

Origins and Dispersal of Cultivated Vanilla (*Vanilla planifolia* Jacks. [Orchidaceae])¹

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Origins and Dispersal of Cultivated Vanilla (*Vanilla planifolia* Jacks. [Orchidaceae]). Vanilla is a clonally propagated crop originating from Mesoamerica. Information regarding the circumstances under which vanilla cultivation began is incomplete. Presumably, the Totonac people of Papantla (north-central Veracruz, Mexico) were the earliest to cultivate vanilla; however, the oldest reports of vanilla use relate to the pre-Columbian Maya of southeastern Mexico/Central America, where vanilla was a cacao-beverage spice. We utilized Amplified Fragment Length Polymorphism (AFLP) marker diversity to infer the origins and relationships among cultivated and non-cultivated vanilla in Mesoamerica and on islands in the Indian Ocean, which comprise today's principal production regions of vanilla. Our results suggest that, genetically, vanilla cultivated outside of Mesoamerica is most closely related to cultivated stock from Papantla; whereas unique clones of *V. planifolia* are found in non-cultivated and cultivated individuals from elsewhere in Mesoamerica. This is consistent with a single origin for cultivated vanilla outside of Mexico, along with multiple origins for cultivated material within Mexico. These data suggest that vestiges of pre-Columbian Maya vanilla cultivars are not found in commercial production today.

Key Words: *Vanilla*; Orchidaceae; Mesoamerica; AFLP; crop origins.

Introduction

Vanilla is a value added spice crop produced in tropical countries and consumed principally in the United States, France, and Germany (FAOSTAT). Most production occurs at the household level and involves tens of thousands of farmers worldwide. Distribution is carried out in a supply chain that starts with local trade at the community and regional level, followed by re-sales to various curers/exporters, international brokers, and wholesalers/extract manufacturers (Ecott 2004; Rain 2004). Two clonally propagated species are commercialized for their cured, fermented fruits: *Vanilla planifolia* Jacks. (i.e., “Mexican” or “Bourbon” vanilla) and *V. tahitensis* J. W. Moore

(“Tahitian” vanilla) (Childers et al. 1959; Correll 1953; Purselglove et al. 1981; Sauer 1993). Of the estimated 5,000 to 10,000 metric tons (Mt) of vanilla-bean production per annum, roughly 95% derives from *V. planifolia*, with most coming from Madagascar and Indonesia (FAOSTAT).

The genus *Vanilla* Plumier ex Miller (ca. 110 species) belongs to one of the basal subfamilies of the Orchidaceae, the Vanilloideae (Cameron et al. 1999). The early origin of *Vanilla* among the orchids is evidenced by its numerous primitive traits such as fruits that are fleshy as well as hard, sclerotic seed coats (Cameron and Soto Arenas 2003). According to molecular clock estimates, *Vanilla* is around 65 million years old (Ramírez et al. 2007). This age supports the hypothesis that the pan-tropical distribution of the genus, which is rare among orchids, has resulted from the separation of continental landmasses (Cameron and Soto Arenas 2003). The only *Vanilla* reported to have aromatic fruits, about 30 species,

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are from the neotropics and the Caribbean, with the exception of *V. tabitensis* (Soto Arenas 1999).

The principal commercial vanilla species, *V. planifolia* ($2n=32$), is a rare, perennial climbing herb endemic to humid evergreen forests in Mesoamerica, on karst substrates between 250 and 750 meters above sea level (masl), in regions that receive more than 2,500 millimeters (mm) of annual precipitation (Hágsater et al. 2005; Portères 1954; Soto Arenas 1999; Tanaka and Kamemoto 1984). It is occasionally sympatric with several other aromatic *Vanilla* species including *V. pompona* Schiede, *V. odorata* C. Presl, *V. insignis* Ames, and *V. bartii* Rolfe, as well as with non-aromatic species like *V. inodora* Schiede (Soto Arenas 1999).

