


Evidence for genetic erosion of a California native tree, *Platanus racemosa*, via recent, ongoing introgressive hybridization with an introduced ornamental species

Matthew G. Johnson¹  · Kylene Lang² · Paul Manos³ · Greg H. Golet⁴ · Kristina A. Schierenbeck²

Received: 9 July 2015 / Accepted: 30 December 2015
© Springer Science+Business Media Dordrecht 2016

Abstract When non-native, genetically diverse species are introduced, hybridization with native congeners may erode the genetic composition of local species, perhaps even resulting in extinction. While such events may lead to adverse consequences at the community and ecosystem level, few studies exist on ecologically important tree species. In the genus *Platanus*, introgressive hybridization is widespread, and one common ornamental species, introduced to California during the late 19th century, is itself a hybrid. Our microsatellite analysis of more than 400 *Platanus* trees from north-central California reveals a complex pattern of invasion and hybridization in an age-structured population. By using size as a proxy for age, we have demonstrated that the *Platanus* population of north-central California has recently gained genetic diversity and effective population size. Principal coordinate analysis (PCoA) and genetic admixture analysis (STRUCTURE) both reveal a strong differentiation of genotypes into two main genetic clusters, with a large number of admixed genotypes. One of the genetic clusters identified is heavily

biased towards younger trees, including samples from locations with relatively recently planted ornamental trees likely to be *P. × hispanica* (formerly known as *P. × acerifolia*). We conclude that the two genetic clusters correspond to the native *P. racemosa* and the introduced invasive hybrid species *P. × hispanica*. Additional hybridization between the invasive ornamental and the native species has occurred in California, and recent hybrid trees are more likely to be younger than trees without admixture. Our findings suggest that the observed increase in genetic diversity among California *Platanus* is due to rampant ongoing introgression, which may be threatening the continued genetic distinctiveness of the native species. This is cause for concern from a conservation standpoint, due to a direct loss of genetic distinctiveness, and a potential reduction in habitat value of associated species.

Keywords Hybrid zone · Admixture · Sycamore · Microsatellite

Electronic supplementary material The online version of this article (doi:[10.1007/s10592-015-0808-z](https://doi.org/10.1007/s10592-015-0808-z)) contains supplementary material, which is available to authorized users.

✉ Matthew G. Johnson
mjohnson@chicagobotanic.org

¹ Department of Plant Sciences, Chicago Botanic Garden, 1000 Lake Cook Road, Glencoe, IL 60626, USA

² Department of Biological Sciences, California State University-Chico, Chico, CA 95929-0515, USA

³ Biology Department, Duke University, 130 Science Drive, Durham, NC 27708, USA

⁴ The Nature Conservancy, 190 Cohasset Rd # 177, Chico, CA 95926, USA

Introduction

Human activities such as introducing non-natives and fragmenting or otherwise degrading natural ecosystems promote hybridization of previously allopatric congeners (Vilà et al. 2000). When hybridization is accompanied by introgression, or the repeated backcrossing to parental species via hybrids, there will be a resultant loss of genetic distinctiveness in the parental taxa. This phenomenon is of particular concern when native species are introgressed with genetic material from a non-native congener (Abbott 1992), and can even result in extinction of the native species altogether (Anttila et al. 1998). Often underrated, human-mediated hybridization is one of the leading causes

of biodiversity loss and documented via numerous examples in plants and freshwater fish (Mack and D'Antonio 1998; Ellstrand and Schierenbeck 2000; Muhlfeld et al. 2009). Hybridization between populations of locally adapted species can lead to outbreeding depression, and the rise of hybrid individuals can limit evolutionary flexibility, increasing potential loss of locally adapted genes within populations that once contained them (Rieseberg 1991; Ellstrand and Elam 1993). Displacement of native species by hybrids is an important topic in conservation biology due to the potential adverse impacts ecosystems. For example, shorebird habitat has been shown to be reduced in San Francisco Bay by invasions of *Spartina alterniflora* × *S. foliosa* hybrids which are able to grow at lower elevations on the mudflats than the native species due to their increased tolerance of salt and inundation (Stralberg et al. 2004).

In plants, wind-pollinated species are at high risk for introgressive hybridization, such as the case of the native red mulberry (*Morus rubra* L.), which is endangered or threatened in several states (USDA, NRCS 2008). It readily hybridizes with the introduced East-Asian white mulberry (*Morus alba* L.) (Burgess et al. 2005; Burgess and Husband 2006) and in a reciprocal transplant and common garden study white mulberry and its hybrids were more fit in all conditions tested, suggesting imminent decline of the red mulberry (Burgess and Husband 2006). The red elm (*Ulmus rubra* Muhl.), native to the eastern United States, is similarly undergoing widespread hybridization with the Siberian elm (*Ulmus minor* Mill.), the latter of which is widely planted as an ornamental (Zalapa et al. 2009).

The Western sycamore (*Platanus* Nutt.) is an integral component of California riparian habitat commonly associated with *Alnus rhombifolia* Nutt., *Acer macrophyllum* Pursh., *Populus fremontii* S. Watson, *Juglans* species, and *Salix* species. Although, *P. racemosa* is not currently listed as an “at risk” species, the riparian habitat in which it occurs has been reduced to less than 5 % of its pre-European distribution (Griggs 2009). As the tallest tree in its native riparian habitat, the abundant shade provided by *P. racemosa* helps to cool streams and riparian zones, and provides habitat and food for nesting birds, is a preferred overwintering species for monarch butterflies, and small animals via trunk cavities. Cavities formed at the base of the *Platanus racemosa* are not found among trees of hybrid origin (Stuart and Sawyer 2001, Smith and Finch 2014).

Seven species and three varieties of *Platanus* are currently recognized (Nixon and Poole 2003; Feng et al. 2005). In addition, the ornamental species, *Platanus* × *hispanica*, was developed in 1670 at the Oxford Botanical Garden in England (Henry and Flood 1919), via hybridization between *P. orientalis* and *P. occidentalis* (Santamour 1969) and has since been introduced widely as

an ornamental. Interestingly, the lineage which contains *P. occidentalis* is estimated to have split from *P. orientalis* and *P. racemosa* in the Paleocene (~60 mya) and the latter two species have been separated since the early Miocene (~24 mya) (Feng et al. 2005).

