

[Genetics and Ethnobotany of *Diospyros riojae*; 5549 words of text]

Population Genetics and Ethnobotany of Cultivated *Diospyros riojae* Gomez-Pompa

(Ebenaceae), an Endangered Fruit Crop from Mexico

Mitchell C. Provance <sup>1,5</sup>, Ignacio García Ruiz <sup>2</sup>, Casper Thommes <sup>3</sup>, Jeffrey Ross-Ibarra <sup>3,4</sup>

<sup>1</sup>Dept. of Botany and Plant Sciences, University of California, Riverside, CA, USA.

<sup>2</sup>Instituto Politécnico Nacional, CIIDIR Jiquilpan, Michoacán, México

<sup>3</sup>Dept. of Plant Sciences, University of California, Davis, CA, USA.

<sup>4</sup>Author for correspondence: rossibarra@ucdavis.edu

## <H1>Abstract

Population Genetics and Ethnobotany of Cultivated *Diospyros riojae* Gomez-Pompa

(Ebenaceae), an Endangered Fruit Crop from Mexico. The traditional cultivation of an endangered species, *D. riojae* Gómez Pompa, in North-Central Mexico, and the sale of its fruit, is described for the first time. This is complemented by the first examination of genetic structure in New World Ebenaceae. Genetic and morphological comparisons are made between *D. konzattii* Standley and *D. riojae* from across its known range of cultivation. *Diospyros riojae* is cultivated in two markedly different climates: semi-arid localities west of the Sierra Gorda, especially near Tecozautla, Hidalgo, and the semi-humid Huasteca Region, east of the Sierra Gorda. Much lower levels of genetic diversity were detected in western populations, where populations are larger, and management intensity is the greatest. Contrary to the most recent revision of these Neotropical *Diospyros*, taxon-level, rather than cultivar-level divergence is suggested for *D. riojae* and *D. konzattii*.

## <H1>Introduction

The genus *Diospyros* (Ebenaceae) is comprised of over 500 species of trees and shrubs best known for producing dense black wood (ebony) and tasty fruit (e.g. persimmons and zapotes). It has a pantropical-subtropical distribution, with several species also in temperate regions of Asia and North America (Duangjai et al. 2009). There are some 100–130 species in the New World (Wallnöfer 2009), with about 35 found in Mesoamerica. Recent phylogenetic work suggests that nearly all of the Mesoamerican species are in a clade that includes many of the species from the Indian Subcontinent, SE Asia, Oceania, and South America (Duangjai et al. 2009).

Morphological studies suggest a suite of species complexes for the Mesoamerican taxa (Provance and Sanders 2006, 2009; Provance, Garcia-Ruiz, and Sanders 2008), but there have not been any population or phylogeographic studies of Mesoamerican *Diospyros*.

Here, we provide the first detailed account of the traditional cultivation of *D. riojae* Gómez Pompa in Queretaro, Hidalgo, and San Luis Potosi, Mexico. We also conduct the first population studies of a Mesoamerican *Diospyros*, based on 26 individuals of *D. riojae* from across its range of cultivation, and one putative wild individual from near the type locality in central Veracruz. Our immediate objective was to explore genetic structure within *D. riojae* using two geographically separated clusters: high elevation populations west of the Sierra Gorda, and low elevation populations east of the Sierra Gorda (Fig. 1). We sought to determine if populations on either side of the Sierra Gorda showed evidence of differentiation, and where unique genotypes and phenotypes occurred that could be important to future germplasm conservation efforts.

Lastly, we investigated whether morphological and genetic evidence suggests cultivar or taxon-level divergences between *D. riojae* and *D. konzattii* Standl.

## <H1>Methods

### <H2>Species Examined:

*Diospyros riojae* is a tree native to oak and mesophytic cloud forest between 100 and 1350 m elevation in central and northern Veracruz. It has rarely been reported as a cultivated tree, and there are indications that it is a rare species. A recent reassessment of herbarium material found that many putative *D. riojae* collections represented a similar species, *D. konzattii* (Provance and Sanders 2006). Since *Diospyros riojae* is considered an endangered species in Mexico, there is considerable interest in its conservation biology (Sosa, Vovides, and Castillo-Campos 1998; Luna Vega et al. 2006). Females bear edible fruits (berries) that are typically globose and 3–5 cm in diameter. The fruits have up to ten seeds, or are seedless. The leaves are elliptic to widely obovate, or sub-oval, and typically have an obtusely rounded apex. The lamina is shiny and relatively thick, with slightly revolute, thickened margins, and prominent 3° and 4° venation. The fruiting calyx is accrescent and firmly attached to the fruit, with 5(–6) spreading to reflexed sepals. Male flowers are relatively short and urceolate-campanulate (Provance and Sanders 2006).

*Diospyros konzattii* is primarily known from cloud and pine-oak forests of west-central Veracruz and nearby Puebla, although the type locality is in the Sierra Madre del Sur of southern Oaxaca (Provance and Sanders 2006; Wallnöfer 2007). *Diospyros konzattii* is known for Oaxaca only from the type collection and trees in the Konzatti Garden in Oaxaca de Juárez, which have likely descended from seeds collected at the type locality. *Diospyros konzattii* has leaves and

flowers that are distinctly different from those of *D. riojae*, but the calyx and edible fruits are similar. Its leaves are lanceolate to elliptic, or ovate, with an apex that is distinctly acuminate, or sometimes tapered to an acute or obtusely rounded point. The lamina is dull, with thin, flat margins, and lacks prominent higher orders of venation (Provance and Sanders 2006). The male trees produce long-urceolate flowers.

