collected on December 18–20, 2018. Seed was collected from single trees at Coyote Creek Golf Course, Henry Coe State Park–North Fork Pacheco Creek, Little Alamitos Creek, and Pacheco Creek, and two trees at Llagas Creek (Figures 1 and 2). Seed was collected from trees that were previously identified by UC Davis and additional trees that were identified as part of this study. The collected seeds were propagated at the Grassroots Ecology Nursery located in the foothills of Palo Alto, California. Approximately half of the seeds were cold stratified and all seeds were sown into seed beds on March 23, 2019. All seedlings were transplanted into 4-inch^2 pots that were 10 inches deep on June 14, 2019. Leaves from 80 seedlings (3–12 seedlings per seed tree) were collected on August 29, 2019.

2.2.2 Leaf Collections

Leaf collection from established trees occurred between August 14, 2019, and September 1, 2019, at Coyote Creek Golf Course, Henry Coe State Park–Hunting Hollow, Henry Coe State Park–North Fork Pacheco Creek, Little Alamitos Creek, Llagas Creek, Pacheco Creek, Uvas Canyon County Park, and Uvas Road. During leaf collection, each tree was mapped and the diameter at breast height (DBH) of each tree was measured using a DBH tape. Eighty leaf collections were made across all of the populations (Figure 1). Two to 10 leaves were collected from each tree using a hand pruner or pole pruner, as necessary. Leaves were immediately placed into a labeled plastic bag, then temporarily stored in a cooler containing ice. At the end of each collection day the sycamore leaves were transferred from the cooler into a freezer at the H. T. Harvey & Associates office in Los Gatos, California.

2.2.3 Sample Prioritization for Genetic Identification

On November 17, 2019, all sycamore leaf collections, including seedling collections, were mailed to Genomeadvisors Inc. for genetic identification. Due to the funds needed to develop the genetic identification method and need to identify 48 samples previously identified by UC Davis for use as reference standards, only 52 unknown samples could be genetically identified (i.e., genotyped) as part of this study. Thus, the seedling and leaf collections were ranked in priority for genetic identification. Identifying seedlings grown from seed collected across varying distances from urban centers in Santa Clara County was the highest priority, and at least three seedlings from each seed tree were identified to capture potential variation of fertilization within the same tree. If seeds were collected from a tree of unknown identity, seedlings were not genetically identified until it was confirmed that the seed tree was a California sycamore. Therefore, the second priority for genetic identification was identifying a subset of seed trees to determine whether their seedlings also merited genetic identification. The testing of young saplings that were presumably recruited from seed in wildland settings was given third priority, and identifying larger trees from which seed was not collected was the lowest priority. In accordance with these priorities, genetic identification was conducted for 39 seedlings grown from collected seed, 5 seed trees, 7 saplings, and 1 larger non-seed tree. This resulted in only identifying seed collected from 5 populations and leaves collected from 6 populations. All seedling and leaf collections that were not genetically identified are being stored at Genomeadvisors Inc. for future identification pending additional funding.



