Int. J. Plant Sci. 170(5):609–626. 2009.

1058-5893/2009/17005-0005$15.00 DOI: 10.1086/597780

GENETIC DIVERSITY AND GENE EXCHANGE IN *PINUS OOCARPA*, A MESOAMERICAN PINE WITH RESISTANCE TO THE PITCH CANKER FUNGUS (*FUSARIUM CIRCINATUM*)

W. S. Dvorak,[1]* K. M. Potter,† V. D. Hipkins,‡ and G. R. Hodge*

*International Tree Conservation and Domestication Program (CAMCORE), Department of Forestry and Environmental Resources, Box 8008, North Carolina State University, Raleigh, North Carolina 27695, U.S.A.; †Department of Forestry and Environmental Resources, Forest Science Laboratory, 3401 Cornwallis Road, Research Triangle Park, North Carolina 27709, U.S.A.; and ‡USDA Forest Service, National Forest Genetics Laboratory, 2480 Carson Road, Placerville, California 95667, U.S.A.

Eleven highly polymorphic microsatellite markers were used to determine the genetic structure and levels of diversity in 51 natural populations of *Pinus oocarpa* across its geographic range of 3000 km in Mesoamerica. The study also included 17 populations of *Pinus patula* and *Pinus tecunumanii* chosen for their resistance or susceptibility to the pitch canker fungus based on previous research. Seedlings from all 68 populations were screened for pitch canker resistance, and results were correlated to mean genetic diversity and collection site variables. Results indicate that *P. oocarpa* exhibits average to above-average levels of genetic diversity ($A = 19.82$, $A_R = 11.86$, $H_E = 0.711$) relative to other conifers. Most populations were out of Hardy-Weinberg equilibrium, and a high degree of inbreeding was found in the species ($F_{IS} = 0.150$). Bayesian analysis grouped *P. oocarpa* into four genetic clusters highly correlated to geography and distinct from *P. patula* and *P. tecunumanii*. Historic gene flow across *P. oocarpa* clusters was observed ($N_m = 1.1$–2.7), but the most pronounced values were found between *P. oocarpa* and *P. tecunumanii* (low-altitude provenances) in Central America ($N_m = 9.7$). *Pinus oocarpa* appears to have two main centers of diversity, one in the Eje Transversal Volcánico in central Mexico and the other in Central America. Introgression between *P. oocarpa* and *P. tecunumanii* populations appears to be common. *Pinus oocarpa* populations showed high resistance to pitch canker (stemkill 3%–8%), a disease that the species has presumably coevolved with in Mesoamerica. Resistance was significantly correlated to the latitude, longitude, and altitude of the collection site but not to any genetic-diversity parameters or degree of admixture with *P. tecunumanii*.

Keywords: Pinus tecunumanii, *Pinus patula*, microsatellite markers, gene flow, biogeography, hybridization.

Introduction

Forty percent of all the pine species and varieties in the world occur in Mexico (Perry 1991; Farjon and Styles 1997). The high pine diversity in the region is thought to be the result of repeated migrations from mid-latitudes in North America to Mexico as early as the Oligocene (33.7–23.8 Ma; see Graham 1999) and subsequent local speciation in the geologically and climatically diverse mountain ranges of the country (Eguiluz-Piedra 1985; Millar 1993; Farjon 1996). One of the pine subsections that are thought to have evolved in Mexico is the Oocarpae (Axelrod 1967; Perry 1991; Millar 1993; Farjon and Styles 1997). Molecular work by Krupkin et al. (1996) shows that the subsection is monophyletic, but alternate views exist (Geada López et al. 2002; Gernandt et al. 2005). The ancestors of the Oocarpae, including the forerunners of *Pinus oocarpa*, were probably in place by the Miocene (23.8–5.3 Ma; Axelrod and Cota 1993). Many of the closed-cone pines included in the subsection (see Price et al. 1998) are important in plantation forestry in the tropics and subtropics (Barnes and Styles 1983).

[1] Author for correspondence; e-mail: dvorak@unity.ncsu.edu.

Manuscript received November 2008; revised manuscript received January 2009.

Pinus oocarpa Schiede ex Schlechtendal var. *oocarpa*, a five-needle hard pine in the Oocarpae subsection, is the most common pine in Mesoamerica. It occurs from southern Sonora, Mexico (28°10′N), to northern Nicaragua (12°40′N), a distance of 3000 km (fig. 1). It is a small (10–13 m), rustic-looking tree on dry sites in the Sierra Madre Occidental of northwestern Mexico but becomes a much taller (20–35 m), better-formed tree in areas of adequate rainfall from southern Mexico through Nicaragua. The presence of *P. oocarpa* in forest ecosystems is very dependent on the frequency and intensity of fires, which suppress more competitive broadleaf species (Deneven 1961; Robbins 1983).

Because of its extensive geographic range, *P. oocarpa* is thought to be the ancestral species of the subsection by some authors (Axelrod and Cota 1993; Dvorak et al. 2000*b*). Analysis of the evolutionary relationships among 10 taxa in the Oocarpae using RAPD markers confirmed that *P. oocarpa* from eastern Mexico/Central America was the core element from which most other species in the subsection evolved (Dvorak et al. 2000*b*). *Pinus oocarpa* from other geographical areas, such as northwestern Mexico in the Sierra Madre Occidental, appears to represent historically recent colonization, a hypothesis consistent with geologic information on volcanism and mountain

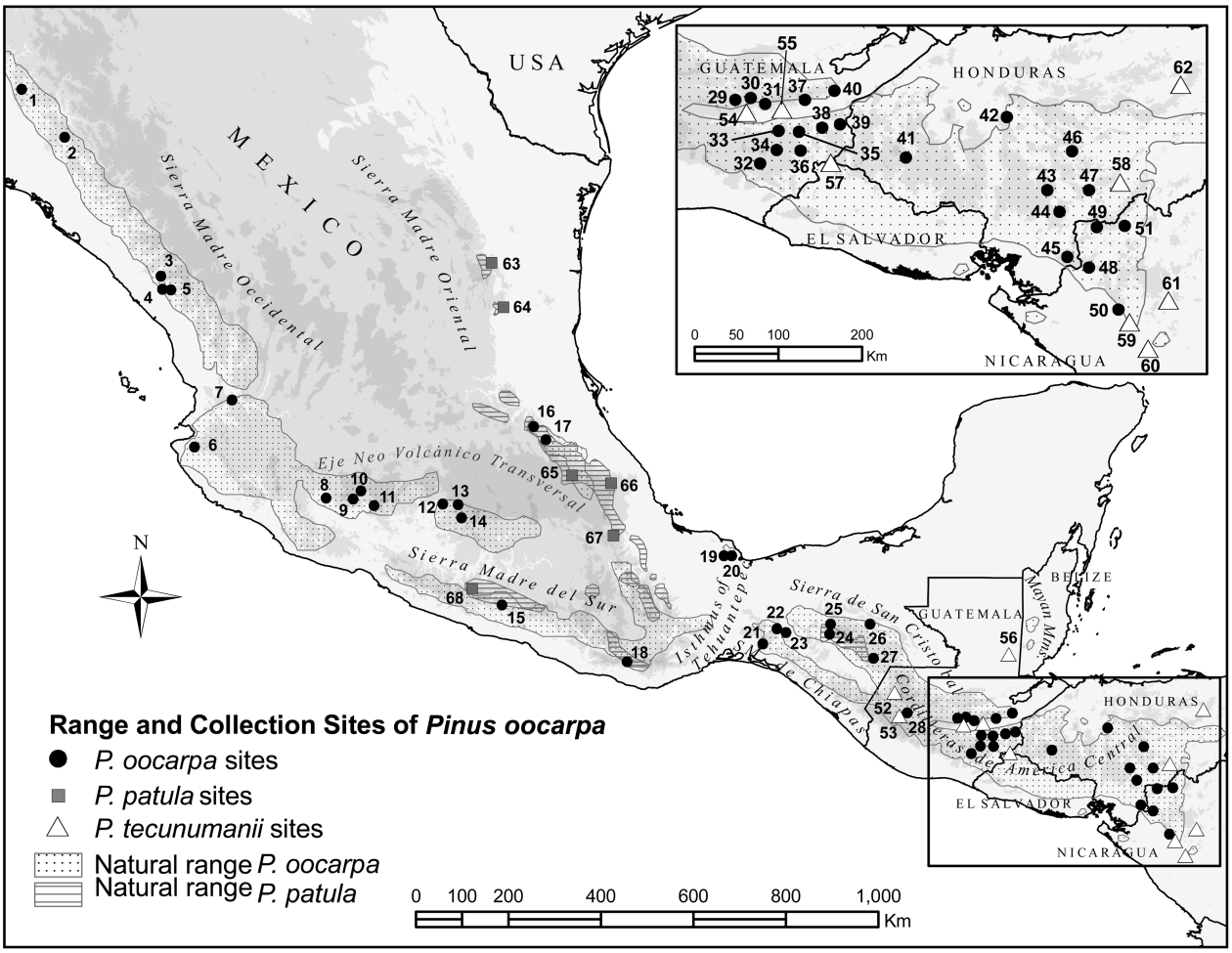

Fig. 1 Provenance locations of *Pinus oocarpa*, *Pinus tecunumanii*, and *Pinus patula*. See table 1 for provenance information.

building in the area (Dvorak et al. 2000*b*). Therefore, a plausible scenario based on the phylogenetic information from the RAPD study is that *P. oocarpa* migrated north into northwestern Mexico and southeast into Central America from some evolutionary center that formed in eastern Mexico/Central America. Questions still remain about the specific geographical center of diversity of *P. oocarpa*, how genetic diversity is structured in the species, and the extent of gene exchange with closely related closed-cone pines, such as *Pinus tecunumanii*.

Interpretations of results from the RAPD study also suggested that *P. tecunumanii* Eguiluz & Perry is a direct and probably recent descendent of *P. oocarpa* (Dvorak et al. 2000*b*) and the only pine species endemic to Central America, with the possible exception of the tropical pine *Pinus caribaea* Morelet var. *hondurensis* (Sénéclauze) Barrett & Golfari. *Pinus tecunumanii* grows sympatrically with *P. oocarpa* from Chiapas southward through central Nicaragua. We have separated *P. tecunumanii* into two major subpopulations for breeding purposes based on the altitude of its occurrence in natural stands, high-elevation (THE, >1500 m) and low-elevation (TLE, <1500 m), because of subtle morphological differences (Dvorak 1986). Seed collectors in the field often have difficulty in determining where *P. tecunumanii* begins and *P. oocarpa* ends because a myriad of intermediate morphologic forms exist in the transition areas of natural stands where both occur. RAPD markers can separate the two species by means of a species bulking technique (Grattapaglia et al. 1993) but cannot readily distinguish between individual populations of the two species (Furman and Dvorak 2005).

Pinus oocarpa is no longer as important to plantation forestry as it once was because it has been replaced by faster-growing *P. tecunumanii* in the tropics and subtropics (Dvorak et al. 2000*a*). However, the question of gene flow and admixture between the two species raises practical concerns in genetic trials when selecting trees in provenances. Research foresters assume that poor growth of *P. tecunumanii* in provenance trials is the result of *P. oocarpa* introgression in natural stands and that good growth of *P. oocarpa* is because of *P. tecunumanii* admixture.

The question of gene admixture between the two species in natural stands also influences questions of disease resistance. *Pinus oocarpa* has recently received great attention as a possible hybrid parent with other species in the subsection because of its high resistance to the pitch canker fungus *Fusarium circinatum* Nirenberg & O'Donnell (Hodge and Dvorak 2000). The pathogen appears to be an opportunistic fungus that represents a benign problem in natural stands of pines in Central America and Mexico (Winkler and Gordon 2000), but it is an important

problem in pine nurseries in several areas in the southern hemisphere on such species as *Pinus radiata* D. Don and *Pinus patula* Schiede ex Schlechtendal & Chamisso (Viljoen et al. 1994; Britz et al. 2001), and it has been found to kill older trees in plantations (Coutinho et al. 2007). On the basis of molecular data, pitch canker is thought to have originated in Mexico (Winkler and Gordon 2000). If this is the case, the disease may have coevolved with *P. oocarpa*. Little information exists on trends in variation for *P. oocarpa* other than for five sources from Guatemala, a seedling bulk of which was found to be highly resistant to pitch canker in greenhouse screening studies (Hodge and Dvorak 2000). Surprisingly, high-elevation populations of *P. tecunumanii* are moderately susceptible to the disease but exhibit great provenance variation, while low-elevation populations of *P. tecunumanii* are, like *P. oocarpa*, mostly resistant to pitch canker (Hodge and Dvorak 2000, 2007). It is important for breeders to quantify the amount of provenance variation in pitch canker resistance in *P. oocarpa* across its 3000-km range and to know whether high-elevation *P. tecunumanii* populations that show the best resistance to the pitch canker disease are simply those with the greatest historic gene exchange with *P. oocarpa*.