We included several other taxa in the study for comparative purposes: *D. digyna* Jaq., which is widely distributed in Mesoamerica; *D. californica* (Brandege) I.M. Johnst., which is endemic to the Cape Region of Baja California Sur; *D. rekoii* Standl and *D. rosei* Standl., which are mainly from western Mexico; and an indefinitely determined species, *D. cf. riojae*, from the Chimalapas region of Oaxaca that is morphologically most similar to *D. riojae*.

## <H2>Study Area and Sampling Method

The study was conducted in four areas of Mexico, including: 1) arid and semi-arid zones west of the Sierra Gorda, in Queretaro and northwestern Hidalgo at 1300-2100 masl, with mean annual precipitation (MAP) of 400-800 mm, mean annual temperature (MAT) of 16-20 °C, long dry periods, and occasionally frosts; 2) the Sierra Gorda of eastern Queretaro and northern Hidalgo at 500-1800 masl, with MAP of 800-1800 mm, MAT of 20-24 °C, and a semi-humid climate; 3) escarpment of the eastern Sierra Gorda, and the North Gulf Coastal Plain in San Luis Potosi and northern Hidalgo, at 60-900 masl, with MAP of 1500-3000 mm, MAT of 20-26 °C, and high humidity, with rain abundant in the summer, or most of the year; 4) Cafetal San Rafael and its vicinity in the Sierra Madre del Sur of the State of Oaxaca, at 800-1200 masl, with MAP of 1000-1200 mm, MAT of 22-24 °C, and a sub-humid atmosphere (Figure 1, inset).

During 2009 and 2010, the first and third authors searched for populations of *D. riojae* at previously recorded localities, and new localities outside of the presumed range for the species. These localities included homegardens, managed forests, managed groves, and putative natural vegetation communities. Gardens adjacent to living quarters or work residences, whether in towns, rural areas, or intermediate settings, were considered homegardens. Community-managed forests were defined as natural settings with high concentrations of indigenous or nonindigenous species of useful trees, and were managed by people living in the area. Community-managed groves were characterized by the predominance of one species of useful tree. Otherwise, they were similar to community-managed forests. Tree occurrences were located by direct observation of trees while walking, or driving, and from leads provided by people. Thus, a mixture of intuitively directed searches and local knowledge was used to locate trees and informants. *Diospyros riojae* fruits were sought at open-air markets in Xilitla (SLP), Huichapan (HID), and Ezequiel Montes (QTO).

When adjacent trees appeared to be clonal, only one individual was sampled. Leaf samples were dried in silica gel for later extraction. Herbarium vouchers, deposited at CIMI, were made for all genetically tested individuals, except those from the market at Ezequiel Montés, Queretaro, for which photos of the fruits were obtained, and dried calyces were preserved. Species determination was made following Provance and Sanders (2006) and by comparison with type specimens. Prior informed consent was obtained verbally from growers before informal conversation interviews, which were conducted in the immediate vicinity of *Diospyros riojae* trees.

## <H2>Genetic Analysis

Primers were designed from sequences of *D. kaki* for the following loci (NCBI Genbank numbers in parentheses): phytoene synthase (PSY, FJ713744.1), lycopene beta-cyclase (LCYB, FJ940723.1), beta-carotene hydroxylase (BCH, GQ265553.1) (Zhao 2011), and squalene synthase (SQS, FJ687954.1) (Zhou 2012). The trnL intron was amplified from primers 'c' and 'd' (Taberlet et al., 1991). The trnL intron sequence for *D. digyna* was obtained from a sequence submitted to Genbank (Duangjai et al. 2009).

Genomic DNA was extracted from lyophilized leaf tissue using a modified CTAB protocol (Saghai-Maroo et al. 1984) that included polyvinylpyrrolidone (PVP). DNA was quantified using nanodrop spectrometry. PCR amplification was done in 25  $\mu$ l reactions containing 6  $\mu$ l of 10 ng/ $\mu$ l DNA solution, 0.25  $\mu$ l of TAQ polymerase, 12  $\mu$ l of sterile water, 1  $\mu$ l of dNTPs, 1  $\mu$ l for each primer at 10  $\mu$ M, 2.5  $\mu$ l for 10x TAQ buffer solution mix, and 1.25  $\mu$ l of DMSO. An initial 1 min pre-melt was followed by PCR for 30 cycles, with denaturation at 94 °C for 30 seconds, annealing at 56 °C for 45 seconds, and extension at 72 °C for 5 minutes. Purified PCR products were obtained by mixing 12  $\mu$ l with 3  $\mu$ l of the exo-SAP purification solution (2 U of exonuclease 1, 2.5 U of Antarctic phosphatase) and placing them in a thermal cycler at 37 °C for 30 min, followed by 80 °C for 15 min, and finally 4 °C for 5 min. Purified PCR products were sequenced at the UC Davis College of Agriculture and Environmental Sciences Genomics Facility on an ABI 3730 XL capillary sequencer. The resulting sequences were aligned using Geneious Pro 5.0.4 (Biomatters) and sequence analysis used the analysis package of libsequence (Thornton 2003).