N:\Projects4100\4109-01\Reports\Fig 1 All Leaf Collection Locations.mxd

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Figure 1. Sample Locations

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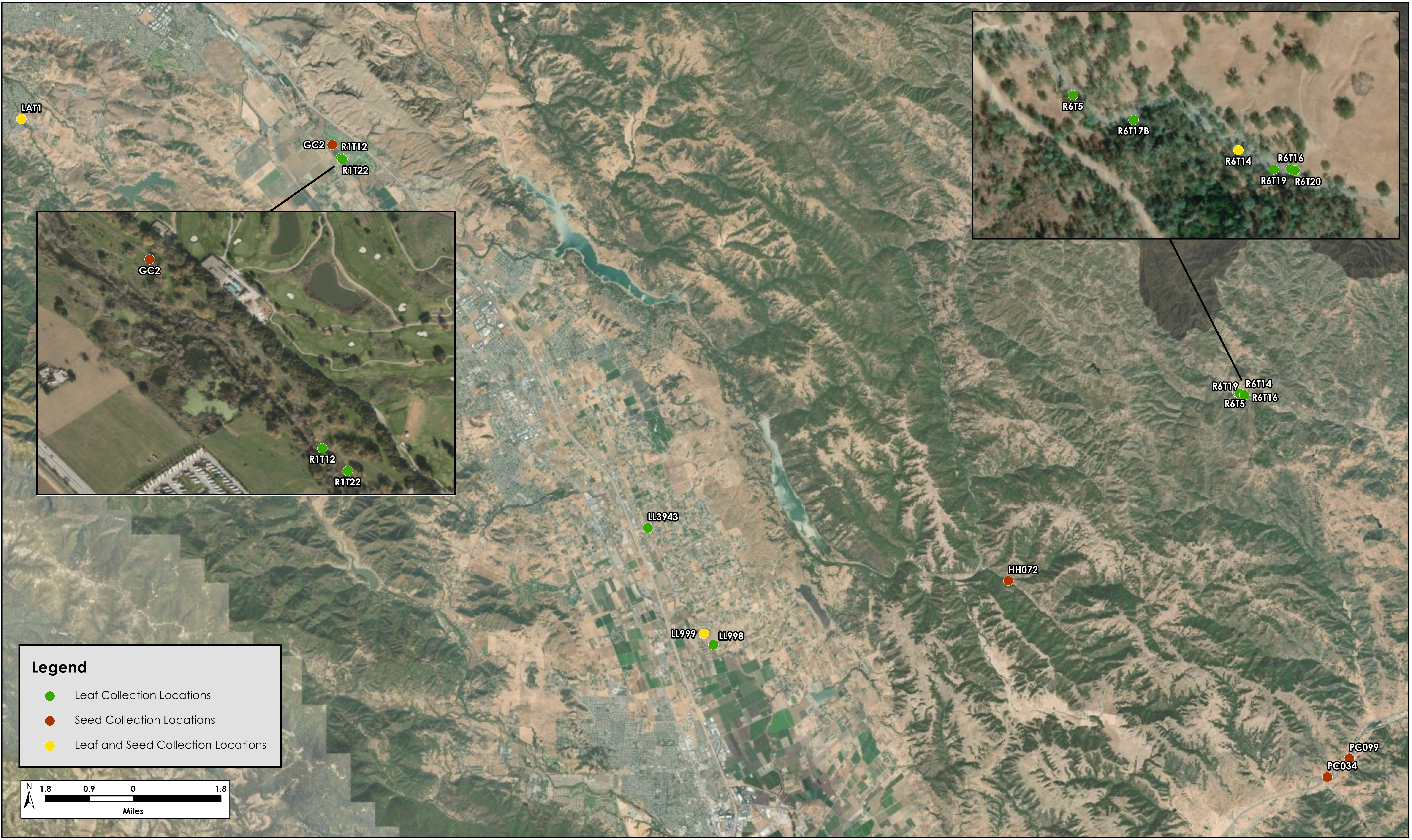

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Figure 2. Genetically Analyzed Seed and Leaf Collection Locations

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Section 3. Results

3.1 Genetic Identification

The initial PCR amplification and genotyping using the multiplex PCR was marginally effective with a successful genotyping rate of 38%. Because all options for improving PCR conditions had been exhausted, additional denaturing reagents were used in the next round of PCR. This improved the genotyping to a success rate of 92%; only 1 reference sample (LL1614) could not be genetically identified (Table 1). The failed genetic identification was determined to be due to the sample's low quality genetic material and not attributable to the genetic identification methods since all microsatellite markers failed for this sample. Thus, Genomeadvisors Inc. was successful in establishing efficient and replicable methods for identifying between California sycamore, London plane, and hybrids. These methods were effectively used to obtain the following results.

Table 1. Assignment of Reference Samples to the Sycamore and London Plane Populations and their Respective Genotype

Sample Name	Reference Genotype (UC Davis)	% London Plane	% California Sycamore	Predicted Genotype
LL1564	California sycamore	0.9%	99.1%	California sycamore
LL1565	California sycamore	0.9%	99.1%	California sycamore
LL1567	California sycamore	0.9%	99.1%	California sycamore
LL1569	California sycamore	0.9%	99.1%	California sycamore
LL1614	California sycamore	NA	NA	NA[1]
LL1616	California sycamore	0.7%	99.3%	California sycamore
LL1654	California sycamore	0.9%	99.1%	California sycamore
LL1656	California sycamore	0.9%	99.1%	California sycamore
LL1662	California sycamore	0.8%	99.2%	California sycamore
LL1700	California sycamore	1.1%	98.9%	California sycamore
LL1702	California sycamore	0.7%	99.3%	California sycamore
PC041	California sycamore	0.8%	99.2%	California sycamore
PC048	California sycamore	7.0%	93.0%	California sycamore
PC063	California sycamore	0.9%	99.1%	California sycamore
PC105	California sycamore	0.8%	99.2%	California sycamore
PC110	California sycamore	0.9%	99.1%	California sycamore
PC159	California sycamore	0.7%	99.3%	California sycamore
PC162	California sycamore	0.7%	99.3%	California sycamore
PC172	California sycamore	0.8%	99.2%	California sycamore
PC183	California sycamore	0.8%	99.2%	California sycamore
PC185	California sycamore	0.7%	99.3%	California sycamore