In this article, we examine population structure and trends in genetic diversity within *P. oocarpa* var. *oocarpa* by assessing 50 natural populations of the species, and we also include one population of *P. oocarpa* var. *microphylla* Shaw (syn. *Pinus praetermissa* Styles & McVaugh) and 17 control lots (provenances) of *P. tecunumanii* and *P. patula* from Mexico and Central America, using nuclear microsatellite markers. We attempt to better define the evolutionary center of *P. oocarpa* to understand its historical migration routes through Mesoamerica. We also screen open-pollinated seedlings from the 51 *P. oocarpa* populations plus the 17 control lots for pitch canker resistance. We quantify provenance variation in pitch canker resistance across the range of *P. oocarpa* in Mesoamerica. We hypothesize that the genetic history of *P. oocarpa* might parallel evolutionary trends in the migration of the pitch canker fungus if, indeed, the two had historically intertwining relationships. We determine levels of gene admixture between *P. oocarpa* and *P. tecunumanii* to better understand growth performance in field trials and pitch canker resistance patterns among populations.

Material and Methods

Provenance Collections

This study encompasses 68 provenances from three associated pine species in Central America and Mexico: *Pinus oocarpa*, *Pinus tecunumanii*, and *Pinus patula*. The *P. oocarpa* collection of 50 provenances sampled the entire natural distribution of the species, from northwestern Mexico to central Nicaragua (fig. 1; table 1), in addition to one population of *P. oocarpa* var. *microphylla*. The 17 additional provenances of *P. tecunumanii* and *P. patula* were chosen for their resistance or susceptibility to the pitch canker fungus based on provenance-screening results summarized by Hodge and Dvorak (2007). Eleven of the 17 provenances were *P. tecunumanii*, five from high-elevation (THE) regions above 1500 m altitude in Mesoamerica (San Jerónimo and Montecristo, considered moderately resistant; Cabricán, Pinalón, and Chiquival Viejo, considered susceptible) and six from low-elevation (TLE) areas below 1500 m altitude (Sacul Arriba, San Esteban, Villa Santa, Yucul, Cerro la Joya, and La Rinconada, all considered resistant). Six provenances of *P. patula* were sampled (Conrado Castillo, El Cielo, and Yextla, considered moderately resistant; Corralitla, Llano de las Carmonas, and Cruz Blanca, considered susceptible). The classification of *P. tecunumanii* THE and *P. patula* provenances as resistant or susceptible is relative to their respective species means.

The Yextla population of *P. patula* was the varietal form *longipedunculata* Loock ex Martínez; its taxonomic classification was confirmed in an earlier study by Dvorak et al. (2001). In addition, a bulk seedlot of *Pinus taeda* L. was included as an outgroup for the construction of neighbor-joining (NJ) trees.

Thirty-five of the *P. oocarpa* provenances, in addition to all the *P. tecunumanii* and *P. patula* provenances, were collected from natural stands by CAMCORE, an international tree conservation and domestication program at North Carolina State University, between 1985 and 2007. Within each provenance, seedlots generally were collected from 10 open-pollinated mother trees located at least 100 m apart, with selections emphasizing trees with better form and volume whenever possible (Dvorak et al. 1999). Another 18 *P. oocarpa* provenances were donated to CAMCORE by the Oxford Forestry Institute (OFI) of England for the study. These included provenances from Mexico and Central America that were part of an international trial series sponsored by OFI and the Instituto Nacional de Investigaciónes Forestales (INIF) Mexico in the late 1970s and summarized by Greaves (1979). The OFI/INIF provenance collections were bulked seedlots from at least 25–50 mother trees spaced 100 m apart. Of the 18 provenances, one provenance was used to supplement CAMCORE collections made at the same location (Mal Paso, Guatemala), and one was an additional provenance (Valle de Angeles, Honduras) represented by 10 individual tree seedlots rather than a single bulked provenance seedlot (table 1).

The latitude and longitude positions of all OFI/INIF collections in the 1970s and of many of the CAMCORE collections in the 1980s and 1990s were determined by using government maps drawn to a scale of 1 : 50,000. Collectors did the best job possible of correctly identifying the location of each provenance, but the coordinates of exact locations were sometimes incorrect. It is apparent, upon review of the provenance list (table 1), that various collectors occasionally used different names and provided slightly different coordinates for the same collection sites. Affected provenances include San Jerónimo (provenance 29 in table 1) and Chuacús (30) from Guatemala; Capilla del Taxte (4) and La Petaca (5) from Sinaloa, Mexico; Taretan (9) and Tzararacua (10) from Michoacán, Mexico; and Ocotal Chico (19) and San Sebastián Solteapán (20) from Veracruz, Mexico (fig. 1). Even though these might represent the same collection area, we decided to leave the apparent duplicates in both the genetic-diversity and pitch-canker-screening studies because seed collection periods in some cases were separated by as many as 30 yr and the exact collection location might have varied in altitude by as much as 300 m.

Microsatellite Study

Tissue harvesting. For each of the provenances included in the study, the seeds were treated with a 24-h water soaking

Table 1

Location and Seed Collection Information for Each of the Provenances in the Study

ID	Taxon	Provenance	State/department, country	*N*	Latitude (°N)	Longitude (°W)	Elevation (m)	Collection type
1	*Pinus oocarpa*	Chinipas	Chihuahua, Mexico	10	27.310	108.597	1460	CAM
2	*P. oocarpa*	Mesa de los Leales	Chihuahua, Mexico	10	26.376	107.765	1305	CAM
3	*P. oocarpa*	Duraznito Picachos	Durango, Mexico	10	23.680	105.894	1615	CAM
4	*P. oocarpa*	Capilla del Taxte	Sinaloa, Mexico	10	23.421	105.865	1260	CAM
5	*P. oocarpa*	La Petaca	Sinaloa, Mexico	10	23.418	105.804	1635	CAM
6	*P. oocarpa*	El Tuito	Jalisco, Mexico	10	20.358	105.245	950	CAM
7	*P. oocarpa* var. *microphylla*	Ocotes Altos	Nayarit, Mexico	9	21.269	104.513	1450	CAM
8	*P. oocarpa*	El Durazno	Jalisco, Mexico	9	19.367	102.683	750	OFI[a]
9	*P. oocarpa*	Taretan/Uruapan	Michoacán, Mexico	10	19.417	102.067	1610	CAM
10	*P. oocarpa*	Tzararacua	Michoacán, Mexico	10	19.417	102.033	1400	OFI[a]
11	*P. oocarpa*	Los Negros	Michoacán, Mexico	10	19.217	101.750	1710	OFI[a]
12	*P. oocarpa*	El Llano	Michoacán, Mexico	10	19.250	100.417	1760	OFI[a]
13	*P. oocarpa*	Valle de Bravo	México, Mexico	10	19.233	100.117	1870	CAM
14	*P. oocarpa*	Tenería	México, Mexico	10	18.983	100.050	1760	CAM
15	*P. oocarpa*	El Campanario	Guerrero, Mexico	10	17.284	99.266	1528	CAM
16	*P. oocarpa*	Chinameca	Hidalgo, Mexico	10	20.750	98.650	1550	OFI[a]
17	*P. oocarpa*	Huayacocotla	Veracruz, Mexico	10	20.500	98.417	1300	CAM
18	*P. oocarpa*	San Sebastián Coatlán	Oaxaca, Mexico	10	16.183	96.833	1750	CAM
19	*P. oocarpa*	Ocotal Chico	Veracruz, Mexico	10	18.250	94.867	550	CAM
20	*P. oocarpa*	San Pedro Solteapán	Veracruz, Mexico	10	18.250	94.850	602	CAM
21	*P. oocarpa*	El Jícaro	Oaxaca, Mexico	8	16.533	94.200	1000	CAM
22	*P. oocarpa*	La Cascada	Chiapas, Mexico	10	16.833	93.833	900	OFI[a]
23	*P. oocarpa*	Cienega de Leon	Chiapas, Mexico	10	16.750	93.750	1100	OFI[a]
24	*P. oocarpa*	El Sanibal	Chiapas, Mexico	10	16.833	92.917	1180	OFI[a]
25	*P. oocarpa*	La Florida	Chiapas, Mexico	10	16.917	92.883	1625	OFI[a]
26	*P. oocarpa*	La Codicia	Chiapas, Mexico	10	16.917	92.117	1200	OFI[a]
27	*P. oocarpa*	La Trinitaria	Chiapas, Mexico	10	16.250	92.050	1450	OFI[a]
28	*P. oocarpa*	Las Peñas–Cucal	Huehuetenango, Guatemala	10	15.200	91.500	1835	CAM
29	*P. oocarpa*	San Jerónimo	Baja Verapaz, Guatemala	10	15.050	90.300	1508	CAM
30	*P. oocarpa*	Chuacús	Baja Verapaz, Guatemala	10	15.033	90.267	1300	OFI[a]
31	*P. oocarpa*	El Castaño	El Progreso, Guatemala	10	15.017	90.150	1130	CAM
32	*P. oocarpa*	Tapalapa	Santa Rosa, Guatemala	10	14.400	90.150	1488	CAM
33	*P. oocarpa*	La Lagunilla	Jalapa, Guatemala	10	14.700	89.950	1635	CAM
34	*P. oocarpa*	San José La Arada	Chiquimula, Guatemala	10	14.667	89.950	788	CAM
35	*P. oocarpa*	El Pinalón	El Progreso, Guatemala	8	14.717	89.767	1350	CAM
36	*P. oocarpa*	San Luis Jilotepeque	Jalapa, Guatemala	10	14.617	89.767	980	CAM
37	*P. oocarpa*	San Lorenzo	Zacapa, Guatemala	10	15.083	89.667	1675	CAM
38	*P. oocarpa*	La Mina	Chiquimula, Guatemala	8	14.800	89.417	895	CAM
39	*P. oocarpa*	Camotán	Chiquimula, Guatemala	10	14.817	89.367	850	CAM
40	*P. oocarpa*	Mal Paso	Zacapa, Guatemala	10	15.183	89.350	1040	OFI/CAM
41	*P. oocarpa*	La Campa	Lempira, Honduras	10	14.467	88.583	1258	CAM
42	*P. oocarpa*	Pimientilla	Comayagua, Honduras	10	14.900	87.500	750	OFI[a]
43	*P. oocarpa*	Valle de Angeles	Francisco Morazán, Honduras	10	14.117	87.067	1300	OFI
44	*P. oocarpa*	Guinope el Paraíso	El Paraíso, Honduras	10	13.883	86.933	1300	OFI[a]
45	*P. oocarpa*	San Marcos de Colón	Choluteca, Honduras	10	13.400	86.850	1120	CAM
46	*P. oocarpa*	Guaimaca	Francisco Morazán, Honduras	10	14.533	86.800	920	CAM
47	*P. oocarpa*	Las Crucitas	El Paraíso, Honduras	10	14.117	86.617	1060	CAM
48	*P. oocarpa*	San José Cusmapa	Madriz-Nuevo Segovia, Nicaragua	10	13.283	86.617	1345	CAM
49	*P. oocarpa*	Dipilto	Nueva Segovia, Nicaragua	10	13.717	86.533	1100	CAM
50	*P. oocarpa*	Cerro Bonete	León, Nicaragua	10	12.833	86.300	950	OFI[a]
51	*P. oocarpa*	San Nicolás	Nuevo Segovia, Nicaragua	10	13.733	86.233	863	CAM
52	*P. tecunumanii* THE	Cabricán	Quetzaltenango, Guatemala	10	15.583	91.633	2590	CAM
53	*P. tecunumanii* THE	Chiquival Viejo	Quetzaltenango, Guatemala	10	15.129	91.543	2300	CAM
54	*P. tecunumanii* THE	San Jerónimo	Baja Verapaz, Guatemala	10	15.050	90.300	1735	CAM
55	*P. tecunumanii* THE	El Pinalón	El Progreso, Guatemala	10	14.983	89.917	2435	CAM
56	*P. tecunumanii* TLE	Sacul Arriba	Petén, Guatemala	10	16.325	89.419	575	CAM
57	*P. tecunumanii* THE	Montecristo	Santa Ana, El Salvador	10	14.412	89.392	1775	CAM
58	*P. tecunumanii* TLE	Villa Santa	El Paraíso, Honduras	10	14.200	86.283	900	CAM
59	*P. tecunumanii* TLE	La Rinconada	Matagalpa, Nicaragua	8	12.700	86.183	950	CAM