The Aztec elite and merchant class esteemed vanilla for its flavor and rarity. For his pleasure garden in Huaxtepec, Moctezuma “dispatched messengers to the tropical coast region with a request to the Lord of Cuertaxtla [San Juan de Ulua, Veracruz] for plants with roots of the vanilla orchid” (Nuttall 1925; p. 456). These plants presumably came from the region of Papantla (Tecolutla river basin), in the north-central section of what is today the state of Veracruz. Papantla and its environs, which include the Classic Period (A.D. 250–900) archaeological site of El Tajín, are home to the Totonac people. In the eighteenth century, Papantla emerged as the first and only region in the world to produce vanilla for international export (Kourí 2004). The sole destination for Papantla vanilla was the European market, which demanded vanilla as an ingredient for cacao-based confections (Coe and Coe 1996). Cultivation of vanilla by the Totonac was integrated into a system of swidden agriculture wherein *milpa* fallows were managed as vanilla agro-forests (Kelly and Palerm 1952; Medellín-Morales 1988; Toledo et al. 2001). Papantla lost its vanilla monopoly in the mid-nineteenth century, when vanilla production took off in French colonies in the Indian Ocean, most notably Madagascar (Kourí 2004). Mexico remains a minor supplier of vanilla today, accounting for less than 1% of global production (FAOSTAT), with 20–30 Mt of cured vanilla being produced annually in the Papantla area.

Although vanilla has been a Papantla cash crop for roughly 250 years, there is deficient evidence to suggest that it was a plant of any internal

cultural value to the pre-Columbian Totonac. In the original 1740s record of vanilla use from Papantla, vanilla is gathered, not cultivated, in the surrounding forests and sold in town (Bruman 1948). While observers claimed that this was “el modo que siempre se ha tenido [the way it has always been],” a 1662 list of cash crops from Papantla only mentions tobacco and chili peppers, without citing vanilla (Bruman 1948).

The earliest suggestions of vanilla gathering and/or cultivation anywhere in pre-Columbian Mesoamerica contain two elements: They come from the Maya lowlands and they are ancillary to observations of cacao (*Theobroma cacao* L.) production (Bruman 1948; Caso Barrera and Fernández 2006; Kourí 2004). Cacao-based beverages (“chocolate”) have been consumed in the Maya lowland area, where cacao grows naturally, for at least 2,500 years (Henderson et al. 2007; Hurst et al. 2002; McNeil 2006; Millon 1955). During the Late Post-Classic (A.D. 1350–1500), a trade in cacao, which had started in the Maya area and among the Maya elite, grew to encompass all of Mesoamerica, with the Aztec rulers of the Valley of Mexico (where cacao does not grow well) serving as the dominant, centripetal force (Bergmann 1969). Plants that were used to spice cacao and that grew sympatric with areas of cacao cultivation in the Maya lowlands became incorporated into cultivation and trade. These included annatto/achiote (*Bixa orellana* L.) and vanilla (Caso Barrera and Fernández 2006).

Several sources document this assemblage of economic plants in the Maya area. In 1618, two Franciscan friars, Bartolomé Fuensalida and Juan de Orbita, commented that in Lucú, a town that is in the Rio Hondo area of northern Belize, “... [there is] much achiote, which is the best that is known in all of New Spain... very good fat cacao that produces a deep red color and is by itself a good flavor; vanillas that they call *cizbiques*, very good and fragrant for chocolate [beverage] (Jones 1983).” Between the 1620s and the 1630s, Thomas Gage reported on the presence of vanilla along the Pacific coast of Guatemala: “The chief commodities which from along that coast are brought to Guatemala, are from the provinces of Soconusco and Suchitepéquez, which are extreme hot, and subject to thunder and lightning, where groweth scarce any remarkable commodity, save only cacao, achiote, “mechasuchil,” vanilla, and other drugs for chocolate” (Thompson 1958;

p. 192). An official order filed in 1678 by the Mayor of Verapaz, Sebastián de Olivera, stated, "Seize from the poor, newly converted [Manché Chol of the eastern Petén] their annatto, cacao and vanillas" (Caso Barrera and Fernández 2006). Finally, in 1699, Marcelo Flores, a Spanish Captain, remarked that in the vicinity of eastern Guatemala/southern Belize, "... there is a town... that belongs to the doctrine of the priests of Santo Domingo, which is the town of Belén, close to Rabinal. And in all of these localities there is evidence that there are Indians using these paths and trails at their own manner and habit, as is evidenced in the care and tidiness of their cacao

and vanilla orchards and other fruits" (Caso Barrera and Fernández 2006).

