

Serendipitous backyard hybridization and the origin of crops

Colin E. Hughes^{*†}, Rajanikanth Govindarajulu[‡], Ashley Robertson^{*§}, Denis L. Filer^{*}, Stephen A. Harris^{*}, and C. Donovan Bailey[‡]

^{*}Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, United Kingdom; and [‡]Department of Biology, New Mexico State University, P.O. Box 30001, Department 3AF, Las Cruces, NM 88003

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Backyard gardens, dump heaps, and kitchen middens are thought to have provided important venues for early crop domestication via generation of hybrids between otherwise isolated plant species. However, this process has rarely been demonstrated empirically. For the majority of polyploid crops, it remains uncertain to what extent hybridization and polyploidization preceded domestication or were precipitated by human activities. Using archaeological, ethnobotanical, geographical, and genetic data, we investigate the extent and significance of predomestication cultivation, backyard sympatry, and spontaneous hybridization for the Mimosoid legume tree *Leucaena*, which is used as a food crop throughout south-central Mexico. We show that predomestication cultivation was widespread, involved numerous independent transitions from the wild to cultivation, and resulted in extensive artificial sympatry of 2–6 species locally and 13 species in total. Using chloroplast and rapidly evolving nuclear-encoded DNA sequences, we demonstrate that hybridization in *Leucaena* has been extensive and complex, spawning a diverse set of novel hybrids as a result of juxtaposition of species in cultivation. The scale and complexity of hybridization in *Leucaena* is significantly greater than that documented for any other Mexican plant domesticates so far. However, there are striking parallels between *Leucaena* and the other major Mexican perennial domesticates *Agave* and *Opuntia*, which show very similar domestication via backyard hybridization pathways. Our results suggest that backyard hybridization has played a central role in Mesoamerican crop domestication and demonstrate that the simple step of bringing species together in cultivation can provide a potent trigger for domestication.

domestication | Leguminosae | *Leucaena* | Mesoamerica

Hybridization between plant species is frequent in many contemporary contexts where species have been brought together in cultivation (1–4). It is also clear that hybridization and polyploidy have played a central role in the origins of many of the world's crops (5–7). Pinpointing where, when, and how hybridization occurred and assessing the extent to which hybrids and polyploids can be attributed to movement of wild and incipiently domesticated species by early proto-agriculturalists are central to understanding the role of hybridization in crop domestication and the processes that prompted and facilitated early domestication more generally. Hexaploid breadwheat and octaploid strawberry provide well documented examples of crops, which are unknown in the wild and are thought to have arisen via spontaneous hybridization in cultivation (7). In contrast, allotetraploid cotton is thought to have originated well before the arrival of humans in the New World (8). However, for the majority of polyploid crops, such as bananas, citrus, potatoes, kiwi fruit, oca, and peanuts, it remains uncertain to what extent hybridization and polyploidization preceded domestication or were precipitated by human activities (9).

To investigate the role of hybridization in crop domestication, we focus on Mesoamerica, which is one of the most important regions where crops were domesticated (10–15). Recent studies pinpoint where, when, how many times, and from what progen-

itors the mainstream crops of this region, such as maize, beans, and squash, were domesticated (11–15). However, the origins of most other Mesoamerican domesticates remain poorly understood (16). Among these is a set of important polyploid crops in the genera *Agave*, *Opuntia*, and *Leucaena* for which there is preliminary evidence to suggest that hybridization between species in cultivation may have been important in driving domestication (2, 17, 18). For all three genera, archaeobotanical data provide evidence of use over the last 6,000–9,000 years (17, 19, 20); utilization involves more than one, and sometimes numerous, species in any one area; for each there are finely differentiated cognitive systems indicative of sophisticated comparative knowledge of species traits (21–23); present-day use suggests that each genus presents a complex mosaic of wild, managed, and cultivated backyard populations often involving species in sympatry; and finally, hybridization and polyploidy are frequent in all three genera.