Hybridization, both ancient and recent (Besnard et al. 2002) is a well-documented phenomenon within *Platanus* (Rhymer and Simberloff 1996; Nixon and Poole 2003; Whitlock 2003; Grimm and Denk 2008). *Platanus* × *hispanica* Mill. Ex Münchh. (London plane tree), a hybrid between *P. occidentalis* L. and *P. orientalis* L., is widely planted as an ornamental in temperate regions (especially in cities), in part due to its disease resistance and tolerance of air pollution (Besnard et al. 2002; USDA, NRCS 2008). Plantings in European cities are extensive, composing 40 % of tree plantations in Paris, even more in London (Besnard et al. 2002), and as much as 60 % in Milano (Anselmi et al. 1994). Ornamental plantings are equally common in the United States. Species within this ancient genus are hypothesized to be threatened with extinction via genetic homogenization mediated via the human dispersal of *P. × hispanica* (Whitlock 2003).

Due to its hybrid background, *P. × hispanica* is variable in both form and growth rate. It can usually be distinguished from *P. occidentalis* by somewhat longer and narrower leaf lobes, and the larger size of its leaves (USDA, NRCS 2008). Also, *P. × hispanica* has only one to two fruiting heads per stalk, and its bark is usually greener than *P. occidentalis* (USDA, NRCS 2008). It can be distinguished from *P. racemosa* by the presence of leaf serrations and shallower lobes. However, in California, the two species cannot reliably be distinguished morphologically, even in residential planted locations, likely due to hybridization. *Platanus* species are known to be highly interfertile despite the long-term genetic isolation of most species (Ernst 1963; Rhymer and Simberloff 1996; Nixon and Poole 2003; Whitlock 2003; Grimm and Denk 2008). Santamour (1972), successfully produced viable seeds between *P. racemosa* and *P. occidentalis* and *P. orientalis* in all possible crosses and regardless of crossing direction. He determined the chromosome number of *P. racemosa*, *P. racemosa* var. *wrightii*, *P. occidentalis*, *P. orientalis*, *P. × hispanica*, and *P. mexicana* all to be $2n = 42$ (Santamour 1969), which supports a lack of chromosomal barriers among the species.

In the Eastern United States and Europe, *P. × hispanica* has been planted within the ranges of both parental species as well as beyond those ranges (Rhymer and Simberloff 1996). In California, *P. × hispanica* is naturalized along many valley and foothill streams in northern California (Oswald 2002). When it co-occurs with its parental species, *P. × hispanica* demonstrates hybrid vigor (heterosis), including a higher resistance to rot diseases compared with

both parental species, as seen in other examples (Rhymer and Simberloff 1996; Rieseberg et al. 2006). The deep phylogenetic context of the parent species makes this system ideal for identifying divergent alleles of the parental species that have flowed into populations of *P. racemosa* via hybridization.

Platanus racemosa has a limited range from California to Baja California and is restricted to perpetual and intermittent streams or moist canyons in well-drained soils (Sudworth 1965; Baldwin 2012). *Platanus racemosa* grows to heights of 10–35 m, with a base of less than one meter in diameter (Sudworth 1967; Hickman 1993). Trees can live to over 200 years and are erect, forked, or leaning, with relatively short, forked trunks and ascending, sometimes crooked branches (Sudworth 1967; Stuart and Sawyer 2001). The crooked nature of the branches leads to a relatively open crown (Sudworth 1967). The leaves of *P. racemosa* are somewhat round, with three or five lobes, acute to acuminate, usually entire, infrequently serrate, and 10–25 cm in diameter (Stuart and Sawyer 2001).

Whitlock (2003) examined the ITS-1 and ITS-2 regions of nuclear ribosomal RNA from individuals of the species, *P. racemosa* (collected from native California populations, N = 8), *P. occidentalis* (collected from Virginia populations, N = 8), and *P. orientalis* (collected in France, N = 7) with sequences collected from suspected hybrid populations (near the Sacramento River, California, N = 22). In that pilot study Whitlock found that the majority of *P. racemosa* individuals sampled near the Sacramento river contained alleles that originated from *P. x hispanica*, consistent with the hypothesis of introgressive hybridization.

Platanus x hispanica may have a reproductive advantage over *P. racemosa* because of its apparent resistance to sycamore anthracnose (Oswald 2002). Sycamore anthracnose is a fungal disease (*Gnomonia platani*) that readily infects native *P. racemosa* (Baldwin 2012), causing a dieback of twigs and flowers in the spring (Stuart and Sawyer 2001; Whitlock 2003). Although it rarely kills an entire tree, sycamore anthracnose can completely defoliate or kill large portions of the tree crown, leaving the tree weakened and unattractive (Stuart and Sawyer 2001; Whitlock 2003). The resistance present in *P. x hispanica* originates from *P. orientalis* (Anselmi et al. 1994), and when crossed with *P. occidentalis*, the resistance is strongly inherited (Whitlock 2003). The resistance is only predictable in first generation hybrids, however and becomes variable with subsequent crosses between F1 individuals (Santamour and McArdle 1986). If resistance to sycamore anthracnose is inherited by *P. racemosa*-*P. x hispanica* hybrids, it could give them a selective advantage over native *P. racemosa* individuals.

A primary motivation for this study was to inform horticultural restoration efforts on the Sacramento River. This is one of North America's largest floodplain restoration projects with >6000 acres of riparian habitats having been planted over the past 25 years (Golet et al. 2013). *Platanus* has been an important component of the restoration pallet, however, some planted individuals, which were propagated from derived cuttings, have been determined to be non-native hybrids.

Here, we expand on the work of Whitlock (2003) to investigate populations of *Platanus* in northern California to determine 1) the age-structure of the invasion of *P. x hispanica*, and 2) the nature and persistence of hybridization and subsequent introgression of alleles between the native and invasive species. We develop hypervariable microsatellite markers and genotype more than 600 trees to assess the population structure and introgression at a finer scale than the nuclear DNA sequences used previously. Finally, we characterize the invasion of the *Platanus* hybrids into this region and discuss the implications that ongoing hybridization with an invasive ornamental may have upon the future conservation of the native species.