Table 1

(***Continued***)

ID	Taxon	Provenance	State/department, country	*N*	Latitude (°N)	Longitude (°W)	Elevation (m)	Collection type
60	*P. tecunumanii* TLE	Cerro la Joya	Matagalpa, Nicaragua	10	12.417	85.983	1050	CAM
61	*P. tecunumanii* TLE	Yucul	Matagalpa, Nicaragua	10	12.933	85.767	1040	CAM
62	*P. tecunumanii* TLE	San Esteban	Olancho, Honduras	10	15.250	85.633	900	CAM
63	*P. patula*	Conrado Castillo	Tamaulipas, Mexico	10	23.933	99.467	1780	CAM
64	*P. patula*	El Cielo	Tamaulipas, Mexico	10	23.067	99.233	1665	CAM
65	*P. patula*	Llano de Carmonas	Puebla, Mexico	10	19.800	97.900	2705	CAM
66	*P. patula*	Cruz Blanca	Veracruz, Mexico	10	19.650	97.150	2500	CAM
67	*P. patula*	Corralitla	Veracruz, Mexico	10	18.633	97.100	2115	CAM
68	*P. patula* var. *longipedunculata*	Yextla	Guerrero, Mexico	10	17.598	99.843	2295	CAM
69	*P. taeda*	Bulk	Rangewide (USA)	10	...	...	...	TIP[a]

Note. THE = high-elevation; TLE = low-elevation; CAM = CAMCORE; OFI = Oxford Forestry Institute; TIP = North Carolina State University Tree Improvement Program.

[a] Bulk collection.

before they were sowed in the greenhouse into Ray Leach super cells using a soil medium that was three parts composted pine bark, one part perlite, and one part coarse sand. Fifty milligrams of fresh leaf tissue was harvested from each of 680 seedlings within 4 mo of germination, with DNeasy Plant Mini Kits (Qiagen, Chatsworth, CA) used to extract genomic DNA from the foliage samples.

Microsatellite analysis. To select a set of microsatellite markers for this study, we first screened 23 microsatellites isolated from *P. taeda* and *Pinus radiata* that had previously demonstrated cross-specific amplification and polymorphism in hard-pine species other than those from which they were isolated (Shepherd et al. 2002; Chagné et al. 2004; Liewlaksaneeyanawin et al. 2004; Shepherd and Williams 2008). The 20 *P. taeda* primers were described by Elsik et al. (2000), Elsik and Williams (2001), Auckland et al. (2002), and Shepherd et al. (2002), while the *P. radiata* primers were described by Fisher et al. (1998) and Chagné et al. (2004). Size homoplasy, which occurs when a high microsatellite mutation rate causes alleles to become similar by state and not descent (Estoup et al. 2002), can lead to biased results among highly divergent species (Selkoe and Toonen 2006). We do not believe this to be the case in this study, however, because microsatellite mutation rates are thought to be low enough for the markers to be applicable among populations or taxa separated by up to a few thousand generations (Jarne and Lagoda 1996) or belonging to the same subgenus (Glaubitz and Moran 2000). Each microsatellite primer set was screened across a set of 15 samples, including eight *P. oocarpa* seedlings, four each from provenances in the northwestern and southeastern parts of the species' range; three *P. tecunumanii* seedlings, one from a high-elevation provenance and two from low-elevation provenances; and one seedling each of *P. patula*, *P. radiata*, *P. taeda*, and *Pinus maximinoi* H.E. Moore. After this screening, we selected 13 polymorphic loci to run across all 680 samples, with two loci later discarded because of difficulty in making consistent allele calls.

Polymerase chain reaction (PCR) amplification was performed in 8-μL reaction volumes containing 20 ng genomic DNA, 0.16 μM of the M13 fluorescent primer label, 0.04 μM of the forward primer (except for 0.04 μM for *PtTX3025*), 0.16 μM of the reverse primer, 0.2 mM dNTPs, 1X *Taq* buffer, 2.0 mM $MgCl_2$ (except for 2.5 mM for *PtTX2080*, *PtTX3024*, and *PtTX3025*), and 0.08 units of HotStar *Taq* DNA polymerase (Qiagen, Valencia, CA). The PCRs were completed with the following protocol on PTC-100 thermal cyclers (MJ Research, Watertown, MA): 15 min at 95°C; three cycles of 30 s at 94°C (denaturation), 30 s at 60°C (annealing), and 1 min at 72°C (extension); three cycles of 30 s at 94°C, 30 s at 57°C, and 1 min at 72°C; and 30 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C; all followed by a final 15 min extension at 72°C and an indefinite hold at 4°C. The resulting PCR products were separated on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA), as recommended by the manufacturer. Peaks were sized and binned, and then alleles were called by using GeneMarker 1.51 (SoftGenetics, State College, PA), with GS(500-250)LIZ as an internal size standard for each sample.

Data analysis. Allele calls from 11 microsatellite loci (table 2) were used to conduct a wide variety of population genetic analyses. FSTAT, version 2.9.3.2 (Goudet 1995), calculated expected heterozygosity (H_E), observed heterozygosity (H_O), and Weir and Cockerham (1984) within-population inbreeding coefficient (F_{IS}) values across loci. In addition, FSTAT generated basic provenance-level measures of genetic diversity, including allelic diversity (A) and mean allelic richness (A_R). It also estimated among-population divergence (F_{ST}) within species as well as pairwise F_{ST} between provenances within species. The Genetic Data Analysis package (Lewis and Zaykin 2001) was used to calculate provenance-level heterozygosity and private alleles. Exact tests for Hardy-Weinberg equilibrium for each locus and provenance were conducted with GENEPOP (Raymond and Rousset 1995), and estimated null allele frequencies for each locus (Brookfield 1996) were estimated with MicroChecker 2.2.3 (van Oosterhout et al. 2004).

We applied a set of Bayesian analysis tools in BAPS 5.1 (Corander et al. 2003) to survey microsatellite variation in *P. oocarpa*, *P. tecunumanii*, and *P. patula*. This kind of analysis has the advantage of combining information from several loci into a single probability model rather than simply averaging across loci, as required in traditional F_{ST} analysis (Corander et al.

Table 2

Description of the 11 Microsatellite Markers Used in the Study and Measures of Genetic Variation, Inbreeding, Deviation from Hardy-Weinberg Equilibrium, and Estimated Null Allele Frequency for Each

Locus	Source	Size range (bp)	A	H_E	H_O	F_{IS}	HWE (P)	Prop. null	Reference
NZPR5	*Pinus radiata*	90–109	12	.732	.562	.165	<.05	.055	Fisher et al. 1998
NZPR114	*P. radiata*	138–196	34	.921	.768	.095	<.05	.119	Chagné et al. 2004
NZPR1078	*P. radiata*	356–396	33	.766	.460	.349	<.05	.155	Chagné et al. 2004
PtTX2123	*Pinus taeda*	216–233	7	.584	.420	.077	<.05	.110	Elsik et al. 2000
PtTX2146	*P. taeda*	163–252	22	.769	.658	.031	<.05	.062	Elsik et al. 2000
PtTX3011	*P. taeda*	168–233	22	.709	.646	.015	ns	.068	Elsik et al. 2000
PtTX3013	*P. taeda*	154–188	13	.768	.579	.074	<.05	.220	Elsik et al. 2000
PtTX3025	*P. taeda*	257–305	25	.804	.438	.390	<.05	.148	Elsik et al. 2000
PtTX3034	*P. taeda*	219–264	35	.904	.749	.092	<.05	.177	Elsik et al. 2000
PtTX3107	*P. taeda*	179–206	13	.714	.496	.122	<.05	.132	Elsik and Williams 2001
PtTX3127	*P. taeda*	190–226	15	.179	.159	.082	<.05	.007	Elsik and Williams 2001
Total			231			.143	<.05		
Mean			21.00	.714	.540	.136		.114	

Note. With the exception of size range, results exclude outgroup species. A = alleles per locus; H_E = expected heterozygosity; H_O = observed heterozygosity; F_{IS} = inbreeding coefficient; HWE = Hardy-Weinberg exact test of heterozygote deficiency; ns = not significant; Prop. null = estimated proportion of null alleles (Brookfield 1996).

2003). In one analysis, we surveyed the spatial genetic structure of the 68 provenances in the study (Corander et al. 2008), testing the likelihood that the provenances could be grouped into a number of genetic clusters (k) between 2 and 68. The k with the minimum log likelihood was then selected. This analysis was then repeated for the provenances within each species to test the consistency of this approach across taxonomic scales, with $k = 2$–51 for *P. oocarpa*, $k = 2$–11 for *P. tecunumanii*, and $k = 2$–6 for *P. patula*. In addition, we investigated the possibility of genetic admixture within provenances by pooling individuals independently of their sample structure (Corander et al. 2003). The proportion of the gene cluster ancestry within each provenance was then displayed graphically in map form with ArcMap 9.2 (ESRI 2006).

To further assess the genetic architecture of the three study species, we conducted analyses of molecular variance (AMOVAs), using Arlequin 3.0 (Excoffier et al. 2005), by partitioning the total microsatellite variation into components to allow for the investigation of differentiation among provenances, groups of provenances, and species. Specifically, we conducted five separate AMOVAs based on taxonomy and the results of the BAPS clustering analyses: (1) provenances within species, (2) provenances within clusters for all species, (3) provenances within *P. oocarpa* clusters, (4) provenances within *P. tecunumanii* clusters, and (5) provenances within *P. patula* clusters. The significance of each variance component was assessed with a test of 1000 permutations.

We generated a set of NJ dendrograms to visualize the relationships among provenances and among clusters, because NJ trees have a greater probability of recovering the true topology when population size has not remained constant over time (Takezaki and Nei 1996). These trees were constructed by using the SEQBOOT, GENDIST, NEIGHBOR, and CONSENSE components of PHYLIP 3.6 (Felsenstein 2005), computed from population allelic frequencies using chord genetic distance (D_C; Cavalli-Sforza and Edwards 1967). Chord distance is based on a geometric model that is less biased by null alleles than other genetic distances in microsatellite analyses (Chapuis and Estoup 2007) and does not require assumptions about the model under which microsatellites mutate (Takezaki and Nei 1996). Branch support associated with the topology of the NJ trees was based on 1000 bootstrap replicates. One tree (fig. 2) encompassed the 68 *P. oocarpa*, *P. tecunumanii*, and *P. patula* provenances, and one (fig. 4) included the genetic clusters determined in the BAPS analysis. In both cases, a *P. taeda* bulk provenance was included as an outgroup.

Finally, we estimated interpopulation gene flow (N_m) in GENEPOP (Raymond and Rousset 1995), using the private-allele method (Barton and Slatkin 1986), corrected for sample size. This was conducted for species, BAPS genetic clusters, pairs of provenances within species, and pairs of genetic clusters.

Pitch Canker Screening Study

Bulk seedlots representing 51 *P. oocarpa* provenances and all of the *P. patula* and *P. tecunumanii* provenances were sent to the U.S. Forest Service Resistance Screening Center (RSC) in Bent Creek, North Carolina, for pitch canker screening. The RSC also included a pitch canker–susceptible seedlot of *Pinus elliottii* Engelmann (FA2), the standard protocol in all of its screening efforts.