Sample Name	Reference Genotype (UC Davis)	% London Plane	% California Sycamore	Predicted Genotype
G001	London plane	99.2%	0.8%	London plane
G002	London plane	99.2%	0.8%	London plane
G003	London plane	99.1%	0.9%	London plane
G004	London plane	97.2%	2.8%	London plane
G005	London plane	99.2%	0.8%	London plane
G006	London plane	99.1%	0.9%	London plane
G007	London plane	99.1%	0.9%	London plane
G008	London plane	99.1%	0.9%	London plane
G009	London plane	99.1%	0.9%	London plane
G010	London plane	99.1%	0.9%	London plane
MH001	London plane	99.1%	0.9%	London plane
MH010	London plane	99.1%	0.9%	London plane
MH012	London plane	99.0%	1.0%	London plane
MH013	London plane	99.1%	0.9%	London plane
MH014	London plane	99.2%	0.8%	London plane
MH015	London plane	99.2%	0.8%	London plane
MH017	London plane	99.1%	0.9%	London plane
MH019	London plane	99.1%	0.9%	London plane
MH020	London plane	99.2%	0.8%	London plane
MH021	London plane	99.2%	0.8%	London plane
MH022	London plane	99.1%	0.9%	London plane
LL3944	Hybrid	50.4%	49.6%	Hybrid
LL4005	Hybrid	37.7%	62.3%	Hybrid
PC004	Hybrid	41.4%	58.6%	Hybrid
PC038	Hybrid	50.6%	49.4%	Hybrid
PC107	Hybrid	55.0%	45.0%	Hybrid
PC153	Hybrid	46.0%	54.0%	Hybrid

[1] This sample did not contain genetic material that was of sufficient quality to use in the final analyses.

3.2 Seed Collection Identification

Of the 39 genetically identified seedlings that were grown from seed, 29 were California sycamores, 7 were hybrids, and 3 could not be identified due to low quality genetic material (Table 2). The hybrid seeds were collected from Llagas Creek, Pacheco Creek, and Henry Coe State Park–North Fork Pacheco Creek. These locations span the complete range of distances from urban centers within Santa Clara County in this study. Three seed trees produced both native sycamore and hybrid seed, demonstrating that fertilization can vary within the same tree (Table 2).

Table 2. Seed Collection Genetic Identification Results

Sample Name	Seed Tree	Collection Location	% London Plane	% California Sycamore	Predicted Genotype
GC2S1	GC2	Coyote Creek Golf Course	1.30%	98.70%	California sycamore
GC2S10	GC2	Coyote Creek Golf Course	1.00%	99.00%	California sycamore
GC2S11	GC2	Coyote Creek Golf Course	1.00%	99.00%	California sycamore
GC2S12	GC2	Coyote Creek Golf Course	7.00%	93.00%	California sycamore
GC2S2	GC2	Coyote Creek Golf Course	1.00%	99.00%	California sycamore
GC2S3	GC2	Coyote Creek Golf Course	1.00%	99.00%	California sycamore
GC2S4	GC2	Coyote Creek Golf Course	0.90%	99.10%	California sycamore
GC2S5	GC2	Coyote Creek Golf Course	NA	NA	NA[1]
GC2S6	GC2	Coyote Creek Golf Course	1.10%	98.90%	California sycamore
GC2S7	GC2	Coyote Creek Golf Course	1.00%	99.00%	California sycamore
GC2S8	GC2	Coyote Creek Golf Course	1.10%	98.90%	California sycamore
GC2S9	GC2	Coyote Creek Golf Course	1.00%	99.00%	California sycamore
HH072S1	HH072	Henry Coe State Park–Hunting Hollow	1.00%	99.00%	California sycamore
HH072S10	HH072	Henry Coe State Park–Hunting Hollow	0.90%	99.10%	California sycamore
HH072S11	HH072	Henry Coe State Park–Hunting Hollow	1.00%	99.00%	California sycamore
HH072S12	HH072	Henry Coe State Park–Hunting Hollow	1.40%	98.60%	California sycamore
HH072S2	HH072	Henry Coe State Park–Hunting Hollow	1.10%	98.90%	California sycamore
HH072S3	HH072	Henry Coe State Park–Hunting Hollow	1.20%	98.80%	California sycamore
HH072S4	HH072	Henry Coe State Park–Hunting Hollow	1.10%	98.90%	California sycamore
HH072S5	HH072	Henry Coe State Park–Hunting Hollow	1.00%	99.00%	California sycamore
HH072S6	HH072	Henry Coe State Park–Hunting Hollow	0.90%	99.10%	California sycamore
HH072S7	HH072	Henry Coe State Park–Hunting Hollow	1.10%	98.90%	California sycamore
HH072S8	HH072	Henry Coe State Park–Hunting Hollow	1.10%	98.90%	California sycamore
HH072S9	HH072	Henry Coe State Park–Hunting Hollow	1.00%	99.00%	California sycamore
LL999S1	LL999	Llagas Creek	20.30%	79.70%	Hybrid
LL999S2	LL999	Llagas Creek	20.40%	79.60%	Hybrid
LL999S3	LL999	Llagas Creek	0.80%	99.20%	California sycamore