Materials and methods

Sampling

We sampled leaf tissue from trees in 27 locations in four counties in California, USA (Tehama, Butte, Glenn, and Colusa) along the Sacramento River (see map, Fig. 1). GPS coordinates were recorded for each tree sampled. Voucher specimens for selected samples are available at the herbarium at California State University, Chico. We sampled both natural populations and stands of planted trees in residential neighborhoods. We also collected size-class data as a surrogate for age; size classes in cm at diameter breast height (dbh) are 1 \geq 1.0–24.0, 2 \geq 25.0–49.0, 3 \geq 50.0–74.0, 4 \geq 75.0–99.0, 5 \geq 100.0–124.0, 6 \geq 125.0–148.0, and 7 \geq 150.0–193.0. We avoided individuals less than 1.0 cm in diameter as it was unclear whether they would fully establish in some areas. Although there is much error associated with the use of dbh as a determinate of age, species-specific multiplication factors have been developed to increase the reliability of this measure. The factor specifically calibrated for *Platanus* species is 3.5 and has been shown to be accurate within 10 years (Avery and Burkhart 1983). It is not been addressed experimentally and growth rates among *Platanus* species are somewhat variable due to site conditions, however, in general, growth rates among *Platanus* species are considered comparable (<http://plants.usda.gov>).

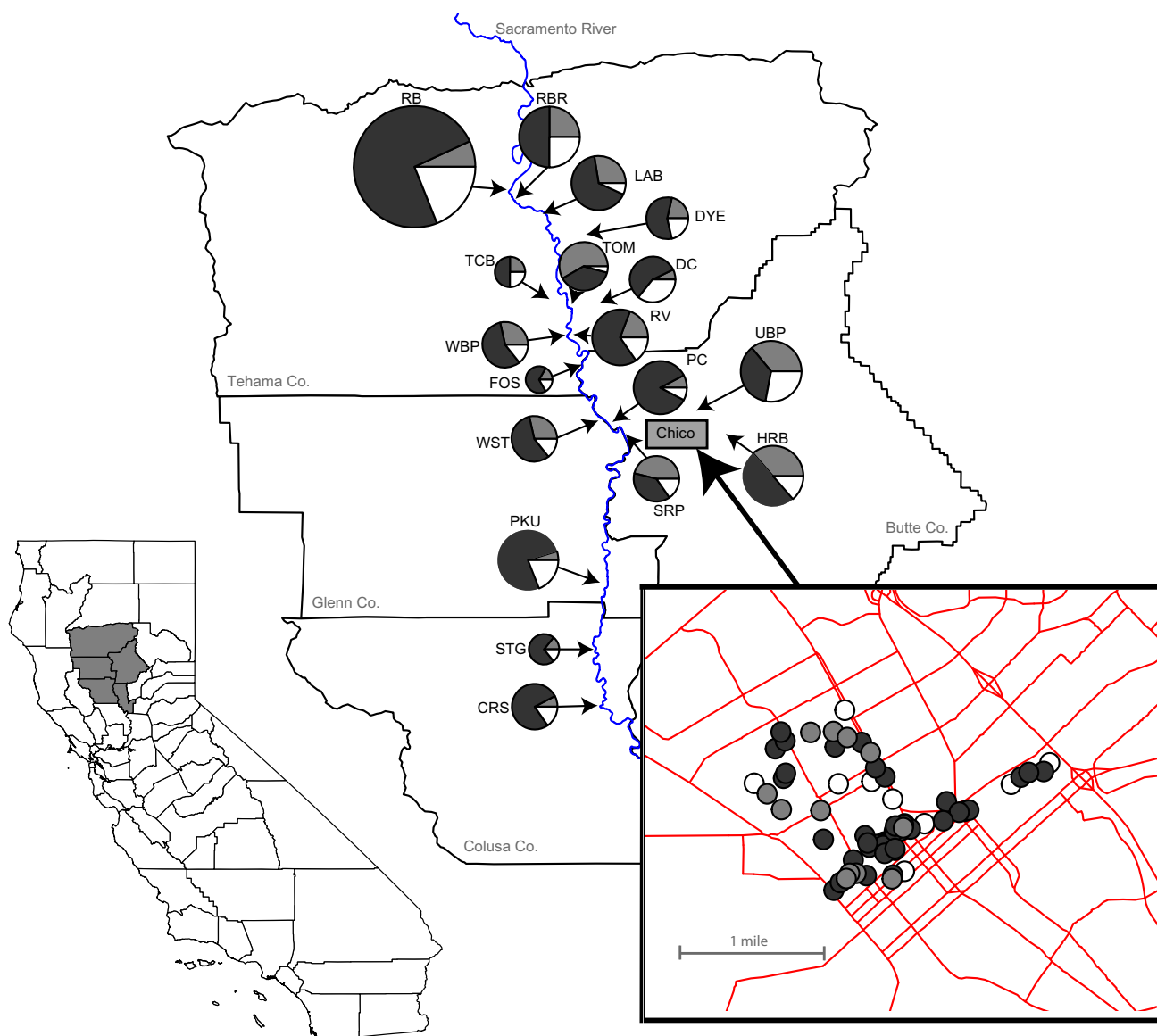


Fig. 1 Sampling locations and distribution of *P. racemosa*, *P. × hispanica*, and hybrids in North-Central California. The four counties sampled (Tehama, Butte, Glenn, and Colusa) are shaded in the bottom left. Pie charts show the percentage of individuals in each location classified as *P. racemosa* (dark gray), *P. × hispanica* (light gray) or

hybrids (white) in the STRUCTURE analysis. The diameter of the pie chart approximates the sample size at each location. *Inset*, bottom-right: Individual trees sampled from the campus of the California State University at Chico. Points represent the classification of trees into the three categories as above

Leaves were harvested from 671 trees using a slingshot and taken to a laboratory at California State University Chico where they were stored at $-80\text{ }^{\circ}\text{C}$ until DNA extraction. Total genomic DNA was extracted from fresh/frozen leaf tissue (40–50 mg) using the DNeasy Plant mini kit (Qiagen). A total of 20 mg of leaf tissue was used for dried specimens.

We developed hypervariable microsatellite markers using the streptavidin bead method (see Supplemental Methods for full details on marker development). Ten loci had primers compatible with both study species. Labeled oligonucleotides were ordered and PCR was carried out on

all individuals. The program used consisted of an initial step of $94\text{ }^{\circ}\text{C}$ for 7 min, 30 cycles of $94\text{ }^{\circ}\text{C}$ 30 s, $61\text{ }^{\circ}\text{C}$ 30 s, $72\text{ }^{\circ}\text{C}$ 1 min, and a final step of $72\text{ }^{\circ}\text{C}$ for 7 min. Amplified fragments were visualized on 1% agarose gels for quality control and samples were sent to the San Diego State University Microchemical Core Facility for purification and genotyping on an ABI Prism sequencer (model 310).