Seeds were soaked in cold water for 24 h before sowing, and seedlings were grown in Ray Leach containers (115 mL). All seedlings were subjected to the pitch canker–screening protocol developed by Oak et al. (1987), in which seedlings are wounded and inoculated with the pitch canker fungus and any resulting stem infection is measured to gauge relative resistance. Seedlings were grown for 12 wk, at which time they were wounded by severing the stem and removing the top just below the apical meristem. The seedlings were then inoculated by atomizing an aqueous spore suspension onto the fresh wounds, with a concentration of 25,000 spores/mL. The atomized spore suspension was sprayed directly on the wound surface from a distance of ~25 cm, passing three times over each tree. A bulk mix of conidia of *Fusarium circinatum* was prepared according to McRae et al. (1985). Single-spore isolates from four locations in Geor-

gia and Florida in the southeastern United States were used to form the mix.

Each provenance was represented by 120 seedlings, with 20 seedlings in each of six replications. After inoculation at 12 wk, the seedlings were returned to the greenhouse, where they were maintained for 20 additional weeks, during which pathogen colonization of the stem occurred. At 12 and 20 wk, the amount of stem dieback was measured in millimeters, and the percentage of the stem killed (stemkill) was calculated. Analyses of the traits dieback and stemkill were conducted with SAS procedure MIXED, with a mixed model including a fixed effect for replication, a covariate for seedling height, and random effects for provenance, provenance × replication interaction, and error. Least squares means were calculated for all seedlots, and species/variety means were compared by using the PDIFF option. Species and provenance rankings for 12- and 20-wk stemkill and dieback were all very similar, so only the results for 20-wk stemkill are reported here. Correlations between mean provenance stemkill values and population genetic-diversity estimates (A, A_R, and H_E) and the latitude, longitude, elevation, and rainfall of the collection site were calculated and examined.

Results

Microsatellite Analysis

General trends. The 11 microsatellite loci included in the analyses were highly polymorphic, totaling 231 alleles, or a mean of 21 alleles per locus (table 2). Although expected heterozygosity was fairly high (mean of 0.712), exact tests for Hardy-Weinberg equilibrium indicated a significant deficit of heterozygotes for all but one of the loci (*PtTX3011*; table 2). The significantly positive F_{IS} inbreeding coefficient across the species in the study (0.143) was indicative of a considerable deficit of heterozygotes and the likely presence of inbreeding (table 2). Similarly, most provenances of the three species were out of Hardy-Weinberg equilibrium and had positive F_{IS} inbreeding coefficients (table 3).

Pinus oocarpa. The *P. oocarpa* provenances with the highest number of alleles per locus (El Tuito, Duraznito Pichachos, Taretan/Uruapan, Huayacocotla, and Los Negros), highest allelic richness (El Tuito, Los Negros, Teneria, Taretan/Uruapan, and San Sebastián Coatlán), and most private alleles (Duraznito Pichachos, La Petaca, Ocotes Altos, Los Negros, and Tzararacua) were generally located across the Eje Volcánico Transversal and the southern half of the Sierra Madre Occidental (table 3). Of the provenances with the highest inbreeding coefficient F_{IS}, two border the Isthmus of Tehuantepec (Cienega de Leon and Ocotal Chico/San Pedro Solteapán) and two are the northernmost provenances included in the study (Mesa de los Leales and Chinipas), both located in the Sierra Madre Occidental of Chihuahua. Interestingly, several of the least inbred provenances are located in the southeasternmost portion of the *P. oocarpa* distribution, in Guatemala and Honduras (e.g., Guaimaca, Tapalapa, San Marcos de Colón, Pimientilla, Valle de Angeles, and Las Crucitas). Many of these were in Hardy-Weinberg equilibrium. The least differentiated *P. oocarpa* provenances, as defined by their mean pairwise F_{ST} values with all other provenances, were all located in Guatemala (San Lorenzo and El Castaño, separated by 52 km in the Sierra de Las Minas or its foothills; La Lagunilla and La Mina, separated by 42 km in the mountains and foothills south of the Montagua Valley; and Las Peñas–Cucal, in the western part of the country). The most differentiated populations were located near the Isthmus of Tehuantepec (Cienega de Leon, El Jícaro, and Ocotal Chico/San Pedro Solteapán) and in the northern Sierra Madre Occidental (Mesa de los Leales, Chinipas, El Durazno, and La Petaca). Ocotes Altos, the only provenance classified as *P. oocarpa* var. *microphylla*, was by far the most differentiated, with a mean pairwise F_{ST} value of 0.263.

Pinus tecunumanii. Among the *P. tecunumanii* provenances, those classified as coming from high-elevation sources always had a higher mean number of alleles per locus and higher allelic richness than those from lower-elevation locations (table 3). The three provenances with the most private alleles (Chiquival Viejo, El Pinalón, and San Jerónimo, Guatemala) were also from high-elevation sources, followed by two low-elevation provenances from Nicaragua (Cerro la Joya and Yucul). El Pinalón and San Jerónimo are separated by 37 km and occupy ranges of mountains—the Sierra de las Minas and the Sierra de Chuacús—that are geologically the same. The most inbred provenance ($F_{IS} = 0.261$) was San Esteban, isolated from the others in northern Honduras. Mean pairwise F_{ST} values for *P. tecunumanii* provenances were generally smaller than those for *P. oocarpa*, with the most differentiated provenance located farthest southeast, Cerro la Joya in Nicaragua, and the least differentiated provenance, Chiquival Viejo, located in the western part of Guatemala.

Pinus patula. Among *P. patula* provenances, the southern provenance of Corralitla had the most alleles per locus and the greatest allelic richness, while the central provenance of Llano de las Carmonas was the most inbred and the least differentiated from the other provenances, on the basis of mean pairwise F_{ST} values (table 3). The lone provenance classified as *P. patula* var. *longipedunculata* (Yextla) was the most differentiated and had the most private alleles.

Dendrogram clustering the three species. The D_C consensus dendrogram of the 68 provenances grouped *P. patula* separately from *P. oocarpa* and *P. tecunumanii* (fig. 2), with the *P. patula* var. *longipedunculata* provenance (Yextla) sister to the *P. patula* var. *patula* provenances. The topology of the dendrogram suggests an association between the evolutionary relationships and the geographic locations of the *P. oocarpa* and *P. tecunumanii* provenances, which clustered into two clades. One clade encompassed all of the *P. oocarpa* provenances in Mexico and one in Guatemala (Las Peñas–Cucal [28]), while the other included the rest of the Central American *P. oocarpa* provenances and all of the *P. tecunumanii* provenances.

In the Mexican clade, the five northernmost *P. oocarpa* provenances (1–5), all in the Sierra Madre Occidental, grouped with high bootstrap support (86.6%) and were in turn clustered with the single *P. oocarpa* var. *microphylla* provenance (7). This group was in turn clustered with 11 provenances from central Mexico, all associated with the Eje Volcánico Transversal (6, 8–17). Six provenances farther south clustered with high bootstrap support: El Campanario in the Sierra Madre del Sur (15); El Jícaro, La Cascada, and Cienega de Leon in western Chiapas (21–23); and Ocotal Chico and San Pedro Solteapán (19, 20). These last two represent the outlier provenances sampled in different years in Veracruz, and they grouped with

Table 3

Measures of Genetic Variation for Each of 51 *Pinus oocarpa*, 11 *Pinus tecunumanii*, and 6 *Pinus patula* Provenances Based on 11 Nuclear Microsatellite Loci

ID	Provenance	Taxon	A	A_R	AP_{sp}	AP_{all}	H_E	F_{IS}	Mean F_{ST}	Cluster[a]	% stemkill
1	Chinipas	*P. oocarpa*	5.18	3.49	0	0	.606	.257	.143	SMO	5.9
2	Mesa de los Leales	*P. oocarpa*	4.27	3.03	0	0	.533	.273	.151	SMO	3.8
3	Duraznito Picachos	*P. oocarpa*	6.00	3.84	6	4	.630	.216	.114	SMO	2.9
4	Capilla del Taxte	*P. oocarpa*	5.27	3.61	0	0	.620	.171	.112	SMO	2.5
5	La Petaca	*P. oocarpa*	5.45	3.58	5	3	.628	.063	.124	SMO	4.4
6	El Tuito	*P. oocarpa*	6.27	3.96	1	1	.678	.154	.074	EVT	3.1
7	Ocotes Altos	*P. oocarpa* var. *microphylla*	3.45	2.69	5	4	.437	.029	.263	MIC	42.2
8	El Durazno	*P. oocarpa*	4.09	3.08	0	0	.575	.018	.138	EVT	6.9
9	Taretan/Uruapan	*P. oocarpa*	6.00	3.91	1	1	.664	.118	.088	EVT	3.8
10	Tzararacua	*P. oocarpa*	5.64	3.77	3	3	.642	.204	.107	EVT	7.0
11	Los Negros	*P. oocarpa*	5.82	3.94	3	3	.689	.136	.076	EVT	7.8
12	El Llano	*P. oocarpa*	5.55	3.66	2	2	.622	.011	.105	EVT	4.0
13	Valle de Bravo	*P. oocarpa*	5.36	3.86	0	0	.697	.149	.088	EVT	5.1
14	Tenería	*P. oocarpa*	5.64	3.92	0	0	.694	.111	.105	EVT	4.2
15	El Campanario	*P. oocarpa*	5.36	3.80	1	1	.670	.177	.088	SMS	3.1
16	Chinameca	*P. oocarpa*	5.55	3.71	0	0	.672	.095	.088	EVT	5.9
17	Huayacocotla	*P. oocarpa*	5.91	3.87	2	2	.670	.212	.091	EVT	4.4
18	San Sebastián Coatlán	*P. oocarpa*	5.55	3.88	1	1	.689	.201	.084	SMS	5.5
19	Ocotal Chico	*P. oocarpa*	4.36	3.24	0	0	.598	.283	.129	SMS	4.6
20	San Pedro Solteapán	*P. oocarpa*	5.00	3.46	1	1	.589	.274	.124	SMS	4.0
21	El Jícaro	*P. oocarpa*	4.55	3.46	0	0	.623	.111	.141	SMS	3.3
22	La Cascada	*P. oocarpa*	4.73	3.32	0	0	.604	.199	.122	SMS	3.8
23	Cienega de Leon	*P. oocarpa*	4.55	3.44	0	0	.605	.431	.149	SMS	5.1
24	El Sanibal	*P. oocarpa*	4.91	3.47	0	0	.631	.186	.113	SMS	4.3
25	La Florida	*P. oocarpa*	4.55	3.31	1	1	.617	.107	.094	SMS	3.8
26	La Codicia	*P. oocarpa*	5.36	3.77	0	0	.698	.109	.076	SMS	3.0
27	La Trinitaria	*P. oocarpa*	4.45	3.40	0	0	.640	.193	.075	SMS	3.2
28	Las Peñas–Cucal	*P. oocarpa*	5.27	3.76	0	0	.696	.185	.065	SMS	6.7
29	San Jerónimo	*P. oocarpa*	5.09	3.64	0	0	.666	.2	.076	CAC	3.0
30	Chuacús	*P. oocarpa*	5.27	3.67	0	0	.666	.114	.087	CAC	3.1
31	El Castaño	*P. oocarpa*	4.91	3.54	1	1	.672	.186	.065	CAC	2.7
32	Tapalapa	*P. oocarpa*	4.91	3.45	1	0	.638	.002	.077	CAC	3.1
33	La Lagunilla	*P. oocarpa*	5.09	3.53	0	0	.654	.13	.068	CAC	3.0
34	San José La Arada	*P. oocarpa*	5.64	3.66	1	1	.637	.191	.078	CAC	3.1
35	El Pinalón	*P. oocarpa*	4.36	3.31	1	1	.604	.089	.097	CAC	2.7
36	San Luis Jilotepeque	*P. oocarpa*	5.09	3.53	1	0	.644	.234	.077	CAC	2.9
37	San Lorenzo	*P. oocarpa*	5.45	3.78	0	0	.692	.126	.057	CAC	3.9
38	La Mina	*P. oocarpa*	4.82	3.70	0	0	.666	.12	.068	CAC	2.8
39	Camotán	*P. oocarpa*	4.55	3.52	1	1	.665	.164	.063	CAC	2.4
40	Mal Paso	*P. oocarpa*	4.73	3.23	0	0	.563	.218	.088	CAC	2.5
41	La Campa	*P. oocarpa*	5.27	3.59	1	0	.644	.201	.083	CAC	3.4
42	Pimientilla	*P. oocarpa*	4.45	3.32	1	1	.605	.089	.094	CAC	4.4
43	Valle de Angeles	*P. oocarpa*	5.00	3.55	1	0	.655	.101	.073	CAC	4.9
44	Guinope el Paraíso	*P. oocarpa*	4.91	3.41	2	2	.617	.16	.082	CAC	3.3
45	San Marcos de Colón	*P. oocarpa*	4.36	3.27	0	0	.636	.029	.084	CAC	3.2
46	Guaimaca	*P. oocarpa*	5.09	3.48	0	0	.642	.001	.093	CAC	3.1
47	Las Crucitas	*P. oocarpa*	4.27	3.13	0	0	.573	.103	.101	CAC	2.5
48	San José Cusmapa	*P. oocarpa*	5.18	3.58	0	0	.659	.15	.082	CAC	4.2
49	Dipilto	*P. oocarpa*	4.55	3.26	1	1	.594	.122	.101	CAC	2.7
50	Cerro Bonete	*P. oocarpa*	4.55	3.38	0	0	.626	.143	.096	CAC	3.4
51	San Nicolás	*P. oocarpa*	4.73	3.44	0	0	.656	.119	.094	CAC	3.3
52	Cabricán	*P. tecunumanii* THE	5.27	3.68	2	0	.654	.159	.055	TCW	51.4
53	Chiquival Viejo	*P. tecunumanii* THE	5.82	3.92	8	2	.680	.118	.027	TCW	32.1
54	San Jerónimo	*P. tecunumanii* THE	5.27	3.58	5	0	.633	.063	.046	TCW	37.2
55	El Pinalón	*P. tecunumanii* THE	5.45	3.67	6	3	.638	.007	.061	TCW	77.7
56	Sacul Arriba	*P. tecunumanii* TLE	4.82	3.39	3	0	.616	.148	.045	TCW	6.1
57	Montecristo	*P. tecunumanii* THE	5.18	3.49	1	0	.593	.078	.053	TCW	12.1
58	Villa Santa	*P. tecunumanii* TLE	4.45	3.25	3	0	.587	.152	.044	TCE	4.6
59	La Rinconada	*P. tecunumanii* TLE	4.27	3.33	1	0	.601	.153	.035	TCE	4.8
60	Cerro la Joya	*P. tecunumanii* TLE	4.64	3.37	4	0	.608	.06	.070	TCE	5.0