## <H1>Results

## <H2>Populations

*Diospyros riojae* is cultivated in two remarkably different climatic regimes, corresponding to areas east and west of the Sierra Gorda. MAP at locations where *D. riojae* was found is between 400 and 500 mm in the arid zone west of the Sierra Gorda, and 2500-3000 mm on the escarpment of the eastern Sierra Gorda and the North Gulf Coastal Plain. We located cultivated *D. riojae* at ten localities in the western arid regions of Queretaro and Hidalgo between 1530 and 1780 m elevation, and four localities east of the Sierra Gorda, on the escarpment at about 820 m elevation, and the coastal plain between 60 and 100 m (Table 1). Western populations represented cultivated trees in homegardens, community-managed forests, community-managed groves, along roadsides, and trees likely naturalized from cultivated settings. Trees east of the Sierra Gorda were only observed in homegardens. We did not observe *D. riojae*, or the closely related species *D. gomeziorum*, in the Sierra Gorda. We located one female *D. conzattii* tree at the Conzatti Garden in Oaxaca City, but were unable to locate this species at the type locality.

The two largest populations of *D. riojae* we found are in the arid region west of the Sierra Gorda in the municipality of Tecozautla, Hidalgo. The largest, roughly comprised of 500 trees, occurs mostly throughout mestizo homegardens in residential and rural areas of Tecozautla and some adjacent communities. Occasional trees of ambiguous ownership occur along roadsides and other public areas. The species also seems to have naturalized in gallery forest along the Rio Tecozautla. The second largest population, comprised of about 100 trees, exists in a community-managed grove in El Paso, a short distance north of Tecozautla. The largest population we observed east of the Sierra Gorda occurs on the coastal plain at Aldzulup (Al d'hulup) Poytzén in San Luis Potosi, where there are about 30 trees in Teenek homegardens. Wild populations apparently do not occur in the adjacent environment, but occasional trees come up spontaneously

in homegardens. Putatively natural populations were reported along the escarpment southeast of San Antonio Xalcuayo Dos, but this was not confirmed.

## <H2>Genetics

High genetic differentiation, calculated as  $F_{st}$  (Hudson, Boos, and Kaplan 1992), was observed between *D. riojae* and *D. conzattii* for three of the nuclear loci, while comparisons of *D. riojae* and *D. cf. riojae* from Oaxaca did not show significant differentiation at any of the four loci (Table 2). TrnL intron sequences were identical for *D. riojae*, *D. californica* and *D. digyna*, while *D. conzattii* had a 2 bp insertion in trnL intron differentiating it from these species. Two 1 bp substitutions in TrnL intron differentiated *D. cf. riojae* from *D. riojae*, *D. californica* and *D. digyna*. Diversity statistics are shown in Table 3.

We found no difference in diversity between males and females (data not shown). Both heterozygosity ( $H$ ) and Simpson's Index of Diversity (SID) were greater in eastern populations than western populations, as was nucleotide diversity at the PSY locus. One tree from a community-managed forest near Toliman had a PSY genotype otherwise detected only in the putative wild tree from Central Veracruz. Samples from Tanjasnec had unique LCYB haplotypes. There was no variation of SQS in *D. riojae*. The BCH sequences of *D. conzattii*, *D. cf. riojae*, and three accessions of *D. riojae*, were each unique.

## <H2>Morphology

Abaxial and adaxial leaf surfaces of typical mature leaves of *D. conzattii* and *D. riojae* are shown in Figure 2. Leaf morphology of our vouchers, which were not exposed to alcohol before



drying, was consistent with that of herbarium specimens that may or may not have been exposed to alcohol. *Diospyros konzattii* leaves were dull on both sides, thin (pergamentaceous to chartaceous) with flat margins, and had inconspicuous higher orders of venation. *Diospyros riojae* leaves were shiny, thick (subcoriaceous to coriaceous), with slightly revolute margins, and had prominent higher orders of venation. Leaf shape differed between the species, and each was consistent with previous descriptions by Provance and Sanders (2006).

## <H2>Ethnobotany

Fruit of *D. riojae* are called **zapote negro** in predominately non-indigenous communities, and sold in late fall and winter for 15–20 pesos/kg at travelling outdoor markets (tianguis) and by roadside vendors in western Hidalgo and western Queretaro. We observed fruit for sale in Ezequiel Montes, Queretaro, and Huichapan, Tecozautla, and La Esquina, in the State of Hidalgo. Several growers stated that fruits were available in other towns as the outdoor markets changed venue.

In Tecozautla, *D. riojae* trees were found to be especially common along roadsides and in homegardens near narrow stone and mortar canals that lined the streets. According to one grower, water flowed through the canals about every forty days, feeding a subterranean system of “caños” that people in residential areas depend upon for homegarden irrigation. In Tecozautla, trees are grown for supplementary food, and other personal reasons. Trees comprise an important component of the landscape, and sometimes people with trees on their property reported having little interest in the fruit. Fruit are usually eaten fresh, but can be prepared in any way that *D.*

*digyna* fruits are prepared. A few people mentioned that an alcoholic drink could be made from the fruit, but they were without further details.

Commercial efforts exploited as few as one tree, and the largest private commercial grove of trees we visited had eleven female and three male trees. This grower reported typical annual fruit yields of 250 kg/tree, with rare yields up to 500 kg/tree. The trees are reported to have a long juvenile stage, with fruit production starting at about ten years of age. Fruit are usually harvested intermittently when olive-green, from late October until January. One grower placed green fruits in direct sunlight until warm, then wrapped them in newspaper, and stored them indoors in wooden, blanket-wrapped bushel crates (Figure 3). Using this treatment, fruit remained undamaged, and ripened in just a few days.