While the Maya area seems to be where vanilla was first used, it is only recently (in the last 20 years) that Maya vanilla has been produced for international export (Schlüter et al. 2007). Earlier diffusions of Maya vanilla were precluded by geographic disadvantage; namely, the relative inaccessibility to overseas markets from the forested, tropical interior. Papantla is much closer to Veracruz and Tuxpan, which were the principal ports linking New Spain to Europe in the eighteenth century (Fig. 1; Kourí 2004). Consequently, if vanilla production worldwide has been

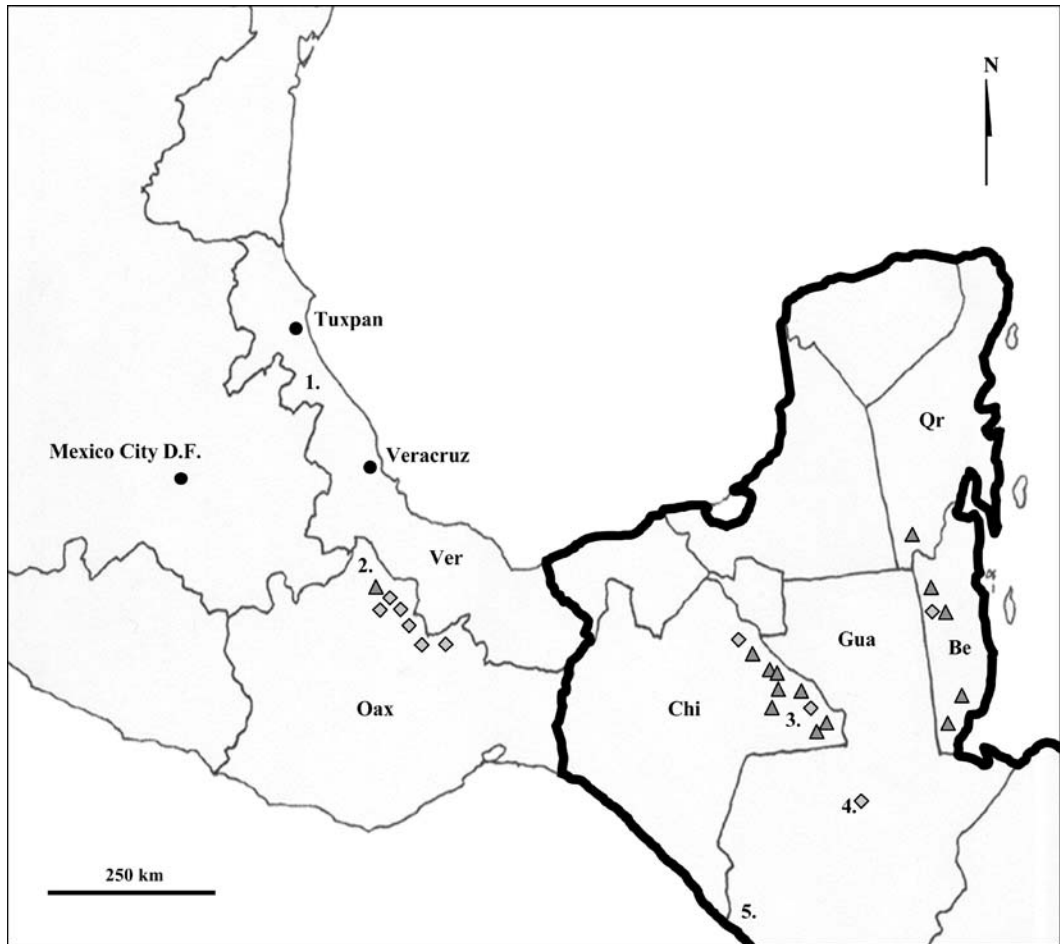


Fig. 1. Map of the Mesoamerican region showing approximate localities where the 24 non-cultivated accessions of Mesoamerican *Vanilla* used in this study were collected (shaded triangles = *V. planifolia*; diamonds = other *Vanilla*). Numbers on the map correspond to cultivation regions: 1. Papantla; 2. Tuxtepec; 3. Montes Azules Biosphere Reserve; 4. Coban; and 5. Retalhuleu. The Maya cultural area is shown in bold outline. [Ver = Veracruz, Oax = Oaxaca, Chi = Chiapas, Qr = Quintana Roo, Be = Belize, Gua = Guatemala].

established strictly from vanilla from Papantla, as seems likely (Smith et al. 1992), it would imply that the vanilla exploited in pre-Columbian Mesoamerica actually is not the equivalent of the vanilla cultivated across the tropics today. In order to test this supposition, i.e., to infer the origin(s) of cultivated vanilla outside of Mesoamerica, this study characterized the genetic relationships among cultivated and non-cultivated vanilla from Mesoamerica, and cultivated vanilla from the Indian Ocean, the principal production region.