Table 3

(*Continued*)

ID	Provenance	Taxon	*A*	A_R	AP_{sp}	AP_{all}	H_E	F_{IS}	Mean F_{ST}	Cluster[a]	% stemkill
61	Yucul	*P. tecunumanii* TLE	4.09	3.00	4	0	.564	.019	.054	TCE	5.5
62	San Esteban	*P. tecunumanii* TLE	4.27	3.03	2	1	.565	.261	.054	TCE	8.5
63	Conrado Castillo	*P. patula*	4.27	3.25	7	1	.583	.084	.041	PAT	57.5
64	El Cielo	*P. patula*	4.18	3.19	2	0	.568	.193	.033	PAT	60.2
65	Llano de Carmonas	*P. patula*	4.73	3.24	6	1	.529	.254	.024	PAT	71.0
66	Cruz Blanca	*P. patula*	4.82	3.23	8	0	.509	.042	.055	PAT	82.3
67	Corralitla	*P. patula*	4.91	3.37	6	2	.570	.158	.028	PAT	89.1
68	Yextla	*P. patula* var. *longipedunculata*	4.55	3.21	10	0	.599	.089	.079	PTL	60.4

Note. Also shown are the mean F_{ST} values of each with the other provenances within the species and the assignment of each provenance to a genetic cluster from the Bayesian structure analysis using BAPS 5.1 (Corander et al. 2003). THE = high-elevation; TLE = low-elevation; *A* = mean alleles per locus; A_R = mean allelic richness; AP = private (unique) alleles, within species (AP_{sp}) and across species (AP_{all}); H_E = expected heterozygosity; F_{IS} = mean fixation index; F_{ST} = mean F_{ST} differentiation with all other interspecific provenances.

[a] Cluster assignment following Bayesian clustering analysis in BAPS. SMO = Sierra Madre Occidental; EVT = Eje Neo Volcánico Transversal; MIC = *P. oocarpa* var. *microphylla*; SMS = southern Mexican Sierras; CAC = Central American Cordilleras; TCW = western *P. tecunumanii*; TCE = eastern *P. tecunumanii*; PAT = *P. patula*; PTL = *P. patula* var. *longipedunculata*.

83.6% bootstrap support. The Central American clade, meanwhile, consisted of the 23 *P. oocarpa* provenances of central and eastern Guatemala, Honduras, and Nicaragua, along with all of the *P. tecunumanii* provenances.

Bayesian clustering. The Bayesian analyses of provenance structure in BAPS 5.1 further clarified the genetic clustering within *P. oocarpa*, *P. tecunumanii*, and *P. patula*. Specifically, the analysis of spatial genetic structure of all 68 provenances in the study (Corander et al. 2008) found an optimum of seven genetic clusters, with a highly significant posterior marginal probability of 1.0. These corresponded to five clusters within *P. oocarpa*: (1) the Sierra Madre Occidental provenances, (2) the Eje Volcánico Transversal provenances, (3) the southern Mexican Sierras provenances, which include the Sierra Madre del Sur and the Sierra Madre de Chiapas, (4) the Central American provenances, and (5) the single *P. oocarpa* var. *microphylla* provenance, as well as one cluster each for *P. tecunumanii* and *P. patula*. (See table 3 for provenance cluster assignments.) One provenance was assigned to a cluster separate from the species in which it was classified. This was the *P. tecunumanii* Cerro la Joya provenance of Nicaragua, which was placed in the genetic cluster of Central American *P. oocarpa* provenances. The number of *P. oocarpa* clusters and the assignment of provenances to clusters did not change when the analysis was repeated separately for the 51 provenances of this species (posterior marginal probability of 0.86). The same analysis for *P. patula* detected two genetic clusters (posterior marginal probability of 1.0), with one cluster consisting of the single *P. patula* var. *longipedunculata* provenance and the other encompassing the other five provenances. For the analysis of the *P. tecunumanii* provenances alone, the Bayesian analysis found an optimum of two clusters (posterior marginal probability of 1.0), with the clusters divided between the western provenances, in Guatemala and El Salvador, and the eastern provenances, in Honduras and Nicaragua (table 3).

Genetic admixtures between clusters. A separate Bayesian analysis in BAPS 5.1, inferring the genetic-cluster ancestry for each sample tree individually (Corander et al. 2003), found evidence for genetic admixture among genetic clusters within some provenances (fig. 3). For example, several *P. tecunumanii* provenances contained a significant portion of ancestry from the Central American *P. oocarpa* cluster, including Cabricán, Montecristo, Villa Santa, La Rinconada, and Cerro la Joya. The last of these contained ~80% *P. oocarpa* ancestry and 18% *P. tecunumanii* ancestry. Several Central American *P. oocarpa* provenances, meanwhile, have apparently received genetic material from *P. tecunumanii*: La Lagunilla, San José La Arada, Guinope el Paraíso, San José Cusmapa, and San Nicolás. A handful of the provenances in the southern Mexican Sierras *P. oocarpa* cluster contained ancestry from other clusters, particularly from the Central American cluster (Las Peñas–Cucal, La Trinitaria, and El Sanibal). Interestingly, in addition to having ~6% of its genetic composition associated with the Central American cluster, the San Sebastián Coatlán provenance in Oaxaca also had ancestry traceable to the Sierra Madre Occidental cluster (11.9%) and the *P. patula* cluster (13.1%). El Campanario also contained ancestry from the Sierra Madre Occidental cluster (3.1%) and the Eje Volcánico Transversal cluster (2%). Among the Eje Volcánico Transversal provenances, three had evidence of multiple-cluster admixture: El Tuito (4.2% Central American Cordilleras and 2.1% *P. tecunumanii*), Valle de Bravo (6.2% Central American, 2% Sierra Madre Occidental), and Huayacocotla (4.6% *P. patula*). Of the Sierra Madre Occidental provenances, two provenances also demonstrated evidence of admixture: Duraznito Picachos (5.5% Eje Volcánico Transversal) and Capilla del Taxte (4.4% *P. patula*). Finally, of the *P. patula* provenances, only Yextla, the lone representative of var. *longipedunculata*, appeared to have experienced admixture, with 5.5% *P. tecunumanii*, 4.7% Central American *P. oocarpa*, and 2.1% Sierra Madre Occidental *P. oocarpa*.

A D_C consensus dendrogram of the relationships among the genetic clusters (fig. 4) was consistent with the provenance dendrogram (fig. 2), grouping *P. patula* separately from the other two species. The two *P. tecunumanii* clusters were grouped together (52.2%) and then joined with the Central American *P. oocarpa*, with high bootstrap support (96.0%). This group was sister to a clade consisting of the remaining four *P. oocarpa* genetic clusters (47.4% bootstrap support). Within this clade, *P. oocarpa* var. *microphylla* grouped with the Sierra Madre Occidental cluster (60.1% bootstrap support). This group was sister to the *P. oocarpa* cluster to the immediate south, the Eje Volcá-

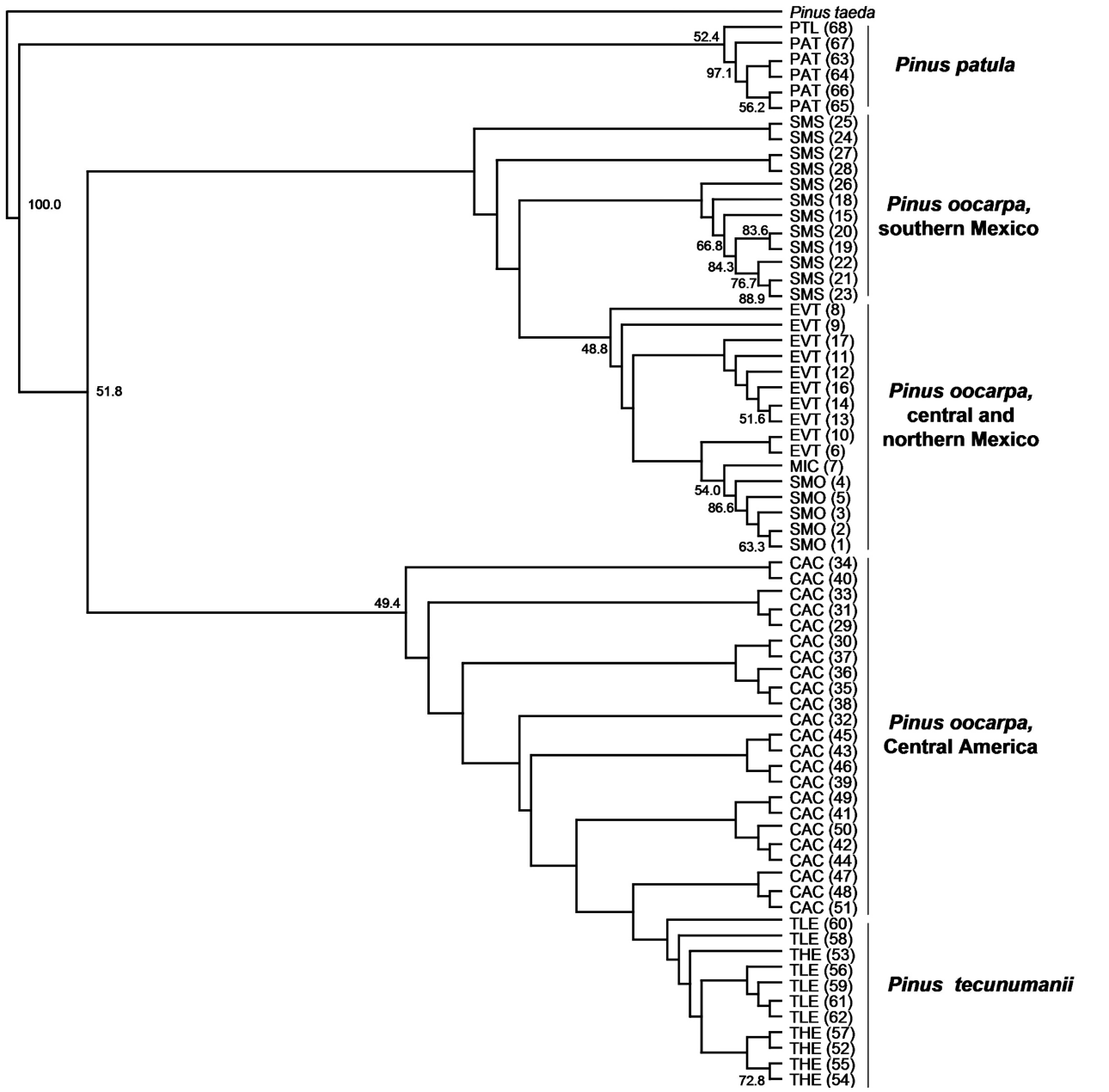

Fig. 2 Neighbor-joining consensus dendrogram depicting chord genetic distances (D_C; Cavalli-Sforza and Edwards 1967) among the 68 provenances of *Pinus oocarpa*, *Pinus tecunumanii*, and *Pinus patula*, with a bulk provenance of *Pinus taeda* as an outgroup. For each provenance, the Bayesian cluster assignment from BAPS 5.1 (Corander et al. 2003) is listed; see table 1 for provenance information. The values represent percent bootstrap support for the nodes of more than 1000 replicates.

nico Transversal cluster; this clade was in turn grouped with the southern Mexican Sierras cluster.