Raw data files were analyzed with GeneMapper v4.0 (Applied Biosystems, Inc.). Auto-binning was used, although each sample was checked manually for accuracy to prevent the calling of false alleles such as stutter peaks.

Admixture analysis

Due to missing data in a large number of samples (>100), we conducted population genetics analysis on a subset of data that had genotypes at all ten loci (434 individuals). We analyzed admixture in the reduced dataset using STRUCTURE (version 2.3.4, Pritchard et al. 2000). We ran STRUCTURE with the admixture model enabled in 10 independent runs of 1 million generations, discarding the first 100,000 iterations as burn-in. No prior information about population identity was used in the analysis. We ran STRUCTURE with the number of a priori clusters (K) varied between 1 and 7, and selected the optimal value of K using the “delta-K” method (Evanno et al. 2005). We summarized the output from the 10 independent runs using CLUMPP (version 1.1.2, Jakobsson and Rosenberg 2007) and visualized the admixture in each individual using a custom R script. The pipeline used for STRUCTURE analysis, including the visualization script, is available online github.com/mossmatters/StructurePipeline.

Because the most likely value of K for our data is 2 (Fig. 2), in some further analyses we assigned cluster membership based on the Q-matrix from STRUCTURE. An individual was placed in one of the two pure clusters if the admixture was less than 10 %; otherwise an individual was classified as “hybrid.” To determine the taxonomic identity of each cluster, we compared the allele frequencies among individuals in the pure clusters to results from an earlier analysis (Lang 2010). In that study, trees of *P. racemosa* were only sampled from old-growth forest, and alleles were identified at several loci that were never found in *P. × hispanica*, *P. occidentalis*, or *P. orientalis*.

The likely identity of the two pure clusters is discussed below.

Population genetics

We calculated several population genetic parameters using the Excel plug-in GenA1Ex (version 6.5, Peakall and Smouse 2012), including allelic diversity (Shannon’s

information index), expected (H_e) and observed (H_o) heterozygosity, and the fixation index ($F = (1 - (H_o/H_e))$). We calculated these parameters by age class and by genetic cluster (identified by STRUCTURE). To visualize the potential hybridization among genetic clusters, we conducted a principal coordinate analysis (PCoA) of the genetic distance among samples (Jaccard’s Distance) using the standardized covariance method.

We calculated effective population size (N_e) using Ne-Estimator (version 0.2, Do et al. 2014) for each age class and genetic cluster, using the linkage disequilibrium (LD) method (Waples and Do 2008), including confidence intervals around each estimate using the non-parametric jack-knife method. In order to avoid bias from rare alleles in the data set, the critical allele value for each estimate of N_e was set to be $1/2 N$, where N is the number of samples in the age class or genetic cluster. All alleles with frequencies less than this percentage were excluded from the analysis.

We calculated the hybrid index in individuals that STRUCTURE identified as admixed, using the method of Buerkle (2005) in GenoDive (version 2.0b27, Meirmans and Van Tienderen 2004). We used non-admixed individuals (less than 10 % admixture) from the STRUCTURE analysis as reference populations. GenoDive calculates a maximum likelihood estimate and of the hybrid-index between 0.0 and 1.0, as well as 95 % confidence intervals. A hybrid index of 0.5 indicates an F1 hybrid, while values closer to 0 or 1 indicate stronger affinities to one of the parental populations.

Results

Microsatellite genotyping

Of the 671 samples genotyped, 434 samples had no missing data at any of the 10 loci, and this reduced “no-missing” dataset was used in all further analysis. Two of the ten loci (plms_136 and plms_92) were monomorphic across all

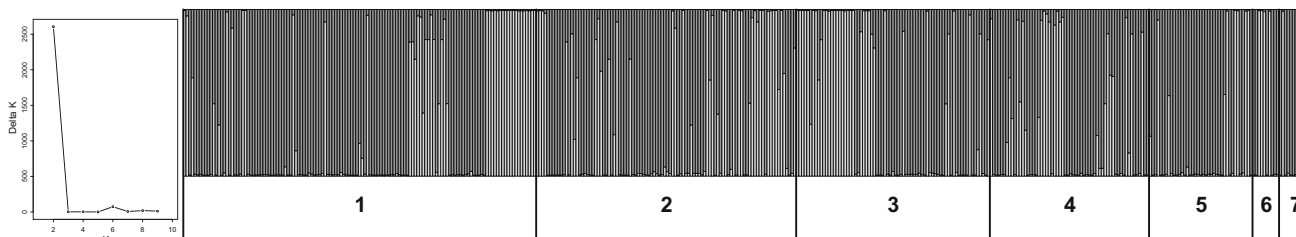


Fig. 2 Admixture analysis using STRUCTURE on the reduced dataset (no missing data). *Left* Evaluation of the most probable number of a priori clusters using the Evanno et al. (2005) method. *Right* Admixture percentage of each individual by age class at $K = 2$.

Each bar is an individual, and the shading of each bar reflects the percentage of admixture between the two clusters. Individuals with no admixture have bars of only one shade. Individuals are sorted by sampling location within each age class

samples (Table 1). At the other eight loci, the number of alleles varied from 4 to 15.

Admixture analysis

We evaluated the population structure in the “no-missing” dataset using STRUCTURE by setting the number of a priori clusters (K) between 1 and 7. Using the “delta-K” method of Evanno et al. (2005), the most likely number of clusters is two (Fig. 2, left). We summarized the admixture probabilities of all 428 samples across 10 independent runs of STRUCTURE, which revealed that most individuals (365) have less than 10 % admixture (Fig. 2, right). A larger number of individuals lacking admixture group into the dark-gray cluster (262) compared to the light-gray cluster (103), while the remaining samples (67) have a level of admixture exceeding 10 % (Fig. 2). When grouped by age-class, there is a clear pattern of cluster membership and admixture percentage: younger samples (age-classes 1 through 4) have a dramatically higher percentage of admixed individuals (Table 2) and a higher percentage of individuals in the light-gray cluster. When grouped into “new trees” (age class 1–4, N = 364 trees) or “old trees” (age class 5–7, N = 64 trees), there are significantly fewer old hybrid trees (3) than new hybrid trees (60, $X^2 = 5.13$, $p = 0.024$). There is not a significantly different percentage of old trees in the light gray cluster (10/64) compared with new trees in the light gray cluster (93/364, $X^2 = 2.42$, $p = 0.12$).