Materials and Methods

PLANT MATERIALS AND SAMPLING

Genetic relationships were assessed among a sample set of 100 accessions of *Vanilla*, consisting of *V. planifolia* (81 individuals), *V. pompona* (four), *V. odorata* (three), *V. tahitensis* (two), *V. insignis* (one), and other unidentified *Vanilla* (nine) (Table 1). The study was designed for multiple species because vanilla cultivation in Mexico has been found to exploit several species concurrently (Soto Arenas 1999).

Per geographic region, the total tally was 25 accessions from Papantla, 24 from the Maya area

(in this case, the Mexican states of Chiapas and Quintana Roo, and countries of Belize and Guatemala), and 25 from islands in the Indian Ocean (La Réunion and Madagascar). An additional sampling of 15 individuals came from Oaxaca (the Tuxtepec region or the Papaloapan river basin), the second most important vanilla producing area in Mexico today after Papantla (Schlüter et al. 2007), as well as a region where *V. planifolia* is native (Schultes 1941). The remaining 11 individuals sampled were of cultivated/feral material from different regions: Three from Hidalgo, Mexico (representing introductions of *V. planifolia* from Papantla), two from Puebla, Mexico (but recently introduced to Papantla; PL855, 1071), two accessions of *V. tahitensis* (one from French Polynesia; the other from Papua New Guinea), and four individuals of *V. planifolia*, two of which are from Ecuador, one of which is from Trinidad & Tobago, and one of which is from Jamaica. This diversity corresponded to plant material from 10 countries, with 25 accessions of non-cultivated *Vanilla* and 75 of cultivated.

Collections in Mesoamerica were made in early spring, March–May, in 2004 and 2005, to coincide with the peak flowering time for the

Table 1. GEOGRAPHIC REGION, QUANTITY, AND CULTIVATION STATUS OF ACCESSIONS USED TO INFER THE ORIGINS OF CULTIVATED VANILLA.

Region	<i>V. planifolia</i> [n/c]	Other <i>Vanilla</i> [n/c]	Total [n/c]
Papantla	25 [0/25] PL827, 830, 832, 833, 835, 836, 844, 846 – 854, 946, 947, 1060 – 1063, 1070, 1072, 1073	0	25 [0/25]
Maya Area	19 [13/6] PL806, 873, 875 – 878, 880, 882, 883, 910, 912, 939, 1000, 1003, 1014, 1015, 1024, 1047, 1048	5 [4/1] PL907, 913, 937, 1026, 1046	24 [17/7]
Oaxaca	4 [1/3] PL750, 754, 824, 826	11 [6/5] PL676, 677, 679, 752, 757, 761, 765, 766, 771, 828, 829	15 [7/8]
Other Mesoamerica	4 [0/4] PL706, 1064, 1065, 1071	1 [0/1] PL855	5 [0/5]
Indian Ocean	25 [0/25] SB1 – 25	0	25 [0/25]
Other Countries	4 [1/3] PL613, 615, 958, 1080	2 [0/2] C01-85, PNG	6 [1/5] 100* [25/75]

Accession numbers are given below each tally.

[n/c] = [non-cultivated/cultivated]; PL = P. Lubinsky (collection number); * = Total tally.

genus, towards the end of the dry season (Soto Arenas 1999). This facilitated positive species identifications, based on the taxonomic keys of Portères (1954) and Soto Arenas (1999), while also providing an opportunity to observe the natural pollination biology of *V. planifolia* (Lubinsky et al. 2006). There are no morphological differences that consistently distinguish “wild” from cultivated *V. planifolia* (Soto Arenas 1999; Schlüter et al. 2007). This is due to a combination of three factors: (1) the life history of the species, which has a sexual generation time on the order of decades, (2) the slow evolution of the crop under domestication that results from clonal propagation and artificially mediated self-pollination, and (3) cultivation history. Vanilla is an incipient crop and non-staple whose cultivation spans centuries rather than millennia (Lubinsky 2007).