A set of AMOVAs indicated that dividing provenances into genetic clusters explained a higher percentage of genetic variation (11.1%, $F_{CT} = 0.111$) than separating them into species units (7.5%, $F_{CT} = 0.075$; table 4). In both cases, the greatest amount of microsatellite variation was the result of variation among provenances (85.9%, with $F_{ST} = 0.141$, and 83.8%, with $F_{ST} = 0.162$, respectively). In analyses of *P. oocarpa* provenances alone, a similar amount of the variation was explained by differentiation among genetic clusters (10.1%, $F_{CT} = 0.101$). Population-level differentiation was high in the AMOVAs run for each species individually ($F_{ST} = 0.131$ for *P. oocarpa*, 0.075 for *P. tecunumanii*, and 0.083 for *P. patula*).

Within *P. oocarpa*, the Eje Volcánico Transversal cluster had the highest values for several genetic-diversity statistics, despite having a population size ($n = 99$) considerably lower than the clusters to its south (table 5). It had the highest allelic richness, the highest expected heterozygosity, the most overall private alleles, and the most private alleles within the species, along with

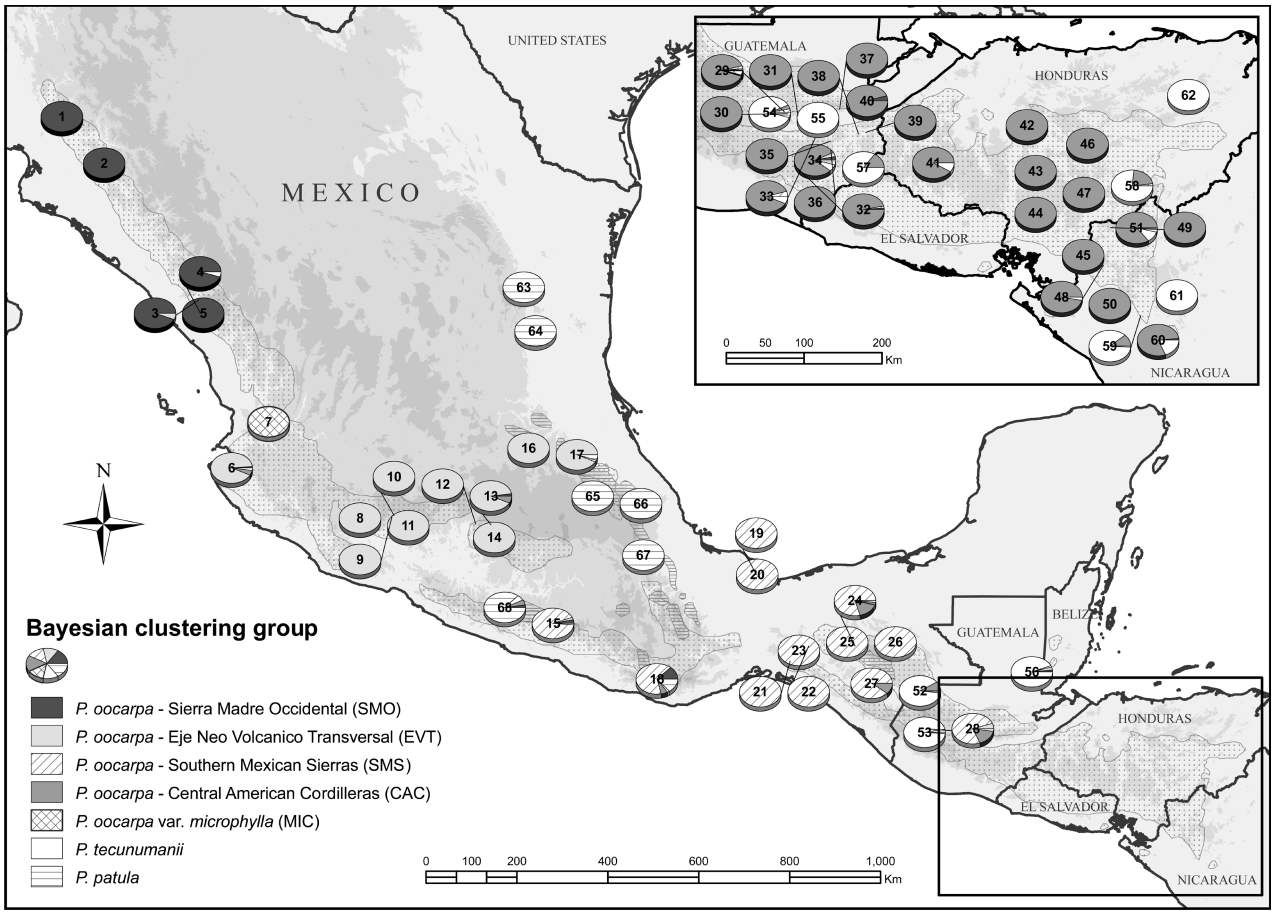

Fig. 3 Estimated proportion of ancestry of each *Pinus oocarpa*, *Pinus tecunumanii*, and *Pinus patula* provenance from the genetic clusters defined in BAPS 5.1 (Corander et al. 2003), with individuals pooled independently of their sample structure. See table 1 for provenance information.

the Central American cluster, which had a much larger population size ($n = 226$). It was also less inbred than most of the other clusters and was the least differentiated, having the lowest mean pairwise F_{ST} value with the other *P. oocarpa* clusters. The two clusters at the center of the *P. oocarpa* distribution, the Eje Volcánico Transversal and the adjacent southern Mexican Sierras, had high estimates of intercluster migration (N_m) compared to the other clusters. Within the *P. oocarpa* clusters, the southern Mexican Sierras provenances were the most differentiated ($F_{ST} = 0.049$) and the Central American provenances were the least differentiated ($F_{ST} = 0.015$). The Central American provenances had the highest N_m (3.84), and the Sierra Madre Occidental had the lowest (2.66). All of the *P. oocarpa* clusters were out of Hardy-Weinberg equilibrium, with the exception of the single-provenance *P. oocarpa* var. *microphylla* cluster, which was also the most differentiated. Within *P. tecunumanii*, the high-elevation provenances had higher values of every genetic-diversity measure, despite having a slightly smaller overall population size than the low-elevation provenances. Interestingly, the low-elevation provenances were more differentiated than the high-elevation provenances ($F_{ST} = 0.035$ vs. 0.019).

Intercluster migration. Estimates of intercluster migration, based on the private-allele method (Barton and Slatkin 1986), were high for several pairs of clusters (table 6, upper diagonal). A particularly high amount of gene exchange was predicted to occur between the southern Mexican Sierras cluster and the Eje Volcánico Transversal and Central American clusters ($N_m = 4.85$ and 3.3, respectively). The Central American cluster was estimated to exchange 9.71 migrants per generation with low-elevation *P. tecunumanii* provenances and 6.52 migrants per generation with high-elevation *P. tecunumanii* provenances, while immigration between the two *P. tecunumanii* groups was estimated at $N_m = 6.75$. The Sierra Madre Occidental cluster had the least overall gene exchange with other clusters, having its greatest amount of migration with the Eje Volcánico Transversal provenances ($N_m = 2.71$). It also was the cluster with the greatest amount of estimated gene exchange with *P. patula* ($N_m = 2.07$), greater even than that between the two *P. patula* clusters ($N_m = 1.64$). The *P. oocarpa* var. *microphylla* cluster, meanwhile, had little estimated gene exchange with any other cluster. Pairwise comparisons of F_{ST} (table 6, lower diagonal) and D_C genetic distance (results not shown) among clusters reflected a similar pattern.

Variation in Pitch Canker Resistance

Significant differences for stemkill were found among the species, as expected (table 7). *Pinus oocarpa* and low-elevation

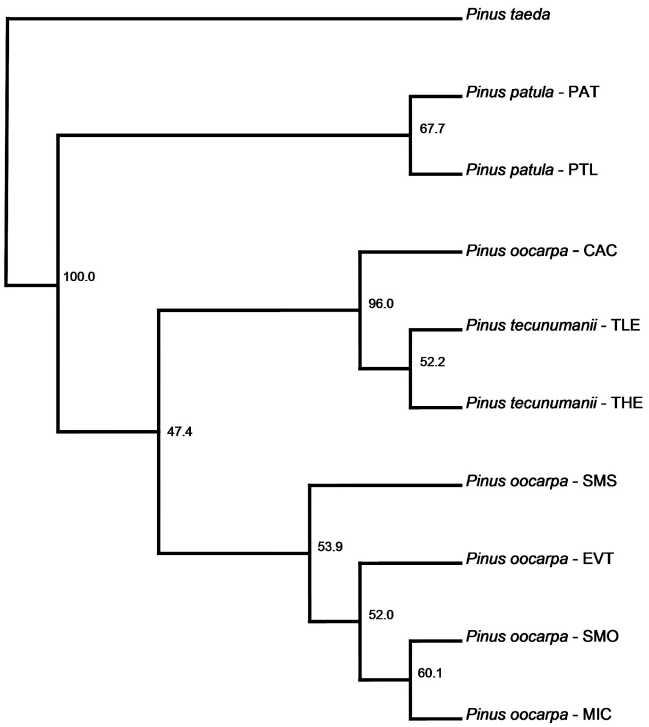

Fig. 4 Neighbor-joining consensus dendrogram depicting chord genetic distances (D_C; Cavalli-Sforza and Edwards 1967) among the clustering groups of *Pinus oocarpa*, *Pinus tecunumanii*, and *Pinus patula*, with a bulk provenance of *Pinus taeda* as an outgroup. The values represent percent bootstrap support for the nodes of more than 1000 replicates. See table 5 for cluster abbreviations.

P. tecunumanii were highly resistant, with stemkills of 4.1% and 5.8%, respectively. High-elevation *P. tecunumanii*, *P. oocarpa* var. *microphylla*, and *P. patula* var. *longipedunculata* were susceptible, with stemkill values that ranged from 42% to 60%. *Pinus patula* var. *patula* and the *Pinus elliottii* control were highly susceptible, with mean stemkill percentages above 70%. The ranks in stemkill percentage of the resistant and susceptible provenances of *P. tecunumanii* and *P. patula* were very similar to results obtained in our previous work (Hodge and Dvorak 2007).

Provenance variation in stemkill percentage within *P. oocarpa* (excluding var. *microphylla*) was significant ($P = 0.0001$). Values of 3% stemkill were common in the Cordilleras of Honduras and Nicaragua in the southern part of the species' range. These values rose in a gentle clinal manner to a maximum near 8% in the Eje Volcánico Transversal in central Mexico before dropping slightly in the northern Sierra Madre Oriental at the extreme of the species' range. Mean stemkill percentage was significantly positively correlated to latitude ($r = 0.35$, $P = 0.014$), longitude ($r = 0.41$, $P = 0.003$), and altitude of the collection site ($r = 0.29$, $P = 0.04$) but not to annual precipitation or any genetic-diversity parameter (A, A_R, H_E). The highest dispersion in mean stemkill percentage within any group was for high-elevation *P. tecunumanii*, with extremes of Montecristo, El Salvador (12%), and Pinalón, Guatemala (77%; table 7).