A nearly homogenous community-managed grove of *D. riojae* trees spans about seven acres of streambed and bottomland along the Río San Francisco near El Paso. The grove is adjacent to some cultivated plots, and is surrounded by terraces and low hills supporting cactus-rich xeric scrub. One fruit collector estimated that there were about 100 zapote negro in the grove. Following our exploration of the groves, we thought that that was a reasonable estimate. Large trees of both sexes were common, as was adventitious growth from roots and crowns. Seedlings and saplings were rarely observed.

Some people, including vendors, mentioned Toliman, Queretaro, as a source of the fruit. We located two trees near Toliman, but neither of them was commercially harvested. One tree in a homegarden was grown from a seed obtained from a fruit bought at the outdoor market in Colón. The other tree was old, and of ambiguous origin, growing in community-managed forest that was enriched with *Juglans*. Our elderly guide's familiarity with this individual tree went back 80 years.

Fruit were not observed being sold at markets east of the Sierra Gorda. *Diospyros riojae* and its fruits are called ***monek'*** by Teeneks in the Huasteca communities of Aldzulup Poytzén and Tanjasnec, San Luis Potosi. Our guide in Tanjasnec described two types of dark zapotes present locally, which were consistent with *D. digyna* and *D. riojae*. He stated that they both were called ***monek'***. The largest eastern population we visited was in Aldzulup Poytzén, a small community with flat topography, and a relatively warm and humid climate. We observed at least 30 trees in at this location. Our guide stated that the species did not occur in the surrounding environs, and that trees were not intentionally planted in the gardens. In Aldzulup Poytzén, tree trunks were sometimes nearly 100 cm in diameter. These trees were presumed by locals to be over 100 years old. Although ambiguous in origin, trees often had adventitious shoots from roots near the ground surface, or coming from the base of the trunk. Some isolated saplings likely to have arisen spontaneously from seeds were observed. Management techniques reminiscent of coppicing and pollarding were noted, though these practices were obviously related to space management within the homegarden.

We also found *D. riojae* trees east of the Sierra Gorda in the mestizo communities of San Antonio Xalcuayo Dos (Mpio. Xilitla), and La Herradura (Mpio. Axtla de Terraza). In San Antonio Xalcuayo Dos, trees of both sexes occurred in intensively managed homegardens. Growers stated the tree was rare in surrounding areas, but that scattered trees in a natural setting grew at lower elevations near Arroyo Seco. In La Herradura, we located one large tree with copious fruit in a homegarden. The origin of the tree was ambiguous, and the owner believed that his tree was locally unique.

Tree origin throughout the range of cultivated *D. riojae* was usually ambiguous. Growers indicated that new trees frequently arose from adventitious root and crown shoots, and

spontaneously from naturally dispersed seeds. Nevertheless, on the west side of the Sierra Gorda some people occasionally spoke about trees that either they, or people that they knew, had intentionally grown from seeds. The seeds are said to be difficult to germinate, and young trees are reportedly slow to establish. We observed one sapling, approximately 30 cm tall, for sale among other species being sold on the west side of the Sierra Gorda. It had been grown from seed.

## <H1>Discussion

### <H2>Genetic Studies

Strong evidence of genetic differentiation exists for *D. konzattii* and *D. riojae*, but there is little evidence for genetic differentiation between *D. cf. riojae* in Oaxaca and *D. riojae*. The sequence of trnL-intron for *D. riojae* is identical to that of *D. californica* and *D. digyna*, but the latter two species differ considerably in morphology from *D. riojae*, and from each another. Conversely, *D. riojae*, *D. konzattii*, and *D. cf. riojae*, each have different trnL-intron sequences, even though they were considered the same species by Wallnöfer (2007). *Diospyros konzattii* has a two base pair insertion that is currently not known to occur in any other species of *Diospyros*, based on Genbank submissions (data not shown). *Diospyros cf. riojae* has two one base pair indels also known to occur in *D. rekoii* and *D. rosei* accessions from Jalisco. Explanations for the co-occurrence of the indels among these taxa include a close phylogenetic relationship and chloroplast capture involving a close relative. *Diospyros cf. riojae* from the Chimalapas of Oaxaca may ultimately be shown to represent an undescribed species.

A genetic analysis of 27 *D. riojae* collections detected higher levels of diversity and heterozygosity in eastern populations than in western populations. This is consistent with the hypothesis that populations of cultivated *D. riojae* are structured on either side of the Sierra Gorda, and may be the result of restricted gene movement or a bottleneck during the colonization of western populations. The minimum distance between eastern and western populations is about 106 km. In the intervening mountain terrain, there are occurrences of *D. gomeziorum*, a rare species that is most similar to *D. riojae*. This species seems to be separated geographically and ecologically from cultivated populations of *D. riojae*. We were unable to locate fresh material of this species for inclusion in our study. Whether or not this species can be genetically differentiated from *D. riojae* warrants further investigation.