In the absence of morphological characters, the designation of non-cultivated (“wild”) vanilla was applied according to setting. Since *V. planifolia* is a naturally rare plant with an estimated abundance of 1 individual/2–10 square kilometers (km²) (Soto Arenas 1999), identification of putatively wild and/or feral individuals (=non-cultivated) was inferred indirectly (e.g., multiple mature individuals in a single locality suggesting feral populations), so long as no positive evidence of recent cultivation was found. In these cases, local guides and informants were queried about whether vanilla cultivation had been practiced. Descriptions of the different vanilla cultivation systems (e.g., agro-forests with *Citrus* as support-trees, etc.) can be found in Hernández Apolinar (1997), Soto Arenas (1999), and Lubinsky (2007).

During sampling, vanilla cultivators were asked about whether they grew different types or varieties of vanilla. In Mesoamerica, 15 common names for cultivated vanilla were recorded, the majority of which were idiosyncratic and not widely recognized by producers. Only three were referred to more than once: “mansa,” “oreja de burro,” and “colibrí.” The term “mansa” (=“tame”) is used most frequently in Papantla. The word is generally employed by cultivators in Mexico to designate a domesticated form of any plant. For example, the pulque agave (*Agave salmiana* Otto ex Salm-Dyck) is called “maguey manso” (Gentry 1982). The cv. “oreja de burro” (=“burro’s ear”) refers to morpho-metrically distinct individuals of *V. planifolia* that abort 70%

or more of their fruits three months after pollination (Castillo and Engleman 1993). The common name “colibrí” is given to some folk cultivars from Oaxaca. Cultivated material from the areas in the Indian Ocean included multiple accessions of three cultivars (“mexique,” “classique,” and “grosse vanilla”) from La Réunion and undesigned material from Madagascar. Accessions were obtained as DNA extracts from CIRAD (La Réunion). P. Lubinsky collected material from Ecuador in February 2004. All other material was obtained through donations of leaf tissue or DNA extracts. Vouchers of accessions are being kept as living material at the INIFAP experimental station in Martínez de la Torre, Veracruz, Mexico.

AFLP GENOTYPING

Genetic analysis was carried out using Amplified Fragment Length Polymorphism (AFLP) markers. AFLPs are fit to appraise highly clonal germplasm because of their ability to rapidly generate and reliably detect numerous polymorphisms distributed widely throughout the genome (Vos et al. 1995). In addition, they have a demonstrated utility for unraveling the origins of crop plants (Spooner et al. 2005; Zerega et al. 2004).

A full description of the protocol used to amplify AFLP size fragments, including primer concentrations, buffer compositions, etc., can be found in Kim et al. (2005). Briefly, total genomic DNA was isolated from fresh and dried leaf tissue using a DNeasy mini-plant kit (Qiagen, Valencia, California). Pre-amplification templates were generated with an IRDye Fluorescent AFLP Kit for Large Plant Genome Analysis (Li-Cor, Lincoln, Nebraska). An initial 4 µL (20 ng/µL) of total genomic DNA was digested in 0.5 µL *EcoRI*/*MseI* mix, 1.25 µL 5× reaction buffer, and 0.5 µL ddH₂O for a final volume of 6.25 µL. This solution was incubated for two hours at 37°C, followed by enzyme inactivation at 70°C for 15 minutes. Ligation was performed by adding 6 µL of adapter mix (containing *EcoRI* and *MseI* double-stranded adapters) and 0.25 µL T4 DNA ligase for a total combined volume of 12.5 µL; reactions were run at 20°C for two hours.

Ligated products were diluted 1:10 in ddH₂O for pre-amplification, which was performed in a total volume of 12.6 µL consisting of 1.4 µL template DNA, 9.75 µL pre-amplification primer

mix, 1.0 μL 10 \times PCR buffer, 0.25 μL MgCl_2 , and 0.2 μL *Taq* polymerase (Promega, Madison, Wisconsin). The following parameters were repeated for 20 cycles: 94°C for 30 sec, 56°C for 30 sec+1 sec/cycle, 72°C for 1 min+1 sec/cycle. The final elongation was set at 72°C for 15 minutes. Selective amplification was performed as multiplex reactions in a total volume of 12.32 μL containing 2.32 μL pre-amplification products (diluted 1:40 in ddH₂O), 4.89 μL ddH₂O, 1.23 μL 10 \times PCR Buffer, 0.74 μL 25 mM MgCl_2 , 0.5 μL each of 1.0 mM 700 and 800 *EcoRI* fluorescent labeled selective primers, 2 μL *MseI* selective primer, and 0.14 μL *Taq* polymerase.