The age-class biased clustering of individuals, and the higher percentage of admixture in the youngest age classes, combined with knowledge of the history of these populations, allows us to make informed predictions on the identity of the genetic clusters identified by STRUCTURE. The dark gray cluster (Fig. 2) is more likely to be the native *P. racemosa*, and the light gray cluster the planted

non-native *P. × hispanica*. An earlier study revealed private alleles at several loci found in *P. racemosa* trees from old-growth forests (Lang 2010). Two of these private alleles are found in high frequency in the dark gray cluster. At locus plms53, allele 325 is found at 74.7 % in the dark gray cluster and is absent in the light gray cluster. Similarly, at locus plms130, allele 202 has a frequency of 68.5 % and is also absent in the light gray cluster.

Individuals with high levels of admixture are likely hybrids of these two species. In addition, one population of young (age-class 1) trees planted on the California State University at Chico campus (Population NW) are completely within the light-gray clade with no admixture. These planted trees are much more likely to be ornamental varieties of *P. × hispanica*. Although there are no fixed private alleles distinguishing the genetic clusters, this analysis suggests that allele-frequency patterns characteristic of *P. × hispanica* can be identified even though *P. × hispanica* is itself a hybrid species. In further analyses, we characterize the genetic diversity and population genetic parameters of individuals with less than 10 % admixture in either the dark gray or light gray clusters, and we will refer to these clusters as *P. racemosa* and *P. × hispanica*, respectively.

Population genetics

When grouped by age-class, we find higher levels of allelic diversity (I, Shannon’s Index) in younger age classes, compared to older ones (Table 2). This coincides with increases in both observed and expected heterozygosity in younger age classes, resulting in a fairly constant fixation index (around 0.5) among age classes. One exception is age-class 6, which has a lower fixation index of 0.22. This age class is also the only one made up entirely of samples from the *P. racemosa* genetic cluster (dark-gray in Fig. 2).

Estimates of effective population size by age-class reveal very small effective population sizes for most classes, all below 7.0 (Table 2). The confidence intervals indicate that younger age-classes (1–2) have three to four times the population size of the next three older age classes. The estimate for age class 6 could not be calculated, as the observed mean r-squared did not exceed the expected r-squared value, and NeEstimator returned a value of “Infinite.” The effective population size of age-class 7 is similar to that of age-classes 3 through 5.

It is clear through principal coordinate analysis (PCoA) that the ordination of the samples that describes the highest variance (axis 1) corresponds to the genetic clusters from STRUCTURE, rather than age-class or sampling location (Fig. 3). The PCoA reveals a large percentage of individuals marked by STRUCTURE as having high percentages of admixture as intermediate between those inferred to

Table 1 Number of alleles (Na), effective alleles (Ne), information index (I), observed heterozygosity (Ho), expected heterozygosity, and fixation index (F) for 434 individuals at ten microsatellite loci

Locus	N	Na	Ne	I	Ho	He	uHe	F
plms_29	434	12	1.587	0.784	0.122	0.370	0.370	0.670
plms_53	434	3	2.002	0.701	0.184	0.500	0.501	0.632
plms_71	434	4	1.082	0.185	0.037	0.076	0.076	0.513
plms_92	434	1	1.000	0.000	0.000	0.000	0.000	#N/A
plms_109	434	7	1.636	0.861	0.164	0.389	0.389	0.579
plms_113	434	11	2.292	1.228	0.336	0.564	0.564	0.403
plms_122	434	4	1.067	0.166	0.005	0.063	0.063	0.927
plms_130	434	9	3.462	1.445	0.196	0.711	0.712	0.725
plms_136	434	1	1.000	0.000	0.000	0.000	0.000	#N/A
plms_176	434	6	1.763	0.896	0.406	0.433	0.433	0.063

Table 2 Population genetic parameters, admixture analysis, and effective population sizes by age class, in 434 samples without missing data. For each age class, %hybrid and %racemosa refer to classification of samples based on the STRUCTURE admixture analysis (see text). Allelic diversity (I-Shannon's diversity index),

Size class	N	%Hyb	%Racemosa	I	Ho	He	F	Ne	CI
1	133	0.12	0.63	0.60	0.13	0.31	0.59	2.8	2.3–3.4
2	98	0.17	0.56	0.65	0.15	0.33	0.60	2.8	2.4–3.2
3	73	0.16	0.53	0.60	0.16	0.32	0.48	1.9	1.6–2.2
4	60	0.25	0.55	0.50	0.14	0.25	0.44	3.6	2.8–5.8
5	39	0.05	0.74	0.68	0.18	0.33	0.55	2.5	2.0–3.1
6	15	0.00	0.87	0.39	0.11	0.20	0.35	0.9	0.7–1.1
7	11	0.10	0.90	0.24	0.10	0.14	0.16	2.3	1.2–12

expected (H_e) and observed (H_o) heterozygosity, and fixation index (F) were calculated in GenAIEx. Effective population size (N_e) was calculated using the linkage disequilibrium method in NeEstimator. Confidence intervals (CI) on N_e estimates were calculated using the non-parametric jack-knife method

have no admixture (Fig. 3). By contrast, there is no association between the PCoA and age-class or sampling location (not shown).

For individuals identified as admixed by STRUCTURE, we calculated a hybrid index (Buerkle 2005), using *P. racemosa* as the “reference” population (Fig. 4). The overall mean hybrid index was 0.63, indicating hybrids had a stronger genetic identity with *P. racemosa* than with *P. × hispanica*. However, all individuals had very high error bars around the maximum likelihood estimate of hybrid index, and many individuals had values that overlapped with the maximum (1.0) or minimum (0.0) values. An analysis of variance revealed no association between mean hybrid index and age class ($df = 4$, $F = 0.568$, $p > 0.6$), and there was no significant between-age-class comparisons in a post hoc Tukey HSD test (results not shown).

Discussion

We developed hypervariable genetic markers that allowed us to distinguish between two *Platanus* taxa with cryptic morphological differences: the native *P. racemosa* and a hybrid, *P. × hispanica*, composed of its sister species (*P. orientalis*) and a more distant relative (*P. occidentalis*).