To gain insight into hybrid origins and the extent to which these can be attributed to predomestication cultivation, we examine one of these domesticates, the Mimosoid legume tree *Leucaena*, which comprises 22 species of small trees distributed from the southern United States to Peru (24). There is clear evidence to suggest that interspecific hybridization has been important in *Leucaena*. Five tetraploid species have been documented (25), and there is preliminary evidence that two of these, *L. leucocephala* and *L. confertiflora*, are allopolyploids (26). Artificial crossing experiments have shown that crossability among species is high (27). In addition, two named hybrids, *L. × mixtec*, a putative sterile triploid between tetraploid *L. leucocephala* and diploid *L. esculenta*, and *L. × spontanea*, a putative fertile hybrid between tetraploid *L. leucocephala* and *L. diversifolia*, have been proposed (26, 28, 29). Furthermore, several other putative hybrids found growing in backyards in south-central (S-C) Mexico (Fig. 1), are investigated here. Previous attempts to disentangle divergent from reticulate relationships in *Leucaena* have relied on chloroplast restriction fragment length polymorphism (30) and internal transcribed spacer (ITS) sequence data (26). These analyses provided evidence for three robustly supported clades of diploid species but lack of resolution within clades, and the occurrence

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Abbreviation: S-C, south-central.

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[†]To whom correspondence should be addressed. E-mail: colin.hughes@plants.ox.ac.uk.

[§]Present address: School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, United Kingdom.

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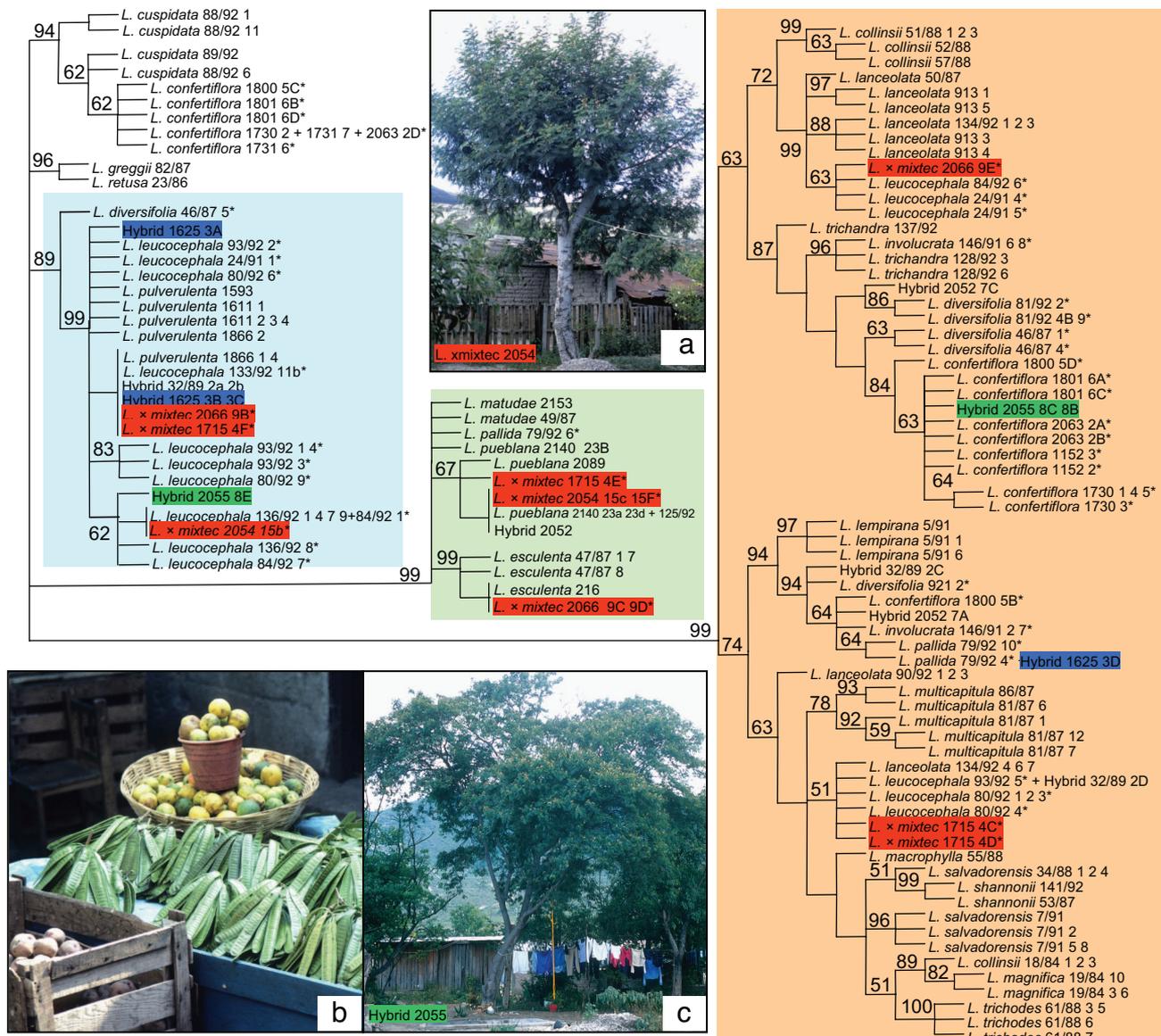