Discussion

Geology and Centers of Diversity

The evolutionary history of *Pinus oocarpa* in Mesoamerica is defined by the geologic events that created the region's mountain ranges, by climatic changes that influenced natural selection, and by the frequency and intensity of fires. Undoubtedly, the geographic range of *P. oocarpa* has expanded and contracted numerous times during its evolutionary history, like that of other pine species (see Millar 1999), but these apparently were never extreme events because there is no evidence of the presence of genetic bottlenecks. The geologic history of the mountain ranges in present-day Mexico and Central America is complex (Farjon and Styles 1997) and greatly understudied (Ferrusquía-Villafranca 1993). Research work such as our study is therefore necessary to present reasonable scenarios about the evolution and migration of *P. oocarpa*.

Our molecular work indicates that *P. oocarpa* has probably had a long evolutionary history, in light of the large number of provenance-level private alleles ($AP_{sp} = 44$) found throughout its natural range, in what is thought to be a relatively young subsection (see Strauss and Doerksen 1991; Krupkin et al. 1996; Willyard et al. 2007). *Pinus oocarpa* appears to have two centers of diversity, one in the Eje Volcánico Transversal in central Mexico and the other in the Central American Cordilleras of southeastern Guatemala, southwestern Honduras, and northwestern Nicaragua. The Eje Volcánico Transversal has always been considered a center of the evolution of the pine diversity whereby today 14–18 taxa can be found in most Mexican states (Perry 1991; Farjon 1996). The mountain range serves as the evolutionary conduit between eastern and western Mexico and provides ecological niches for pine speciation and hybridization (Eguiluz-Piedra 1985). From central Mexico, *P. oocarpa* presumably migrated north into the Sierra Madre Occidental and south and east across the Isthmus of Tehuantepec into Central America.

Genetic Diversity

Pinus oocarpa appears to possess average to above-average levels of genetic diversity, as would be expected for a tree species with a large geographic range. It has high levels of alleles per polymorphic locus ($A = 19.8$) and allelic richness ($A_R = 11.9$) and average levels of expected heterozygosity ($H_E = 0.711$) and population differentiation ($F_{ST} = 0.131$) relative to other conifers assessed with nuclear microsatellite markers (Al-Rabab'ah and Williams 2002; Boys et al. 2005; Wang et al. 2005; Karhu et al. 2006; Potter et al. 2008). We observed no significant changes in genetic diversity as *P. oocarpa* migrated into Central America; by contrast, its subtropical cousin *Pinus maximinoi* exhibited reduced genetic diversity with distance from its evolutionary center in central Mexico when assessed with RAPD markers (Dvorak et al. 2002).

Genetic diversity, as measured by number of alleles, allelic richness, and expected heterozygosity, was significantly correlated to elevation of the collection site in *P. oocarpa*. The same trend was found for *Pinus tecunumanii*: high-elevation populations were more diverse than low-elevation ones. We do not know why genetic diversity increases with altitude. One hy-

Table 4

Results of Five Analyses of Molecular Variance (AMOVAs) Using 11 Polymorphic Microsatellite Loci from the Three Mesoamerican Pine Species

Source of variation	df	Sum of squares	Variance components	% of variation	*F* statistics
Species:					
Among all species	2	181.6	.298	7.5	$F_{CT} = .075$
Among provenances within species	65	653.9	.343	8.7	$F_{SC} = .094$
Within provenances	1272	4202.8	3.304	83.8	$F_{ST} = .162$
Total	1339	5038.3	3.945		
Clusters:					
Among all clusters	8	507.2	.427	11.1	$F_{CT} = .111$
Among provenances within clusters	59	328.4	.115	3.0	$F_{SC} = .034$
Within provenances	1272	4202.8	3.304	85.9	$F_{ST} = .141$
Total	1339	5038.3	3.846		
Pinus oocarpa clusters:					
Among *P. oocarpa* clusters	4	294.3	.391	10.1	$F_{CT} = .101$
Among provenances within *P. oocarpa* clusters	46	258.4	.115	3.0	$F_{SC} = .033$
Within *P. oocarpa* provenances	953	3196.9	3.354	86.9	$F_{ST} = .131$
Total	1003	3749.6	3.860		
Pinus tecunumanii clusters:					
Among *P. tecunumanii* clusters	1	20.6	.139	3.9	$F_{CT} = .039$
Among provenances within *P. tecunumanii* clusters	9	51.9	.127	3.6	$F_{SC} = .037$
Within *P. tecunumanii* provenances	205	672.0	3.278	92.5	$F_{ST} = .075$
Total	215	744.5	3.544		
Pinus patula clusters:					
Among *P. patula* clusters	1	10.7	.185	5.8	$F_{CT} = .058$
Among provenances within *P. patula* clusters	4	18.0	.079	2.5	$F_{SC} = .026$
Within *P. patula* provenances	114	334.0	2.929	91.7	$F_{ST} = .083$
Total	119	362.7	3.193		

Note. Significance levels of variance components, based on 1000 permutations, were all $P < 0.001$.

pothesis would suggest that at the high-elevation sites, the chances for hybridization between *P. oocarpa* and *P. tecunumanii* increase, which in turn influences diversity, but this cannot be definitely confirmed by our study. In fact, our results suggest a greater amount of gene exchange and less genetic differentiation between *P. oocarpa* and the low-elevation provenances of *P. tecunumanii* than between *P. oocarpa* and the high-elevation provenances (table 6).

Bayesian Clustering

The formation of four clusters of *P. oocarpa* var. *oocarpa* defined by the Bayesian analysis—(1) Sierra Madre Occidental, (2) Eje Volcánico Transversal, (3) southern Mexican Sierras, and (4) Central America—is highly correlated to geography and is consistent with the RAPD grouping that separated *P. oocarpa* in eastern Mexico and Central America from populations in the Sierra Occidental in northwestern Mexico (Dvorak et al. 2001). The affinity between *P. oocarpa* populations in Sierra Madre del Sur and its neighbors in the western highlands of Chiapas is especially interesting because the mountain ranges have physiographic and geologic-tectonic features distinctive enough to separate them (Ferrusquía-Villafranca 1993) and because the area between them is bisected by the Isthmus of Tehuantepec. Generally, the pine forests of Chiapas appear more similar to those in Central America than to those in the rest of Mexico.

Relationship of P. oocarpa *with Other Pine Species*

This microsatellite assessment confirms that *P. tecunumanii* evolved from Central American *P. oocarpa* in Honduras and Nicaragua and that *Pinus patula* is a sister species genetically different from both taxa. Microsatellite markers were more effective in distinguishing differences between populations of *P. oocarpa* and *P. tecunumanii* than RAPD markers (see Furman and Dvorak 2005). The BAPS analysis clustered high- and low-elevation populations of *P. tecunumanii* into two distinct groups, with the exception that the low-elevation provenance Sacul Arriba, Guatemala, clustered with the high-elevation provenances. The monoterpene composition of low-elevation populations of *P. tecunumanii* have moderate to high levels of α-pinene and are more similar to *P. oocarpa* in this respect than are high-elevation populations of *P. tecunumanii* that have moderate to high levels of β-phellandrine and low levels of α-pinene (Squillace and Perry 1992). This is consistent with our microsatellite results, which show higher gene exchange and lower genetic differentiation between Central American *P. oocarpa* and the low-elevation populations of *P. tecunumanii* than between those *P. oocarpa* and high-elevation *P. tecunumanii*. Because both *P. tecunumanii* ecotypes presumably evolved from *P. oocarpa* at the same time, these distinct monoterpene and microsatellite differences are intriguing and possibly suggest different migration histories.

The large genetic separation of the *P. oocarpa* var. *microphylla* population of Ocotes Altos from other *P. oocarpa*

Table 5

Measures of Genetic Variation for Within-Species Clusters of *Pinus oocarpa*, *Pinus tecunumanii*, and *Pinus patula*, Based on 11 Nuclear Microsatellite Loci

										Intragroup		Intergroup mean	
Species, cluster	*n*	*A*	A_R	AP_{sp}	AP_{all}	H_E	H_O	F_{IS}	HWE (*P*)	F_{ST}	N_m	F_{ST}	N_m
Pinus oocarpa	502	19.82	11.86	...	82	.711	.545	.150	<.05	...	2.49	...	...
Sierra Madre Occidental (SMO)	50	10.27	4.11	13	8	.619	.492	.193	<.05	.02	2.66	.121	1.58
Eje Neo Volcánico Transversal (EVT)	99	11.91	4.51	19	16	.688	.584	.123	<.05	.036	3.62	.087	2.62
Southern Mexican Sierras (SMS)	118	12.55	4.34	11	8	.678	.517	.202	<.05	.049	2.81	.114	2.66
Central American Cordilleras (CAC)	226	12.73	3.97	19	10	.652	.559	.131	<.05	.015	3.84	.152	1.65
var. *microphylla*	9	3.46	2.93	5	4	.437	.426	.029	ns	...	...	.195	.76
Pinus tecunumanii	108	11.36	9.49	...	7	.646	.549	.109	<.05	...	3.14	...	...
High-elevation (THE)	50	9.45	9.41	35	5	.651	.587	.086	<.05	.019	2.66	...	...
Low-elevation (TLE)	58	8.18	7.83	21	2	.611	.516	.131	<.05	.035	2.65	...	...
Pinus patula	60	9.36	9.09	...	5	.586	.486	.138	<.05	...	2.26	...	...
var. *patula* (PAT)	50	8.45	4.64	53	5	.567	.475	.148	<.05	.025	1.81	...	...
var. *longipedunculata* (PTL)	10	4.55	4.27	10	0	.599	.548	.089	ns	...	...	...	...

Note. Genetic clusters were defined with BAPS 5.1 (Corander et al. 2003; see table 3 for provenance assignments). Values of F_{ST} (population differentiation) and N_m (migrants per generation) are reported for provenances within each cluster. Mean pairwise F_{ST} differentiation and N_m migration values are reported between each *P. oocarpa* cluster and the other clusters within the species. A = mean alleles per locus; A_R = mean allelic richness; AP = private (unique) alleles within species (AP_{sp}) and across species (AP_{all}); F_{IS} = mean fixation index; H_E = expected heterozygosity; H_O = observed heterozygosity; HWE = Hardy-Weinberg exact test of heterozygote deficiency; ns = not significant.

provenances and its significantly higher susceptibility to the pitch canker fungus (see "Pitch Canker Resistance") seem to support its elevation from varietal (var. *microphylla*) to specific rank (*Pinus praetermissa* Styles and McVaugh). As described, *P. praetermissa* has rounded cones very similar in shape to those of *P. oocarpa* but shares very few other external or internal needle and cone morphologic traits (Shaw 1909). Styles and McVaugh (1990) suggest that it exhibits some taxonomic similarities to trees in the Pseudostrobus group (Ponderosae subsection); Pérez de la Rosa (2001) believes that it possesses morphologic characteristics of *Pinus greggii* Engelmann ex Parlatore (Oocarpae subsection). Our two NJ dendrograms place this provenance firmly within a clade with northern *P. oocarpa* provenances (fig. 2) and as a well-supported sister cluster to the Sierra Madre Occidental *P. oocarpa* cluster (fig. 4). However, its ancestral origin remains unclear.

Intercluster migration. Historic gene flow (N_m) among most *P. oocarpa* clusters appears to have been common, even across great geographic distances in Mesoamerica, and it explains the relatively small population differentiation found in the species. The southern Mexican Sierras cluster apparently has served as a conduit for pollen flow between the Eje Volcánico Transversal and Central American clusters.