Our genetic analysis, particularly the admixture analysis using STRUCTURE, demonstrates the feasibility of distinguishing *P. racemosa* from *P. × hispanica* using only a few microsatellite markers. Specifically, we have the corroboration of two main genetic clusters (Fig. 3), the demographic evidence that the dark-gray cluster contains predominantly older individuals, and the genetic identity of planted populations likely to be *P. × hispanica* from a college campus. These all suggest that the dark-gray genetic cluster in Fig. 3 is the genetic identity of *P. racemosa*. This allows us to assign each sample to a genetically

“pure” cluster representing a parental species and identify hybrids using microsatellite markers alone.

It is important to note that our characterization of samples cannot be made based on specific alleles at any one marker or combination of markers. This is because there are no alleles at any of our loci that are private to one genetic cluster at high frequency. The presence of such alleles would allow us to definitively call the genetic parentage of individuals from pure genetic backgrounds, as well as identify whether hybrids are F1, F2, or even backcrosses (Zalapa et al. 2009). Instead, we show genetic similarity through admixture analysis, which summarizes allele frequencies at the population level. While we are not able to further classify hybrids, our age-class information reveals that hybrids are much more likely to be younger trees—hybrids are almost completely absent older than age-class 5 (Table 2; Figs. 2, 3). The high incidence of hybrid individuals among intermediate age classes (4 and 5) indicates that hybridization between *P. × hispanica* and *P. racemosa* was immediate upon arrival of the former in California. The hybrids also appear to have a higher affinity for our “reference” genotypes of *P. racemosa* (i.e. a mean hybrid index >0.5) across all age classes. However, the hybrid indices for each individual had very large error bars, and the affinity for *P. racemosa* may reflect the more homogenous genotypes in that species.

Introgressive hybridization may do more than simply cloud the genetic identity of *Platanus* in northern California. *Platanus × hispanica* is known to be resistant to several fungal diseases, including Sycamore anthracnose, a fungal disease that defoliates the more susceptible trees of *P. racemosa* (Oswald 2002). Resistance to fungal diseases has resulted in higher fitness for the *P. × hispanica* compared with its own parental species in the eastern United States and Europe (Oswald 2002). Hybrids between *P. racemosa* and *P. × hispanica* may therefore gain a fitness advantage and outcompete the native species, hastening its decline.

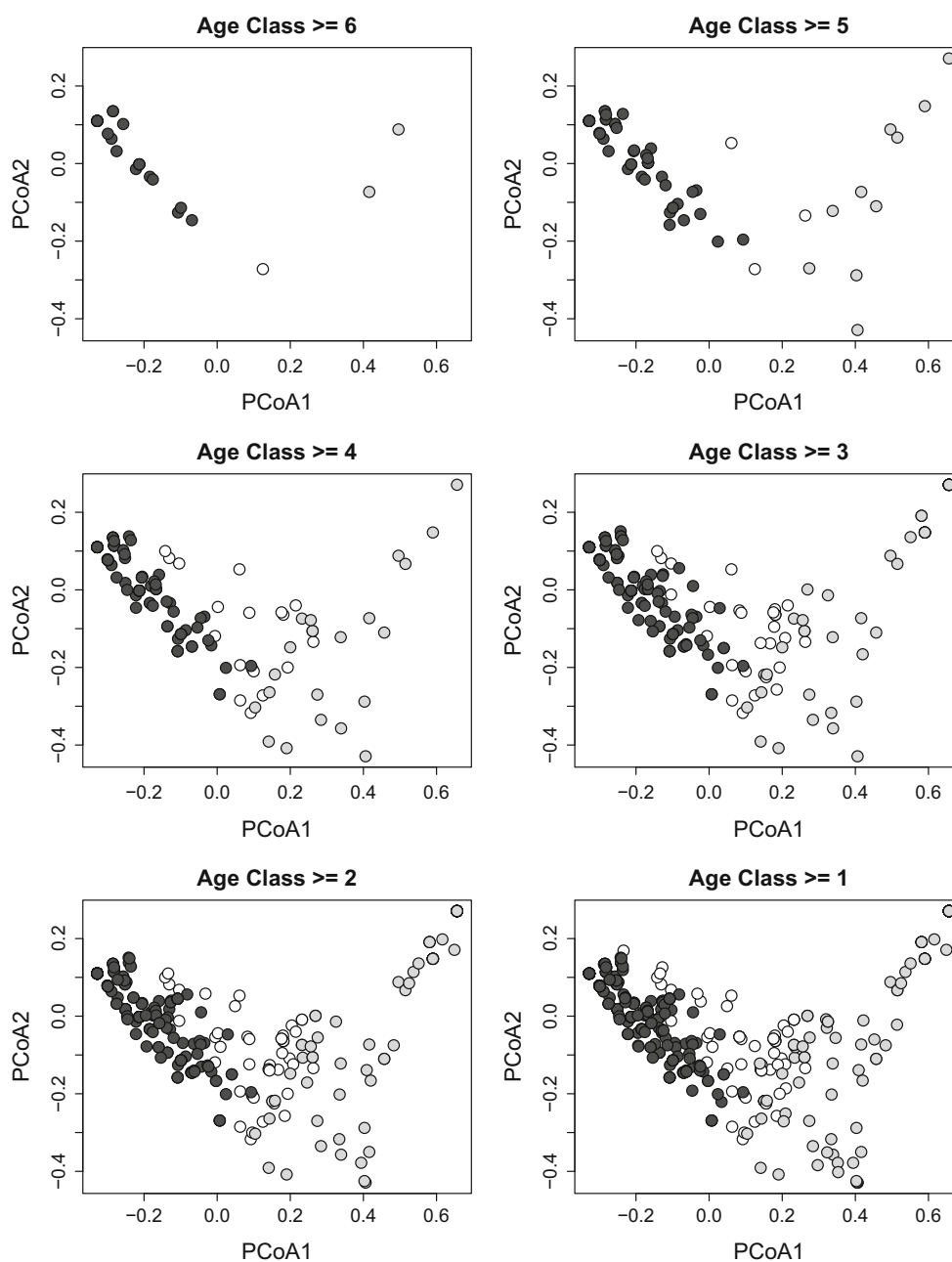


Fig. 3 Principal Coordinate Analysis (PCoA) of the pairwise distance matrix in the reduced dataset (no missing data), organized by age-class. Axis 1 (49 % of total variance) and Axis 2 (9.5 % of total variance) are shown in each plot. In the top-left, only individuals classified as age-class 6 or older are shown; colors correspond to cluster membership from STRUCTURE analysis (admixture less than