## <H2>Morphological Studies and Taxonomic Status

Provance and Sanders (2006) examined the taxonomy of *D. riojae* and *D. conzattii*, and discovered several differences in leaf and flower morphology that could be used to differentiate the species. Based on herbarium specimens, they recognized three additional geographically coherent taxa. Subsequently, Wallnöfer (2007) treated all of these species as *D. conzattii*. He considered their morphological differences cultivar-level, ecologically induced, or artifacts of short-term alcohol preservation techniques. He also suggested that morphological differences among these species had resulted from gene flow between ancient cultivars and wild populations. Differences in flower size led Wallnöfer to suspect that changes in ploidy had also occurred. He considered the type of *D. conzattii* to be an ancient cultivar, and the distribution of the species to be distorted by relict cultivars surviving the decline of indigenous tribes following the colonization of Mexico by Europeans.

Previously, Provance and Sanders demonstrated the taxonomic utility of male flower size: long, urceolate flowers occur in *D. konzattii* and *D. costaricensis*, and short, urceolate-campanulate flowers occur in *D. riojae* and *D. gomeziorum* (2006). We contend that changes in ploidy level, which can result in barriers to reproduction, and concomitant changes in morphology, argue in favor of recognizing multiple taxa. Ploidy levels are currently unknown for most Mesoamerican *Diospyros*, however, as cytological studies have only included *D. digyna* and *D. xolocotzii* Madrigal and Rzed. Both species produce large edible fruit and long male flowers, but have typical ploidy levels of  $2n = 30$  (Calderón-Olascoaga 2011).

Wallnöfer explained striking differences in leaf brilliance between *D. riojae* and *D. konzattii* as artifacts of alcohol preservation, and differences in vein prominence induced by the local ecology. We eschewed alcohol while collecting and all of our herbarium vouchers were air-dried in the same oven. Higher orders of venation were not prominent in our collection of *D. konzattii*, and consistently prominent in our *D. riojae* collections, even though we sampled at various elevations from a large geographical area, and from across local landscapes in both arid and humid climates. Consistent with findings of Provance and Sanders (2006), leaf brilliance, leaf shape, and leaf margin structure is considerably different in these two taxa, while rather constant among members of the same taxon. Genetic and morphological evidence strongly suggests that *D. konzattii* and *D. riojae* are not cultivars of the same species, but species unto themselves. Some of the morphological and genetic differences observed in *D. riojae* and *D. konzattii* may reflect a long history of human cultigen selection, but we doubt this model (or an ecological one) adequately explains all of the observed morphological and genetic variation.

*Diospyros* cf. *riojae* from Oaxaca resembles *D. riojae*, differing primarily in leaf morphology. Although differences in morphology and the trnL-intron of these two species

increase our suspicion that *D. cf. riojae* is distinct from *D. riojae*, differentiation was not demonstrated quantitatively by our data. Wallnöfer (2007) included this specimen in his concept of *D. conzattii*. Our results suggest that the Oaxaca specimen of *Diospyros cf. riojae* has less to do with Oaxaca populations of *D. conzattii* than with *D. riojae* populations from North-Central Mexico. *Diospyros aff. riojae* Gomez-Pompa, is probably the best determination for the specimen from the Chimalapas of Oaxaca at this time.

## <H2>Ethnobotany

The gathering of useful trees into protected areas can be viewed as an early step in the process of tree domestication (e.g. Casas et al. 2007). Here, we present the first detailed description of *D. riojae* cultivation, occurring primarily in homegardens and community-managed forests and groves in North-Central Mexico. The fruits have an appearance, taste, and flesh texture similar to *D. digyna* fruits. In its stronghold, Tecozautla, Hidalgo, a number of growers reported that the species was in decline. They attributed this drop in the population to increasing disinterest in the fruit by younger generations. One grower remarked that today spontaneous trees are less likely to be conserved, and that mature trees are more likely to be cut down than in previous years. It was further stated that growers are increasingly apt to cut down zapote negro trees to create space for more popular crops, such as wild pecans known locally as criollas (*Carya* sp.).

Narrow canals attributed to the Toltec were used to irrigate residential gardens 60 km southeast of Tecozautla at Tula, Hidalgo, as early as ca. A.D. 950–1200 (Doolittle 1990:97). Early references to irrigation canals in Tecozautla suggest they existed before 1600 (Melville

1997:107–110), though who constructed the putative canals is unknown. Reports of an early canal system that is no longer apparent raises the suspicion that the current canal system was built over an earlier, and perhaps indigenous canal system, as occurred in other parts of Mexico (Doolittle 1990:168–174). If it is true, then the distribution of *D. riojae* in Tecozautla may at least in part be explained as an artifact of indigenous plantings along canals.

Alternatively, the current distribution may be a result of post-contact enrichment. However, pre-contact establishment seems more likely, since seeds and seedlings seem to require some human management in the Municipio of Tecozautla, even if minimal or indirect, to achieve adulthood. Remarkably, healthy mature trees clearly survive in this area without supplemental water, as they are occasionally encountered in the rural outskirts along the fringes of non-irrigated fields, at the sites of deserted homes, and other semi-natural settings where supplemental water is not available. The population in Tecozautla seems too extensive to have been cultivated into its present existence post-contact without some kind of historical account of enrichment. Thus, we doubt the zapote negro population in Tecozautla has come about entirely post-contact. Rather, the species seems to have been introduced by people relatively early to arid lands west of the Sierra Gorda, especially around Tecozautla.