The number of migrants per generation (N_m) among *P. oocarpa* provenances was 2.49, while the average pairwise N_m

Table 6

Pairwise Gene Exchange Estimates and Genetic Differentiation among Genetic Clusters of Three Mesoamerican Pine Species, Based on 11 Polymorphic Microsatellite Loci

	Pinus oocarpa					*Pinus tecunumanii*		*Pinus patula*	
Cluster	SMO	EVT	SMS	CAC	MIC	HE	LE	PAT	PTL
SMO		2.71	1.67	1.05	.88	1.39	1.06	2.07	1.27
EVT	.051		4.85	1.91	1.00	1.96	1.36	1.58	1.61
SMS	.110	.057		3.3	.80	2.84	2.67	1.82	1.26
CAC	.145	.100	.092		.37	6.52	9.71	1.13	.88
MIC	.178	.139	.195	.269		.67	.41	.54	.45
HE	.168	.117	.123	.064	.297		6.75	1.32	1.43
LE	.194	.15	.143	.052	.323	.048		.88	.93
PAT	.165	.125	.133	.166	.313	.135	.174		1.64
PTL	.127	.097	.102	.093	.303	.077	.099	.070	

Note. Upper diagonal: number of migrants per generation (N_m), estimated with the private-allele method; lower diagonal: cluster pairwise F_{ST}, with all differences significantly different from 0 at $P = 0.05$. See table 5 for cluster definitions.

Table 7

Stemkill Percentages (Least Squares Means ± SE and Ranges) for Five Pine Species and Varieties Screened for Pitch Canker

Species, cluster	*n*	Stemkill (%)[a]	
		LS mean ± SE	Range
Pinus oocarpa	50	4.1 ± 1.0^{A}	2.4–7.8
Sierra Madre Occidental (SMO)	5	3.9 ± 1.3	2.5–5.9
Eje Neo Volcánico Transversal (EVT)	10	5.1 ± 1.5	3.1–7.8
Southern Mexican Sierras (SMS)	12	4.2 ± 1.1	3.0–6.7
Central American Cordilleras (CAC)	23	3.2 ± .6	2.4–4.9
Pinus oocarpa var. *microphylla*	1	42.2 ± 7.3^{BC}	...
Pinus tecunumanii low-elevation (TLE)	6	5.8 ± 3.0^{A}	4.6–6.5
Pinus tecunumanii high-elevation (THE)	5	42.1 ± 3.2^{B}	12.1–77.7
Pinus patula	5	60.4 ± 7.3^{CD}	57.5–89.1
Pinus patula var. *longipedunculata*	1	71.5 ± 3.3^{D}	...
Pinus elliottii (control)	1	70.1 ± 7.2^{D}	...

Note. Results are also provided for genetic subclusters of *P. oocarpa* based on Bayesian structure analysis (see text for details).

[a] Least squares means for species and varieties not followed by the same letter are significantly different from one another, according to multiple-comparison significance tests.

among *P. oocarpa* clusters was 2.58 (not including the single provenance of *P. oocarpa* var. *microphylla*). For most pine species, N_m values of 10 are average (Ledig 1998). Values for the tropical and subtropical Mesoamerican pines *Pinus caribaea* (seven populations, two countries) and *P. maximinoi* (five populations, four countries), assessed using isozymes, were 10 and 15, respectively (Dvorak et al. 2002, 2005). Gene flow values of 6.52 and 9.71 between the high-elevation and low-elevation *P. tecunumanii* clusters, respectively, and the Central American *P. oocarpa* cluster are noteworthy and cause us to speculate how these two species with high levels of historic gene flow have evolved such different mechanisms for such traits as fire resistance and site adaptability. *Pinus oocarpa* resprouts at its base after fires, while *P. tecunumanii* does not. Instead, *P. tecunumanii* survives fires by rapid growth and the development of a thick bark at the base of the tree. *Pinus tecunumanii* predominates in moist but well-drained soils in fertile highlands and valleys. *Pinus oocarpa* commonly occurs on shallow, infertile soils on the southern and eastern slopes of mountains.

Genetic admixture between clusters. The Bayesian analysis (fig. 3) confirmed what foresters have been seeing in the field for years, that gene exchange exists between *P. oocarpa* and *P. tecunumanii* in Central America, explaining why delineation only by morphologic analysis is difficult and sometimes not appropriate. *Pinus tecunumanii* from Cabricán, Montecristo, Villa Santa, and La Rinconada are all closely surrounded by *P. oocarpa* in natural stands, and gene flow between the two is expected. The designation of Cerro La Joya as *P. oocarpa* and not *P. tecunumanii* is consistent with observations in genetic field trials and supports our original doubts about the authenticity of species when making the seed collections in the field. The Cerro la Joya population exhibited growth development like *P. oocarpa*'s and was 34% below the average in volume production when compared to the mean of other sources of *P. tecunumanii* planted in a number of different countries (Hodge and Dvorak 1999). Likewise, the finding that some *P. oocarpa* populations have *P. tecunumanii* admixtures is consistent with field observations. *Pinus tecunumanii*-like trees have been found at the altitudinal extreme of a predominantly *P. oocarpa* stand at La Lagunilla, Guatemala. At San Jerónimo, *P. oocarpa* occurs sympatrically with *P. tecunumanii* at ~1600 m elevation, admixture is expected, and trees intermediate between the two abound. Interestingly, no gene admixture was found in the population of Chuacús, which we believe to be approximately the same site as San Jerónimo, collected by OFI 8 yr before the CAMCORE collections. The area of *P. tecunumanii* at San Jerónimo has been reduced by 70% by wood cutters in the past 25 yr. Possibly selectively harvesting in the *P. tecunumanii* stand promoted pollen production (more sunlight) and increased air flow to move pollen longer distances into the *P. oocarpa* stand.

In some cases, the Bayesian assessment was not concordant with our field observations in Central America. We have seen no morphologic evidence in natural stands or results from genetic field trials (growth and productivity) to suggest that the *P. oocarpa* provenances of San José La Arada, Guinope, San José Cusmapa, and San Nicolás have *P. tecunumanii* admixture, even though low-level hybridization and introgression is certainly possible from long-distance pollen flow. The *P. oocarpa* stand at San Lorenzo adjacent to a 5-ha natural stand of *P. tecunumanii* exhibited no admixture. *Pinus oocarpa* progeny from San Lorenzo have characteristics of *P. tecunumanii* when grown in field trials (Dvorak et al. 2000*a*).

In Mexico, the admixture of *P. oocarpa* and *P. patula* makes sense at Huayacocotla because both species occur in the area, though at different elevations. Artificial crosses between the two species have also been successfully completed in South Africa. However, west of the Isthmus of Tehuantepec in southern and central Mexico, there are as many as nine pine species and varieties in the Oocarpae subsection, all which supposedly can naturally hybridize with the others. Therefore, the

Bayesian cluster coancestry assessments must be interpreted with some caution, especially with regard to the admixture of *P. tecunumanii*. One of our working hypotheses in years past was that *P. tecunumanii* might have originated in the Sierra Madre de Sur of Guerrero and migrated into Central America (Dvorak 2008). We based this scenario on the fact that 3% of the trees studied in morphologic analysis of *P. patula* var. *longipenduculata* in Oaxaca grouped more closely with Central American *P. tecunumanii* than with other closed-cone pines (Dvorak and Raymond 1991) and that the southern Cordilleras of Guatemala are geologically an extension of the Sierra Madre del Sur. However, we have never been able to confirm the existence of *P. tecunumanii* west of Chiapas with species-specific RAPD markers (Dvorak et al. 2001). We have examined a number of trees from the provenance of Juquila (Oaxaca) classified by Farjon and Styles (1997) as *P. tecunumanii*, but detailed morphologic and marker studies indicate that they are an atypical form of *Pinus herrerae* Martínez, or possibly *Pinus pringlei* Shaw, or a mixture (Dvorak et al. 2001; Dvorak 2008). Whereas species admixture east of the isthmus could be the result only of introgression by either *P. tecunumanii* or *P. oocarpa*, west of the isthmus it could be the result of introgression by a host of Oocarpae pines other than *P. tecunumanii*, with nonhomologous microsatellite alleles of lengths similar to those of *P. tecunumanii*. More comprehensive molecular-marker studies of the Oocarpae are needed to confirm admixtures west of the isthmus. The natural pine stands of the Sierra Madre del Sur continue to provide forest taxonomists their greatest professional challenge in Mexico.

Pitch Canker Resistance

Levels of species susceptibility to pitch canker found in this study correspond to results obtained by Hodge and Dvorak (2000, 2007). *Pinus oocarpa* and low-elevation populations of *P. tecunumanii* were resistant, high-elevation populations of *P. tecunumanii* and *P. praetermissa* (*P. oocarpa* var. *microphylla*) were moderately susceptible, and *P. patula* was highly susceptible.

At the provenance level, *P. oocarpa* exhibited high levels of resistance to the pitch canker fungus throughout its entire geographic range of 3000 km. This is contrary to what has been found for susceptible and moderately susceptible species like *P. patula* and high-elevation *P. tecunumanii* (Hodge and Dvorak 2007), which exhibit significant provenance variation in greenhouse screening studies. We could find no clear trends between the genetic structure and evolutionary history of *P. oocarpa* and resistance patterns to pitch canker.

Even though the range in provenance variation in *P. oocarpa* was small and might not be biologically important to breeders, the clinal trend of increasing pitch canker susceptibility from southeast, in the Cordilleras of Honduras/Nicaragua, to northwest, in the Eje Volcánico Transversal of Mexico, is intriguing. It would suggest the possibility that the pitch canker fungus evolved in Central America and not in Mexico. As far as we know, there has never been a complete survey of pitch canker in Central America.

As we have found for high-elevation *P. tecunumanii* and *P. patula* in our earlier studies (Hodge and Dvorak 2007), there is a positive correlation between pitch canker susceptibility in *P. oocarpa* and the altitude of the collection site. One hypothesis is that at higher altitudes needles are thinner and more flexible (softer tissue), regardless of species, and therefore are possibly more susceptible to wounding for entrance of the disease. A second hypothesis is that the higher-elevation populations of *P. tecunumanii*, which are susceptible, form natural hybrids with highly resistant *P. oocarpa* to produce high-elevation populations with more resistance than populations with no admixture. Alternatively, natural *P. oocarpa* stands at the limits of their altitudinal gradients may introgress with susceptible high-elevation *P. tecunumanii* to produce populations with less-than-average resistance for the species. Gene admixture between the two groups has been confirmed in this study (see above).

Analyses of the effects of admixture on pitch canker resistance in natural stands were inconclusive. *Pinus oocarpa* populations that exhibit *P. tecunumanii* admixture had stemkill of 3.4%, versus 3.1% for populations with no admixture. Low-elevation *P. tecunumanii* populations that showed introgression with *P. oocarpa* exhibit stemkill of 5%, while those with no introgression had 7% stemkill. High-elevation *P. tecunumanii* populations that had *P. oocarpa* admixture exhibited stemkill of 27.5%, versus 77% for those with no introgression. The last comparison is somewhat tentative because of small sample size. We do know that artificial hybrid crosses made in South Africa between susceptible *P. patula* and highly resistant *P. oocarpa* or low-elevation *P. tecunumanii* produce progeny that are often intermediate in resistance between the two (Roux et al. 2007). More studies are needed to determine why resistance genes do not express themselves in high-elevation populations of *P. tecunumanii* and why populations of *P. oocarpa*, *P. patula*, and *P. tecunumanii* at high altitudes are generally more susceptible than those at low altitudes.

Acknowledgments

We would like to thank the two anonymous reviewers for their comments, which improved the manuscript. We would also like to thank Elmer Gutiérrez, Mike Tighe, Juan Luís Lopez (CAMCORE), and José Romero (formerly with CAMCORE) and our counterparts in Mexico and Central America for making seed collections throughout Mesoamerica. We would also like to thank David Boshier of the OFI for the donation of additional provenances of *Pinus oocarpa* seeds; Josh Bronson of the U.S. Forest Service, Rust Screening Center, for conducting the *Fusarium* testing; and Willi Woodbridge, Robert Jetton, and Andy Whittier (CAMCORE) for assistance on the maps and tables. We also appreciate the advice from Claire Williams (Duke University) and Merv Shepherd (Southern Cross University) on the selection of microsatellite loci for the study and the laboratory assistance of Ricardo Hernandez and Jennifer DeWoody (National Forest Genetics Laboratory). Finally, we appreciate the financial support of the CAMCORE membership in 17 countries for funding this research project. K. M. Potter's research was supported in part through Research Joint Venture Agreement 08JV11330146078 between the USDA Forest Service Southern Research Station and North Carolina State University.

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