10 %, Fig. 2). In the top-right, individuals from age-class 5 have been added, and now includes individuals with more than 10 % admixture from the STRUCTURE analysis (*open circles*). Each successive plot adds one more (younger) age-class; the full dataset is at the bottom-right

Whitlock (2003) also suggests that resistance to disease, while a desirable characteristic in an ornamental street tree, could actually have negative ecological consequences such as reduction of the prevalence of deadwood and trunk cavities. These cavities are important nesting sites for wood ducks (*Aix sponsa*) and ringtail (*Bassariscus astutus*). Lack of deadwood also could have detrimental effects on numerous detritivorous natives. Ecological impacts of

native species displacement by non-native congeners, or hybrids thereof, may be subtle and not easily recognized, especially when they result in characteristics that are desirable to humans. For example, when native prickly pear cactus (*Opuntia littoralis* (Engelm.) Cockerell), hybridizes with non-native mission cactus (*Opuntia ficus-indica* (L.) Mill.), it produces a plant that with much smaller spines. While this may be an attractive outcome to

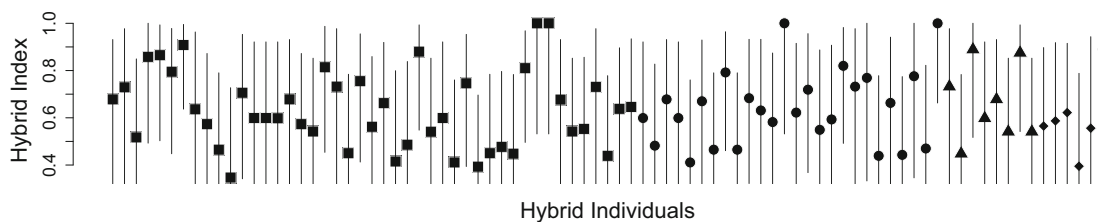


Fig. 4 Hybrid indices for individuals identified as admixed in the STRUCTURE analysis, calculated via the method described by Buerkle (2005). Error bars indicate 95 % CI of maximum likelihood estimate for each individual. Values can range between 1.0 (more

humans, it negatively impacts the cactus wren (*Campylorhynchus brunneicapillus* Lafresnaye) which depends upon the large spines for nest protection and concealment (Solek and Szijj 2004).

While the genetic diversity of *Platanus* in central California has remained relatively stable across age classes, we do observe an increase in effective population size in younger plants. The invasive *P. × hispanica* may be receiving alleles from the native *P. racemosa* through frequent and ongoing hybridization. Future studies may be able to distinguish the specific direction of hybridization and whether *P. racemosa* is more likely to be the pollen or seed parent, perhaps using chloroplast microsatellite loci. It has been previously suggested that invasive species may gain further competitive advantage through hybridization by increasing their genetic diversity and gaining new genotypes for local adaptation, potentially resulting in the extinction of the native species (Ellstrand and Schierenbeck, 2000). Rapid extinction of native plants in response to introgression from invasive species has been extensively documented (e.g. Vilà et al. 2000, Fant et al. 2010, Balao et al. 2015). One important consideration for *P. racemosa* is the magnitude and direction of gene flow; asymmetric introgression, especially if backcrosses occur more frequently with the invasive species, may hurt the survival chances of the native species. In the present study, we do not find evidence that the gene flow is biased in either direction: the hybrid indices are not indicative of backcrosses (near 0 or 1). Therefore, it is possible that *P. racemosa* may also receive alleles for disease resistance, though it may destroy the genetic identity of the native species in the process.

Conservation implications

The consequences of introgressive hybridization may be disastrous for *Platanus racemosa*, especially if heterosis encourages continued back-crossing with *P. × hispanica*. Fortunately for *P. racemosa*, the timeline for acting on conservation concerns in tree species may be extended relative to invasive grasses or other plants, due to the longer generation time in trees (Zalapa et al. 2009). A

similar to *P. racemosa*) and 0.0 (more similar to *P. × hispanica*). Symbols indicate the age-class of the individual: 1 squares; 2 circles; 3 triangles; 4 diamonds

genetic database of tree species in California should be maintained to serve as a reference for the conservation strategy—the genotypic information from this study will be submitted to the Dryad data repository, and can serve as a starting point for such a database. To minimize genetic erosion of the respective native taxa, city arborists should be educated on the potential implications of *P. × hispanica* planting, with the goal of limiting introductions to stands of currently native *P. racemosa*, or alternatively in areas where *P. occidentalis* and *P. orientalis* are native. Further, information could be provided at nurseries to private citizens regarding the impact of planting *P. × hispanica* as “problematic in California and potentially harmful to native species.” Planting native *P. racemosa* saplings may be encouraged instead, although care should be given to ensure that the genetic stock matches that of pure *P. racemosa*, rather than a hybrid individual. A number of larger trees, putatively “pure” *P. racemosa* have been identified in this study and should be used for propagation. GPS coordinates for each tree sampled can be obtained from the authors. Finally, restoration of native *P. racemosa* populations and conservation of riparian habitats should be encouraged, to ensure the health and potential for this important native shade tree species.

Acknowledgments This project was funded by a grant from The Nature Conservancy to KAS. We greatly thank Evan Padgett, Celia Eckert, Clara Buchholtz, and Camellia Boutros for assistance with the collection of the plant material and DNA extractions, and Shannon Still for help creating maps in R. We also thank two anonymous reviewers for their comments on an earlier version of the manuscript. The entire dataset for this study, including microsatellite alleles, GPS coordinates, PCoA results, and admixture analysis, can be found in Supplementary Online Material.