Fig. 2. Biparentally inherited nuclear 23L gene tree for *Leucaena*. Tetraploid species names are indicated by *. The major clades (color-shaded) are congruent with previous studies (26, 30, 31) and the chloroplast gene tree (SI Fig. 3). Highlighted terminals show divergent placements of sequence types recovered from *L. × mixtec* (red), hybrid 2055 (green) between the tetraploids *L. leucocephala* and *L. confertiflora*, and hybrid 1625 (blue) between the tetraploids *L. leucocephala* and *L. pallida*. Strict consensus of 168 equally parsimonious trees is shown. Length = 268 steps; consistency index = 0.66; retention index = 0.93. The 23L matrix comprises 909 aligned characters with 130 parsimony informative substitutions and 30 informative gaps. Numbers at nodes are bootstrap values. (a–c) Photos illustrate backyard hybrids and marketing of *Leucaena* pods. (a) Sterile triploid *L. × mixtec* in a backyard in San Pedro Chapulco, Puebla. (b) Pods of *L. leucocephala*, the most popular and widespread species for food use, for sale in San Cristobal de las Casas, Chiapas. (c) Hybrid 2055 (highlighted in green) between *L. leucocephala* and *L. confertiflora* in a backyard in San Pedro Chapulco, Puebla.

Leucaena are clearly apparent. Archaeobotanical data from sites across S-C Mexico (19, 34–36) suggest that *Leucaena* seeds have provided a minor, but constant, food source in this region for the last 6,000 years and provide evidence for early cultivation and translocation. Seed remains in caves in Tamaulipas and Oaxaca, and the earlier remains from Tehuacán, correspond to *L. pulverulenta*, *L. trichandra*, and *L. pueblana*,[¶] respectively (19) that grow naturally, but not in cultivation, in the vicinity of these caves today, suggesting that these resulted from archaic foraging in free-living populations (35, 36). The first appearance of seeds and pod fragments of *L. esculenta* from ≈3,200 B.P. in the

Coxcatlán and Purrón caves has been interpreted as marking the start of cultivation of *Leucaena* in the Tehuacán Valley (19). Seeds of *L. leucocephala*, the most widely cultivated species today, were first identified in the Coxcatlán and Purrón sequences between 2,300 B.P. and 1,200 B.P. (19). These two species are abundantly cultivated in the Tehuacán Valley today, but there is no evidence of natural populations to suggest that either species is native there (19). These records are compatible with field observations suggesting that the present-day distribution of *L. esculenta* is much wider than the original native range (19, 24). Archaeological evidence thus suggests that *Leucaena* was brought into cultivation soon after the wider transition from incipient cultivation to village agriculture that started once fusion of the Mesoamerican mainstream crop complex was

[¶]*L. pueblana* and *L. trichandra* were treated as *L. diversifolia* subsp. *stenocarpa* by Zárate (19). Taxonomy here follows Hughes (24).

established $\approx 4,000$ B.P. (11, 37). This sequence fits with the idea that tree cultivation lags behind domestication of annual crops because of longer life cycles and the need for sedentary agriculture before tree cultivation (38).

A total of 1,631 3-min grid cells were recorded containing *Leucaena* from the United States to Peru. Of these, 1,474 contain just a single species, reflecting the predominance of allopatric species distributions (SI Figs. 4 and 5). Backyard sympatry between pairs of species is common across S-C Mexico (Fig. 1a and SI Figs. 4 and 5) with 31 of the 120 possible species combinations (of the 16 species in that area) detected. Although the widely cultivated *L. esculenta*, *L. leucocephala*, and *L. pallida* dominate sympatry, all 13 species used for food were found in sympatry somewhere (SI Table 1). Mixtures of three species occur commonly in northern Oaxaca and southern Puebla and sporadically in Chiapas, Morelos, and Veracruz. Higher levels of sympatry with four to six co-occurring species are found in villages around the upper Tehuacán Valley (Fig. 1a). The substantially lower levels of sympatry found when records representing cultivated trees are excluded (SI Fig. 4) demonstrate that sympatry in *Leucaena* is predominantly artificial and attributable to cultivation. Cultivation of multiple species together has been driven by pursuit of diverse seed and pod qualities and production seasons and the potential for year-round pod production (SI Table 1).