Although we think *Diospyros riojae* is not indigenous to Tecozautla, we cannot be certain of this without further research. Although it seems less likely than our anthropogenic origin hypothesis, areas along the Río Tecozautla may have served as refugia for a formerly more widespread indigenous *D. riojae* populations in the Tecozautla region. These refugia may have, or had, ecological and climatological similarities to the cloud and pine-oak forests on steep terrain in Veracruz and Puebla, of which *D. riojae* has typically been considered native. It is noteworthy that one grower reported that *D. riojae* used to occur in gallery forests at Cañón del



Infiernillo, a location now underwater following the construction of the Presa de Zimapan. Interestingly, a hypothesis of drastic post-contact environmental change in Western Hidalgo, largely induced by grazing livestock, has been proposed (Melville 1997:107–110).

Opossums (*Didelphis* sp., locally tlacuache) are common in Tecozautla courtyards, and growers closely associate them with *D. riojae* fruits. The reputation of the Virginia opossum (*Didelphis virginiana*) as a disseminator of American persimmon (*D. virginiana* L.) is controversial (e.g. Worth 1975), since it has also been reported that they do not swallow the seeds when they eat the fruit (Reynolds 1945). Nevertheless, it appears that opossums do eat large amounts of *D. riojae* fruit, and we suspect they occasionally disperse the seeds through endozoochory or may encourage seed germination by consuming flesh. Thus, local distribution patterns may partly reflect this relationship.

Our guide in Tanjasnec provided the word *monek'* for *D. riojae* and *D. digyna* trees, and for their fruits. Our spelling of the word reflects his insistence on writing the correct spelling of the word down for us. Alcorn reported the Teenek name *munek'* for *Diospyros digyna* (page 630 in Alcorn 1984). Alcorn did not mention the occurrence of two species of *Diospyros* in the Huasteca flora, but her collections included some specimens that would later be determined as *D. riojae*, which suggests that her data for *D. digyna* is a composite. Our guide was familiar with two species, including *D. riojae* and *D. digyna*, which he explained are known by the same name. Clearly, more-in-depth studies related to the use and cultivation of *D. riojae* and *D. digyna* are warranted.

The climate east of the Sierra Gorda is warmer, and receives more rain than the cloud forests at and near the type locality in Veracruz where *D. riojae* is putatively native (sub-humid, with MAP = 1000-1800 mm and MAT = 16-22 °C). The high levels of genetic diversity observed in

eastern populations, raise the suspicion that *D. riojae* could be native to low elevations east of the Sierra Gorda, including the gulf coastal plain in San Luis Potosi. Further studies will be needed to address this question. Our sample was too small to strongly advocate conservation of individual genotypes. However, the higher genetic diversity and unique haplotypes found in Tanjasnec and Aldzulup Poytzén suggest germplasm conservation should emphasize eastern populations. Finally, the *Diospyros riojae* orchard in El Paso might have a particularly high conservation value. It supports a large population of *D. riojae*, and similar groves of semi-domesticated trees have been shown to harbor diverse assemblages of wildlife (Reitsma, Parrish, and McLarney 2001; Perfecto 1997; Estrada, Coates-Estrada, and Meritt 1993).

## <H1>Acknowledgments

We thank UC MEXUS for supporting this research, and acknowledge the contributions and extend our appreciation to the fruit growers that contributed local knowledge. We are grateful to the Conzatti's of Oaxaca for access to precious living material at the Conzatti Garden. The second author would like to thank COFAA of the Instituto Politécnico Nacional for their support. Finally, much appreciated assistance in Hidalgo was provided by Moses Ramirez.

## <H1>Literature Cited

Alcorn, J.B. 1984. Page 630 in *Huastec Mayan Ethnobotany*. University of Texas Press.

Calderón-Olascoaga, J.A. 2011. Análisis citogenético comparativo entre dos especies del genero *Diospyros* (Ebanaceae). Thesis. Universidad Michoacana de San Nicolás de Hidalgo, Michoacan, Mexico.

- Casas, A., A. Otero-Arnaiz, E. Pérez-Negrón, and A. Valiente-Banuet. 2007. In situ management and domestication of plants in Mesoamerica. *Annals of Botany*. 100:1101-1115.
- Doolittle, W.E. 1990. Pages 97, 168-174, *in* Canal irrigation in prehistoric Mexico: the sequence of technological change. University of Texas Press, Austin.
- Duangjai, S., S.R. Samuel, J. Munzinger, F. Forest, B. Wallnöfer, M.H.J. Barfuss, G. Fischer, and M.W. Chase. 2009. A multi-locus plastid phylogenetic analysis of the pantropical genus *Diospyros* (Ebenaceae), with an emphasis on the radiation and biogeographic origins of the New Caledonian endemic species. *Molecular Phylogenetics and Evolution* 52:602-620.
- Estrada, A., R. Coates-Estrada, and D. Meritt Jr. 1993. Bat species richness and abundance in tropical rain forest fragments and in agricultural habitats at Los Tuxtlas, Mexico. *Ecography*. 16:309-318.
- Hudson, R.R., D.D. Boos, and N.L. Kaplan. 1992. A statistical test for detecting geographic subdivision. *Molecular Biology and Evolution*. 9:138-51.
- INEGI. 2012. Instituto Nacional de Estadística, Geografía e Informática. Consulted X. <http://www.inegi.org.mx/geo/contenidos/recnat/clima/default.aspx>
- Luna Vega, I., O. Alcántara Ayala, R. Contreras-Medina, and A. Ponce Vargas. 2006. Biogeography, current knowledge and conservation of threatened vascular plants characteristic of Mexican temperate forests. *Biodiversity and Conservation*. 15:3773-3799.
- Melville, E.G.K. 1997. Pages 107-110 *in* A plague of sheep: environmental consequences A plague of sheep: environmental consequences of the conquest of Mexico. Cambridge University Press, Cambridge, UK.