References

- Abbott RJ (1992) Plant invasions, interspecific hybridization and the evolution of new plant taxa. *Trends Ecol Evol* 7:401–405
- Anselmi N, Cardin L, Nicolotti G (1994) Plane decline in European and Mediterranean countries: associated pests and their interactions. *Bull OEPP/EPPO* 24(1):159–171

- Anttila CK, Daehler CC, Rank NE, Strong DR (1998) Greater male fitness of a rare invader (*Spartina alterniflora*, Poaceae) threatens a common native (*Spartina foliosa*) with hybridization. *Am J Bot* 85:1597–1601
- Avery TE, Burkhart HE (1983) *Forest Measurements*. McGraw-Hill Inc, New York
- Balao F, Casimiro-Soriguer R, García-Castraño JL, Terrab A, Talavera S (2015) Big thistle eats the little thistle: does unidirectional introgressive hybridization endanger the conservation of *Onopordum hinojense*? *New Phytol* 206:448–458
- Baldwin B (2012) *The Jepson manual: higher plants of California*. University of California Press, Berkeley
- Besnard G, Tagmount A, Baradat P, Vigouroux A, Berville A (2002) Molecular approach of genetic affinities between wild and ornamental *Platanus*. *Euphytica* 126:401–412
- Buerkle CA (2005) Maximum-likelihood estimation of a hybrid index based on molecular markers. *Mol Ecol Notes* 5:684–687
- Burgess KS, Husband BC (2006) Habitat differentiation and the ecological costs of hybridization: the effects of introduced mulberry (*Morus alba*) on a native congener (*M. rubra*). *J Ecol* 94:1061–1069
- Burgess KS, Morgan M, Deverno L, Husband BC (2005) Asymmetrical introgression between two *Morus* species (*M. alba*, *M. rubra*) that differ in abundance. *Mol Ecol* 14:3471–3483
- Do C, Wapels RS, Peel D, Macbeth GM, Tillet BJ, Ovenden JR (2014) NeEstimator V2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Mol Ecol Res* 14:209–214
- Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size: implications for plant conservation. *Annu Rev Ecol Syst* 24:217–242
- Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? *Proc Natl Acad Sci USA* 97:7043–7050
- Ernst WR (1963) The genera of Hamamelidaceae and Platanaceae in the southeastern United States. *J Arnold Arbor* 44:193–210
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* 14:2611–2620
- Fant JB, Banai A, Havens K, Vitt P (2010) Hybridization between the threatened plant, *Lespedeza leptostachya* Englem. and its co-occurring congener *Lespedeza capitata* Michx.: morphological and molecular evidence. *Conserv Genet*. doi:10.1007/s10592-010-0105-9
- Feng Y, Oh SH, Manos PS (2005) Phylogeny and historical biogeography of the genus *Platanus* as inferred from nuclear and chloroplast DNA. *Syst Bot* 30:786–799
- Golet GH, Brown DL, Carlson M, Gardali T, Henderson A, Holl KD, Howell CA, Holyoak M, Hunt J, Kondolf GM, Larsen EW, Luster RA, McClain C, Nelson C, Paine S, Rainey W, Rubin Z, Shilling F, Silveira JG, Swagerty H, Williams NM, Wood D (2013) Successes, failures and suggested future directions for ecosystem restoration of the Middle Sacramento River, California. *San Francisco Estuary and Watershed Science* 11(3):1–29. <http://www.escholarship.org/uc/item/0db0t6j1>
- Griggs FT (2009) *California riparian habitat restoration handbook*, Second edition, River Partners, Chico. http://www.water.ca.gov/urbanstreams/docs/ca_riparian_handbook.pdf
- Grimm GW, Denk T (2008) ITS evolution in *Platanus* (Platanaceae): homeologues, pseudogenes and ancient hybridization. *Ann Bot* 101:403–419
- Henry A, Flood MG (1919) The history of the London plane (*Platanus acerifolia*). Notes on the genus *Platanus*. *Proc Royal Ir Acad Sect B* 35:9–28
- Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23:1801–1806
- Lang K (2010) *Microsatellite development in Platanus for documenting gene flow among species*. Thesis. California State University, Chico
- Mack MC, D'Antonio CM (1998) Impacts of biological invasions on disturbance regimes. *Trends Ecol Evol* 13:195–198
- Meirmans P, Van Tienderen P (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Mol Ecol Notes* 4:792–794
- Muhlfeld CC, Kalinowski ST, McMahon TE, Taper ML, Painter S, Leary RF, Allendorf FW (2009) Hybridization rapidly reduces fitness of a native trout in the wild. *Biol Lett* 5:328–331
- Nixon KC, Poole JM (2003) Revision of the Mexican and Guatemalan species of *Platanus* (Platanaceae). *Lundellia* 6:103–137
- Oswald VH (2002) *Selected plants of Northern California and adjacent Nevada*. California State University, Chico
- Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in excel. Population genetic software for teaching and research—an update. *Bioinformatics* 28:2537–2539
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155:945–959
- Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. *Annu Rev Ecol Syst* 27:83–109
- Rieseberg LH, Wood TE, Baack A (2006) The nature of plant species. *Nature* 440:524–527
- Santamour FS Jr (1969) New chromosome counts in *Ulmus* and *Platanus*. *Rhodora* 71:544–547
- Santamour FS Jr (1972) Notes: interspecific hybridization in *Platanus*. *For Sci* 18(3):236–239
- Santamour FS, McArdle AJ (1986) Checklist of cultivated *Platanus* (Plane tree). *J Arboric* 12:78–83
- Smith DM, Finch DM (2014) Use of native and non-native nest plants by riparian-nesting birds along two streams in New Mexico. *River Res Appl* 30:1134–1145
- Solek C, Szijj L (2004) *Cactus wren (Campylorhynchus brunneicapillus)*. In: *The Coastal Scrub and Chaparral Bird Conservation Plan: a strategy for protecting and managing coastal scrub and chaparral habitats and associated birds in California*. California Partners in Flight. <http://www.prbo.org/calpif/htmldocs/scrub.html>
- Stralberg D, Toniolo V, Page GW, Stenzel LE (2004) Potential impacts of *Spartina* spread on shorebird populations in South San Francisco Bay. In: *Proceedings of the third international conference on Invasive Spartina*. 175–183
- Stuart JD, Sawyer JO (2001) *Trees and shrubs of California*. University of California Press, Berkeley
- Sudworth GB (1967) *Forest trees of the Pacific slope*. Dover Publications Inc, New York
- USDA, NRCS (2008) *The PLANTS Database*. National Plant Data Center, Baton Rouge (<http://plants.usda.gov>). Accessed 24 Oct 2008
- Vilà M, Weber E, Antonio CMD (2000) Conservation implications of invasion by plant hybridization. *Biol Invasions* 2:207–217
- Waples RS, Do C (2008) LNDE: a program for estimating effective population size from allele frequency changes. *Genetics* 121:379–391
- Whitlock DL (2003) *The hybridization of California sycamore (Platanus racemosa) and the London plane tree (Platanus × acerifolia) in California's riparian woodland*. Thesis. California State University, Chico
- Zalapa JE, Brunet J, Guries RP (2009) Patterns of hybridization and introgression between invasive *Ulmus pumila* (Ulmaceae) and native *U. rubra*. *Am J Bot* 96:1116–1128