The overall geography of *Leucaena* food use and the concentration of sympatry and spontaneous hybrids in the upper Tehuacán Valley (Fig. 1 and SI Fig. 5) are consistent with the archaeological data suggesting early cultivation in that area and with the emerging focus on S-C Mexico as a center for early agricultural development (11, 37). After initial cultivation, it is clear that *Leucaena* has been brought into cultivation many times independently, at scattered localities across a broad swathe of S-C Mexico, involving at least nine species and, for three species, extensive translocation. Cultivation in the absence of domestication is not unusual among Neotropical fruit trees (16, 39). For *Leucaena*, predomestication cultivation was extensive and involved numerous repeated and temporally and spatially isolated wild to cultivated transitions of a range of species.

Spontaneous Hybridization. The phylogenetic analyses suggest that all five polyploid species, *L. confertiflora*, *L. diversifolia*, *L. involucreta*, *L. leucocephala*, and *L. pallida* may be allopolyploids (Fig. 2) and provide hypotheses for their parentage. Robustly supported placement of divergent 23L sequences from polyploid individuals with specific diploids suggest *L. cuspidata* and *L. trichandra* as parents of *L. confertiflora*, *L. pulverulenta* and *L. lanceolata* are parents of *L. leucocephala*, and *L. pulverulenta* and *L. trichandra* are probable parents of *L. diversifolia* (Fig. 2). With the exception of the evidence for a hybrid origin of *L. diversifolia* and multiple independent origins of *L. leucocephala*, the results are consistent with previous data (26, 30) and a chloroplast DNA gene tree (SI Fig. 3). Anthropogenic origins of two of the five allopolyploid species, *L. leucocephala* and *L. pallida*, are suggested by several lines of evidence, and notably by the lack of known natural populations of these species. The case for *L. leucocephala* is particularly compelling. Despite numerous field collection efforts for this species, no natural populations (apart from weedy occurrences in Yucatan) have been located. Year-round pod production, abundant pod set, seed set on isolated trees caused by self-compatibility, and sweet seeds make *L. leucocephala* outstanding for food production compared with other species (Fig. 2b). These attributes mean that *L. leucocephala* is now the most widely cultivated species of *Leucaena* throughout Mexico. Despite this present-day abundance in cultivation and a 6,000-year archaeological record of *Leucaena* seed fragments, seeds of *L. leucocephala* first appeared only $\approx 2,300$ years ago (19). Attempts to establish *L. leucocephala* at

or beyond its drought and cold tolerance limits using irrigation or even potted trees are evidence of the continued drive to cultivate this species. A spontaneous origin of *L. leucocephala* would have been immediately noticed and seized on by *Leucaena*-conscious farmers. Such a scenario is compatible with the lack of natural populations, low levels of genetic variation among Mexican accessions of the widely cultivated subsp. *glabrata* favored for food use (40), and a possible origin in central Veracruz where *L. leucocephala* occurs sympatrically with sporadically cultivated trees of *L. pulverulenta* in areas where *L. lanceolata* is abundant.

The analyses also provide evidence for the parentage of a number of individuals that were identified as putative morphological hybrids (Fig. 2). Notably, three 23L sequence types placed in strongly supported divergent clades were recovered from putative *L. × mixtec* hybrids (1715 and 2066 in Fig. 2). This finding is as expected for triploids between tetraploid *L. leucocephala*, itself with two divergent sequence types, and one of two diploid species from the *L. esculenta* clade (26, 28). Spontaneous backyard hybridization in *Leucaena* was first suggested by the sporadic, but widespread, occurrence of this hybrid in six states in S-C Mexico (28) (Figs. 1b and 2a). As these hybrids are sterile and *Leucaena* is only propagated by seed and not vegetatively, all individuals must represent independent F₁ hybrids and all are found as backyard trees. *Leucaena × mixtec* is recognized as the “*guaje macho*” because of its lack of pods but remains relatively common despite culling of trees, probably caused by how frequently this hybrid arises and inadvertent cultivation. Many *L. × mixtec* trees are reported to have grown from seed purchased as “*guaje verde*” (*L. leucocephala*), which were either sown deliberately or discarded in backyards. This finding is in line with evidence that most *L. × mixtec* individuals had *L. leucocephala* as the female parent (28). Although it is of no use for food, this hybrid provides tangible evidence of spontaneous backyard hybridization and the complex and serendipitous pathways by which such spontaneous hybrids may be disseminated, often unwittingly, via backyards and kitchen middens.