- Perfecto, I., J. Vandermeer, P. Hanson, and V. Cartín. 1997. Arthropod biodiversity loss and the transformation of a tropical agro-ecosystem. *Biodiversity and Conservation*. 6:935-945.
- Provance, M.C. and A.C. Sanders. 2006. More American black sapotes: new *Diospyros* (Ebenaceae) for Mexico and Central America. *Sida*. 22:277-304.
- Provance, M.C., I. Garcia-Ruiz, and A.C. Sanders. 2008. The *Diospyros salicifolia* complex (Ebenaceae) in Mesoamerica. *Journal of the Botanical Research Institute of Texas*. 2:1009-1100.
- Provance, M.C. and A.C. Sanders. 2009. An overview of the *Diospyros campechiana* complex (Ebenaceae) and description of three new species. *Journal of the Botanical Research Institute of Texas*. 3:85-11.
- Reitsma, R., J.D. Parrish, and W. McLarney. 2001. The role of Cacao plantations in maintaining forest avian diversity in southeastern Costa Rica. *Agroforestry Systems*. 53:185-193.
- Reynolds, H. C. 1945. Some aspects of the life history and ecology of the opossum in central Missouri. *Journal of Mammalogy*. 26:361-379.
- Saghai-Maroofo, M.A., K.M. Soliman, R. A. Jorgensen, and R. W. Allard. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences*. 81:8014-8018.
- Sosa, V., A.P. Vovides, and G. Castillo-Campos. 1998. Monitoring endemic plant extinction in Veracruz, Mexico. *Biodiversity and Conservation*. 7:1521-1527.
- Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*. 17:1105-1109.

- Thornton, K. 2003. Libsequence: a C++ class library for evolutionary genetic analysis. *Bioinformatics*. 19:2325-2327.
- Wallnöfer, B. 2007. A revision of neotropical *Diospyros* (Ebenaceae): part 1. *Annalen des Naturhistorischen Museums in Wien, Serie B*. 108:207-247.
- Wallnöfer, B. 2009. A revision of neotropical *Diospyros* (Ebenaceae): part 2. *Annalen des Naturhistorischen Museums in Wien, Serie B*. 110:173-211.
- Worth, C.B. 1975. Virginia opossums (*Didelphis virginiana*) as disseminators of the common persimmon (*Diospyros virginiana*). *Journal of Mammalogy*. 56:517.
- Zhao, D., Zhou, C., Sheng, Y., Liang, G., and J. Tao. 2011. Molecular cloning and expression of phytoene synthase, lycopene beta-cyclase, and beta-carotene hydroxylase genes in persimmon (*Diospyros kaki* L.) fruits. *Plant Molecular Biology Reporter*. 29:345-51.
- Zhou, C., Zhao, D., Sheng, Y., Liang, G. and J. Tao. 2012. Molecular cloning and expression of squalene synthase and 2,3-oxidosqualene cyclase genes in persimmon (*Diospyros kaki* L.) fruits. *Molecular Biology Reports*. 39:1125-1132.

Figure 1. A map of the *Diospyros riojae* Gomez-Pompa collection sites in Mexico used in genetic analyses (circles), and the locations of *D. riojae* fruit vendors (squares). Toliman vicinity (1), Ezequiel Montes (2), Pathé (3), Tecozautla vicinity (4), Huichapan (5) San Antonio Xalcuayo Dos (6), La Herradura (7), Tanjasnec (8), Aldzulup Poytzén (9). Collection sites for: *D. riojae*, west (1) and east (2) of the Sierra Gorda, and Cruz Blanca, Veracruz (3); *D. conzattii* Standley, Cafetal San Rafael (source of the material in the Conzatti Garden, City of Oaxaca, Oaxaca) (4); *D. cf. riojae*, Chimalapas Region, Oaxaca (5).

Figure 2. Comparison of mature, air-dried leaves of *Diospyros konzattii* (A, B) and *D. riojae* (C, D). Both the adaxial (A, C) and abaxial (B, D) surfaces are shown.

Figure 3. Some recently ripened *Diospyros riojae* fruit in a crate in Tecozautla, which will likely be sold to a vendor in Huichapan, Hidalgo.

Table 1. Location, taxon, herbarium voucher (collector, collection number, and herbarium), elevation, region, management setting, and tree origin of Mesoamerican *Diospyros* sp. material analyzed. R = *D. riojae*, C = *D. konzattii*, cfR = *D. cf. riojae*; IGR = I. Garcia-Ruiz, with M.C. Provance, MCP = M.C. Provance, with Moses Ramirez, IGR & C = I. Garcia-Ruiz et al., \* = included in genetic analyses; HG = Homegarden, N = natural vegetation communities, CMF = community-managed forest, R = streets/old fields/pastures/vacant lots, AHG = abandoned homegardens, CMG = community-managed groves, BG = botanical gardens. U = unknown; GBO = germinated by owner, U = unknown, s = spontaneous.