Sample Name	Seed Tree	Collection Location	% London Plane	% California Sycamore	Predicted Genotype
PC034S1	PC034	Pacheco Creek	0.80%	99.20%	California sycamore
PC034S2	PC034	Pacheco Creek	0.80%	99.20%	California sycamore
PC034S3	PC034	Pacheco Creek	42.10%	57.90%	Hybrid
PC099S1	PC034	Pacheco Creek	26.90%	73.10%	Hybrid
PC099S2	PC034	Pacheco Creek	38.00%	62.00%	Hybrid
PC099S3	PC034	Pacheco Creek	40.10%	59.90%	Hybrid
R6T14S1	R6T14	Henry Coe State Park–North Fork Pacheco Creek	14.30%	85.70%	Hybrid
R6T14S2	R6T14	Henry Coe State Park–North Fork Pacheco Creek	NA	NA	NA[1]
R6T14S3	R6T14	Henry Coe State Park–North Fork Pacheco Creek	0.90%	99.10%	California sycamore
R6T14S4	R6T14	Henry Coe State Park–North Fork Pacheco Creek	0.90%	99.10%	California sycamore
R6T14S5	R6T14	Henry Coe State Park–North Fork Pacheco Creek	NA	NA	NA[1]
R6T14S6	R6T14	Henry Coe State Park–North Fork Pacheco Creek	1.00%	99.00%	California sycamore

[1] These samples did not contain genetic material that was of sufficient quality to use in the final analyses.

3.3 Leaf Collection Identification

Of the 13 genetically identified leaf collections, 6 were identified as California sycamores, 4 were identified as hybrids, and 3 could not be identified due to low quality genetic material (Table 3). Hybrids were located at Little Alamitos Creek, Lower Llagas, and Henry Coe State Park–North Fork Pacheco Creek, spanning the distance from urban centers in Santa Clara County. Hybrids were observed to have DBHs between less than 1 inch and 20 inches. However, it should be noted that no trees analyzed as part of this study had a DBH larger than 25 inches.

Of the 5 seed trees that were genetically identified, 3 were California sycamores and 2 were hybrids. The hybrid seed trees were collected from Little Alamitos Creek and Llagas Creek, both of which are near urban centers. Of the 7 saplings that were genetically identified, 3 were California sycamores, 1 was a hybrid, and 3 could not be identified based on low quality genetic material. The hybrid sapling that was identified was collected from Henry Coe State Park–North Fork Pacheco Creek which is the farthest population from urban centers in our study. The larger non-seed tree analyzed was identified as a hybrid and was collected from Llagas Creek which is near an urban center. This tree was near a seed tree that was also identified as a hybrid.

Table 3. Leaf Collection Genetic Identification Results

Sample Name	Collection Location	DBH (inches)	Notes	% London Plane	% California Sycamore	Predicted Genotype
GC2	Coyote Creek Golf Course	Multistemmed (25)	Seed Tree	1.00%	99.00%	California sycamore
R1T12	Coyote Creek Golf Course	2.5	Sapling	NA	NA	NA[1]
R1T22	Coyote Creek Golf Course	1.5	Sapling	NA	NA	NA[1]
LAT1	Little Alamitos Creek	22.6	Seed Tree	31.40%	68.60%	Hybrid
LL3943	Llagas Creek	20		55.60%	44.40%	Hybrid
LL998	Llagas Creek	20	Seed Tree	48.80%	51.20%	Hybrid
LL999	Llagas Creek	Multistemmed (15)	Seed Tree	0.70%	99.30%	California sycamore
R6T14	Henry Coe State Park–North Fork Pacheco Creek	19	Seed Tree	0.80%	99.20%	California sycamore
R6T16	Henry Coe State Park–North Fork Pacheco Creek	3	Sapling	8.30%	91.70%	California sycamore
R6T17B	Henry Coe State Park–North Fork Pacheco Creek	<1	Sapling	22.10%	77.90%	Hybrid
R6T19	Henry Coe State Park–North Fork Pacheco Creek	3	Sapling	0.80%	99.20%	California sycamore
R6T5	Henry Coe State Park–North Fork Pacheco Creek	2.5	Sapling	0.90%	99.10%	California sycamore
R6T20	Henry Coe State Park–North Fork Pacheco Creek	1	Sapling	NA	NA	NA[1]

[1] These samples did not contain genetic material that was of sufficient quality to use in the final analyses.