Individuals 2055 and 1625 (Fig. 2) are hybrids between tetraploid *L. leucocephala* and the tetraploids *L. confertiflora* and *L. pallida*, respectively, in line with sympatric occurrences of these species in backyards in San Pedro Chapulco, Santiago Acatepec, and Santa Catalina Oxolotepec where they are found (Fig. 1b). That these hybrids have *L. leucocephala* as one parent reflects the very widespread cultivation of this species. Current evidence suggests that these hybrids are sporadic and uncommon. However, field survey of *Leucaena* hybrids has only scratched the surface so far; only four villages were surveyed in detail for hybrids in this study. Furthermore, backyard plants are often neglected by botanical collectors, and it is likely that backyard *Leucaena* hybrids are more common than current collections suggest. Evidence for hybrids arising in natural populations of *Leucaena* is lacking, with the exception of putative hybrids between *L. collinsii* and *L. magnifica* in southeast Guatemala (26) (SI Fig. 6).

Conclusions

Edgar Anderson (41, 42) was among the first to point out the importance of disturbed sites such as kitchen middens and backyard gardens as suitable habitats for spontaneous hybridization, where otherwise isolated plant species were brought into sympatry in cultivation. Anderson (42) suggested that these sites were important venues for the generation of hybrids and polyploids involved in crop domestication, but this process has rarely been demonstrated in practice. For *Leucaena*, we have shown just how prevalent and influential the bringing together of species, casually or intentionally, in cultivation in backyards can be. Predomestication cultivation of *Leucaena* species has resulted in extensive artificial sympatry and a complex series of

geographically dispersed spontaneous hybrids and at least one and possibly more polyploids. The scale and complexity of spontaneous hybridization in *Leucaena* is far greater than that documented for any other Mexican plant domesticated so far (2) to our knowledge.

Two other genera that have produced important Mexican plant domesticates, *Agave* and *Opuntia*, show striking similarities to *Leucaena*. Although genetic studies of hybrids and polyploids in *Agave* and *Opuntia* and surveys of their cultivation are patchy, the evidence for high levels of sympatry in cultivation and spontaneous interspecific hybridization for both these genera is nonetheless compelling (17, 20). For example, in the Bajío region of Guanajuato fruits and *nopales* are harvested from 16 species of *Opuntia* that are wild, tolerated in fields, encouraged in “*nopalera*,” and cultivated in gardens (43). At least five species are widely cultivated alongside other minor cultivated and wild species (20). Ease of hybridization and vegetative propagation are well known in *Opuntia* (18), and it has been suggested that the most widely cultivated domesticated, *Opuntia ficus-indica*, an octoploid, arose as a spontaneous hybrid in cultivation (18). The so-called Man-Agave symbiosis (17) is similar. Ease of vegetative propagation and a long archaeological record suggest that Agaves were among the earliest cultivated plants in Mexico. At least 15 species of *Agave* are widely used and cultivated, and high levels of sympatry in cultivation are common across S-C Mexico. A suite of putative hybrids has been identified (17), including possible spontaneous hybrid origins for the three polyploid domesticates *Agave fourcroydes*, *Agave sisalana*, and *Agave tequilana* after translocation and cultivation (17, 44).

Flannery (45) suggested *Agave*, cactus fruits, and legume seeds as three of six fundamental procurement systems involved in archaic foraging in Mesoamerica. Taken together *Leucaena*, *Agave*, and *Opuntia* comprise three of the dominant perennial plants cultivated in S-C Mexico today. In all three genera, domestication has apparently been facilitated by spontaneous hybridization after extensive predomestication cultivation. In each case there is evidence that the prominent species in cultivation, *L. leucocephala*, *O. ficus-indica* and *A. sisalana*/*A. fourcroydes*/*A. tequilana*, have hybrid origins most likely after cultivation. There is also evidence to suggest that hybridization has been important in many other Mesoamerican crops, such as *Cucurbita*, *Phaseolus*, *Capsicum*, *Lepidium*, *Hyptis*, and *Panicum* (2, 46). It seems that, as suggested by Anderson (42), the simple step of bringing species together, consciously or casually, in dump heaps and informal backyard orchards has played a central role in Mesoamerican crop domestication. As we unravel the domestication history of other crops in Mesoamerica and elsewhere, we can expect other examples where hybridization triggered by early cultivation has been instrumental in generating much of the diversity that we see today.