Species	Location	Herbarium voucher	UTM	Altitude	Region	Setting	Origin
<i>D. riojae</i>	La Herradura, SLP	IGR 8314 CID, MPR19 UCR*	14 Q 505240 2371150	88 m	East	HG	U
<i>D. riojae</i>	S. Antonio Xal. Dos, SLP	IGR 8310-8318 CID, MPR20-MPR24 UCR*	14 Q 498360 2362940	834 m	East	HG	U
<i>D. riojae</i>	Tanjasnec, SLP	IGR 8316 CID, MPR25-MPR26 UCR*	14 Q 510910 2392350	271 m	East	HG	U
<i>D. riojae</i>	Aldzulup Poytzen, SLP	IGR 8317-8319 CID, MPR11-MPR18 UCR*	14 Q 501490 2396490	60 m	East	HG	S, U
<i>D. riojae</i>	Cruz Blanca, VER	T. Vázquez 98-045 UCR*	14 Q 748030 2185800	1072 m	East	N	S
<i>D. riojae</i>	La Cañada, QTO	IGR 8301 CID, MPR09 UCR*	14 Q 406290 2314950	1535 m	West	HG	GBO
<i>D. riojae</i>	Ezequiel Montes, QTO	IGR MP06-MPR07 UCR*	West of Sierra Gorda	Unknown	West	U	U
<i>D. riojae</i>	A. Pequeño Salitre, QTO	IGR 8300 CID, MPR10 UCR*	14 Q 405960 2314220	1535 m	West	CMF	U
<i>D. riojae</i>	Pathé, QTO	IGR 8322 CID, MPR08 UCR*	14 Q 426850 2278090	1774 m	West	HG	GBO
<i>D. riojae</i>	West Tecozautla, HDG	IGR 8323 CID, MPR01-MPR05 UCR*	14 Q 432240 2270820	1710 m	West	HG	S, U
<i>D. riojae</i>	Rancho Viejo, HDG	MCP 17334 UCR	14 Q 428110 2273270	1676 m	West	R	U
<i>D. riojae</i>	La Esquina, HDG	MCP 17336 UCR	14 Q 427050 2272000	1687 m	West	HG	GBO
<i>D. riojae</i>	San Antonio, HDG	MCP 17335 UCR	14 Q 426250 2271210	1712 m	West	AHG	U
<i>D. riojae</i>	Rio Tecozautla, HDG	MCP 17308-17310B UCR	14 Q 434440 2270460	1693 m	West	HG	S

<i>D. riojae</i>	Tecoautla, HDG	MCP 17303-17307, 17311-17322 UCR	14 Q 433420 2271160	1705 m	West	HG, R	GBO, S, U
<i>D. riojae</i>	El Paso, HDG	MCP 17323-17332 UCR	14 Q 433750 2275360	1616 m	West	CMG, R	S, U
<i>D. konzattii</i>	Conzatti Garden, OAX	IGR & C 8343 UCR*	14 Q 742490 1888670	1566 m	South	BG	S, U
<i>D. cf. riojae</i>	El Peñasco, OAX	S. Maya 3885 UCR*	15 Q 363150 1843120	1500 m	South	N	S
<i>D. digyna</i>	Marie Selby B.G., Florida	Chase 212 K*	17 R 347656 3023648	3 m	Unk.	BG	U
<i>D. digyna</i>	UCR B.G., California	M.C. Provance s.n. UCR*	11 S 469918 3758759	400 m	Unk.	BG	GBO
<i>D. californica</i>	Cult. Riverside, CA	M.C. Provance s.n. 2 UCR*	11 S 463042 3758428	300 m	West	HG	GBO
<i>D. rekoii</i>	San Jeronimo, JAL	M.C. Provance 8152 UCR*	13 Q 605740 2256490	1430 m	West	HG	S
<i>D. rosei</i>	Chamela, JAL	Quigley 621 UCR*	13 Q 491480 2159390	90 m	West	N	S

Table 2. Observed Fst and permutation p-values for comparisons between *Diospyros riojae*

Gomez-Pompa and *D. konzattii* Standley, for four nuclear genes. BCH = beta-carotene

hydroxylase, LCYB = lycopene beta-cyclase, SQS = squalene synthase, PSY = phytoene

synthase.

Comparison	Fst observed	P (1000 replicates)
<i>D. riojae</i> vs. <i>D. konzattii</i> (BCH)	0.6	0.033
<i>D. riojae</i> vs. <i>D. konzattii</i> (LCYB)	0.14942	0
<i>D. riojae</i> vs. <i>D. konzattii</i> (SQS)	1	0.001
<i>D. riojae</i> vs. <i>D. konzattii</i> (PSY)	-0.0119219	0.514
<i>D. riojae</i> vs. <i>D. cf. riojae</i> (BCH)	0.3	0.067
<i>D. riojae</i> vs. <i>D. cf. riojae</i> (LCYB)	0.0108828	0.334
<i>D. riojae</i> vs. <i>D. cf. riojae</i> (SQS)	0	--
<i>D. riojae</i> vs. <i>D. cf. riojae</i> (PSY)	0.00843939	0.191

Table 3. Summary statistics for populations of *Diospyros riojae* west of the Sierra Gorda, east of the Sierra Gorda, and for the populations combined.

Locus	Region	nsam	bp	Number of polymorphisms	Polymorphisms occurring in a single individual	Number of haplotypes	Haplotype diversity	Nucleotide diversity
LCYB	East	16	177	3	0	6	0.758065	0.00613951
PSY	East	18	408	15	1	11	0.852941	0.0128582
SQS	East	14	174	0	0	1	0	0
LCYB	West	10	177	1	1	2	0.1	0.000564972
PSY	West	6	408	10	10	3	0.318182	0.00410509
SQS	West	9	174	0	0	1	0	0