Section 4. Discussion

Genetic identification of sycamore trees has been troublesome and expensive in the past. However, the new process developed by Genomeadvisors Inc. can now be used as a routine tool for future sample identification efforts. This new method makes it possible to quickly and accurately test nursery container stock grown from seed prior to installation to avoid outplanting hybrids. Additionally, this approach will allow for the continued identification of unknown sycamore trees that will augment the Santa Clara County database of known native sycamores, which can be used for collecting seed and/or cuttings for propagation, and hybrids which should be avoided during collections.

Nineteen percent (19%) of the genetically identified samples propagated from seed were identified as hybrids. These hybrid seeds were collected from locations that cover the range of distances from urban centers in this study. Based on these results, it can be speculated that pollen from London plane trees may travel great distances. However, it should be noted that London plane trees on private property, particularly in more rural areas, may be a pollen source that could have fertilized the hybrid seed at Henry Coe State Park–North Fork Pacheco Creek that was collected in this study. These findings bring into question the use of wild-collected seed, even from remote stands of documented native California sycamores, unless seedlings are genetically identified before outplanting. Our study makes it seem unlikely that California sycamore populations may exist within Santa Clara County that are not susceptible to fertilization by London plane trees. However, a substantial amount of additional sampling and testing would need to be completed before concluding that stands of California sycamore that produce solely native sycamore seeds do not exist in Santa Clara County.

Forty percent (40%) of the leaf collections were identified as hybrids in this study. While this percentage is higher than what has previously been observed in Santa Clara County, this study has a small sample size (13 trees) and was focused on leaves from younger trees and trees that were producing seed. The hybrid trees were located at varying distances from urban centers and ranged in size from young saplings to a mature tree with a 20-inch DBH that is the largest documented hybrid in Santa Clara County. However, it should be noted that trees with a DBH larger than 25 inches were not genetically identified in this study. Thus, there are not sufficient data to identify a size to use to identify California sycamore trees that are not likely to be hybrid, and additional research is needed to use size for identification purposes.

The previous research conducted by H. T. Harvey & Associates and UC Davis concluded that the identified hybrids were most likely installed from nursery container stock as part of a mitigation project (H. T. Harvey & Associates et al. 2019). This study shows that recruitment from natural seed fall is also likely to be occurring because there is a hybrid at the Henry Coe State Park–North Fork Pacheco Creek site that is in a remote location where no known habitat restoration or active planting has occurred. Thus, hybrids may be more common than was originally thought where sycamores are naturally recruiting from seed.

Section 5. Key Findings

- An efficient and replicable method for identifying California sycamore, London plane, and hybrids was established.
- Hybrid seed was collected from genetically identified California sycamore trees from locations that cover the range of distances from urban centers, where London plane trees are common. Thus, it can be inferred that London plane pollen likely travels and successfully fertilizes California sycamore seeds over long distances and that there may not be a distance from urban centers that is safe for California seed collection without the use of genetic identification.
- A hybrid sapling was identified in a location where no habitat restoration or active planting has ever occurred. Thus, hybrids may be present in other locations where natural regeneration from seed is occurring.

Section 6. Next Steps

1. Encourage restoration nurseries, designers and other practitioners to utilize the process developed by Genomeadvisors Inc. as an expedient tool for the California sycamore/hybrid identification process.
2. Record the tree location and genetic identification of each seed tree. This information can assist in further discerning if there is a "safe" distance to urban centers and will build data on frequency of hybridization.
3. California sycamores grown from seed should be genetically tested early in the propagation process to avoid growing hybrid trees.
4. Conduct additional studies to genetically identify established sycamore trees and continue to build the database of California sycamore and hybrid trees in Santa Clara County.
5. Assess the degree of genetic diversity of California sycamore trees in Santa Clara County. Gathering data on the the genetic diversity could facilitate understanding population viability, as well as help propagate a diverse population. The STRUCTURE program used in this study can test for genetic relatedness and help guide the collection and propagation of genetically diverse California sycamores for use in restoration and mitigation projects.

Section 7. References

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