Methods

Ethnobotanical Survey. We assessed present-day use, marketing, cultivation, and translocation of *Leucaena* throughout its native range from Peru to the United States from published studies (22, 23, 33) and new data assembled from interviews with farmers and people harvesting, processing, and selling pods during 14 field collection expeditions that have allowed us to observe all 22 species and hybrids (SI Table 1). In addition, four villages in southern Puebla, San Pedro Chapulco, Santa Catalina Oxolotpec, Santiago Acatepec, and Santa Ana Teloxtoc, where high levels of sympatry were found, were surveyed for hybrids in detail in 2001 and 2003.

Geographic Data. Present-day species and hybrid distributions were mapped from specimens in 21 herbaria geo-referenced where necessary from 1:250,000 maps and supplemented by records of species occurrences observed by the authors. These

provided a total of 2,617 data points for the 22 species and two named hybrids. Further details on each specimen are available at <http://herbaria.plants.ox.ac.uk/bol/?leucaena>. A further 332 herbarium records were discarded because of the lack of detailed locality data. Of the 2,617 records, 802 represented trees in cultivation based on conservative assessment of field observations, herbarium specimen notes, and data from local informants. Although distribution maps inevitably reflect collection effort, this problem is less serious for *Leucaena* where the very large number of records for a genus of this size reflect intensive collecting of *Leucaena* as forage genetic resources (47). Nevertheless, high levels of sympatry among species and the occurrence of hybrids are likely to be more frequent in S-C Mexico than currently depicted in Fig. 1. Taxonomy follows Hughes (24). Sympatry among species was mapped by using a circular neighborhood with a radius of 5 km to assign records to a grid cell by exporting data points from BRAHMS (www.brahmsonline.com) to the DIVA-GIS software (www.diva-gis.org). This approach produces a smoother grid that is less biased by the origin of the grid and less sensitive to errors in coordinate data than standard grid-cell approaches (www.diva-gis.org). This neighborhood size reflects estimated distances for pollen flow facilitated by *Xylocopa* bees that visit *Leucaena* flowers and groom for pollen. Separate maps were assembled in this way including (Fig. 1*a* and SI Fig. 5) and excluding (SI Fig. 4) cultivated records.

Sequence Data and Phylogenetic Analysis. Seventy-nine accessions representing all diploid and tetraploid species (most with multiple accessions), 15 putative hybrids, and two outgroups (chloroplast data only) were sampled. Three noncoding chloroplast loci (*psbA-trnH* and the two *trnK* introns) were sequenced by using standard protocols. In addition, one anonymous nuclear DNA sequence locus, termed 23L, was selected as the most informative of a set of sequence characterized amplified region-based loci developed for *Leucaena* (31). For finding resolution within *Leucaena* this approach worked well (31), but with the limitation that 23L fails to amplify in outgroup taxa. The 23L gene tree is thus rooted internally (Fig. 2 and SI Fig. 6). The major clades highlighted in Fig. 2 and SI Fig. 6 are consistent with previous data (26, 30, 31). For all hybrid, polyploid, and the majority of diploid accessions, 23L PCR products were cloned by using standard protocols to sample intraindividual sequence diversity attributable to hybridization or heterozygosity. Voucher details and GenBank accession numbers are provided in SI Table 2. Numbers of distinct sequence types recovered from individuals are generally in line with expectations for different ploidy levels (SI Fig. 6). With two notable exceptions, both probably caused by hybridization, sequences of diploids group according to species (SI Fig. 6). Extensive cloning and sampling in the 23L gene tree and congruence with previous internal transcribed spacer and chloroplast restriction fragment length polymorphism gene trees (26) provide no indications of underlying paralogy problems attributable to multiple copies of the 23L locus (SI Fig. 6). The assumption in phylogenetic analyses of multiple DNA alignments that all sites share the same evolutionary history is violated if genomic or PCR-mediated recombination has occurred among sequences, causing errors in tree estimation (48, 49). We identified five potential recombinant sequences, all from polyploid or hybrid accessions, based on excess homoplasy on terminal branches (50) and the ϕ test (51). These were excluded from the analysis. Sequences were aligned by using CLUSTALX and manually adjusted. Indels were coded by using a simple gap-coding method (52). Separate parsimony analyses of the chloroplast and 23L data sets were conducted with NONA (www.cladistics.com) by using 1,000 random addition sequences, tree bisection and reconnection, holding 100 trees per replication and swapping to completion.

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