Eight primer combinations were chosen for analysis after a survey of 24 combinations were screened for maximizing the total number of loci, and amount of polymorphic loci [E-AAG(700)/E-ACG(800) + M-CTT, E-AAC(700)/E-ACG(800) + M-CAT, E-ACA(700)/E-ACG(800) + M-CTA, E-ACA(700)/E-ACG(800) + M-CAC]. Touch-down PCR for selective amplification was performed according to the following conditions: An initial round of 12 cycles (94°C for 15 sec, 65°C for 30 sec -0.7°C/cycle, 72°C for 1 min) was followed by a later round of 29 cycles (94°C for 15 sec, 56°C for 30 sec+1 sec/cycle, 72°C for 1 min +1 sec/cycle), and concluded with a final elongation at 72°C for 15 min. Afterwards, 5 μL of STOP dye were added to selective amplification products, and 1.2 μL of this solution were loaded on a Li-Cor sequencer (Global Edition IR2 4100) for electrophoretic separation and visualization of fragment length differences.

Band presence/absence was scored using SAGA software (version 3.3, Li-Cor, Lincoln, Nebraska). Loci with faint bands were considered unreliable and were excluded. Pairwise differences were calculated according to Nei and Li (1979), using PAUP (version 4.0b10 for Macintosh; Swofford 2002), and relationships depicted as Neighbor-Joining (NJ) and UPGMA dendrograms. Bootstrap values based on 1,000 replicates of 50% majority rule were used as statistical support. *Vanilla odorata*, *V. insignis*, and *V. pompona* were designated as out-groups, and missing data were treated as gaps.

Results

Reproducibility of AFLP markers was verified by comparing banding patterns between two

separately isolated repeats of three accessions (PL766, 875, SB25) that showed no variation. Band intensity was also highly conserved between 1:10 and 1:40 dilutions of pre-selective amplification products in one accession (PL875). Only AFLP fragments between 75 and 450 bp were scored resulting in 185 loci from the eight primer pair combinations. The average number of loci yielded per primer pair was 23, ranging from 20 [E-ACG(800) + M-CAT] to 32 [E-ACA(700) + M-CAC]. A total of 3.2% of the reactions failed, including a high rate (more than 20%, or the equivalent of two primer-pair combinations) for the following seven accessions: PL677, 757, 761, 771, 827, 1014, and 1015.

Phenetic relationships among accessions are depicted in an NJ tree in Fig. 2. In agreement with previous molecular studies of *Vanilla* (Cameron and Soto Arenas 2003; Soto Arenas 1999), rough species boundaries were evident and supported by bootstrap values greater than 50%. Relationships within *V. planifolia* were less resolvable, but revealed a deep structural division that essentially divided non-cultivated and cultivated accessions into distinct clusters. "Non-cultivated" vanilla consisted of relatively more diverse, geographically widespread, and predominantly non-cultivated/feral accessions of *V. planifolia* in addition to several unidentified *Vanilla* (PL676, 855, 752, 1026, 1046) and the two accessions of *V. tabitensis*. The presence of *V. tabitensis* may be due to its putative hybrid origin involving *V. planifolia* as a paternal donor (Lubinsky 2007). The only cultivated *V. planifolia* in this cluster were two of the three accessions of the Oaxaca cultivar "colibrí" (PL754, 826), an accession from the Maya area (PL873), and two accessions recently introduced to the Papantla area from Puebla (PL855, 1071).

The other cluster ("cultivated," Fig. 2) of *V. planifolia* was composed mostly of cultivated accessions with relatively short branch lengths indicative of low levels of diversity. Most notable in this grade was the presence of all cultivated individuals from Veracruz, save for PL855 and 1071, together with all Indian Ocean region accessions. The poor resolution characterizing these accessions suggests a very recent phylogenetic divergence. The small genetic distances may in fact represent clonal rather than non-clonal (i.e., sibling) diversity, a possibility supported by a recent AFLP study that correlated phenotypic

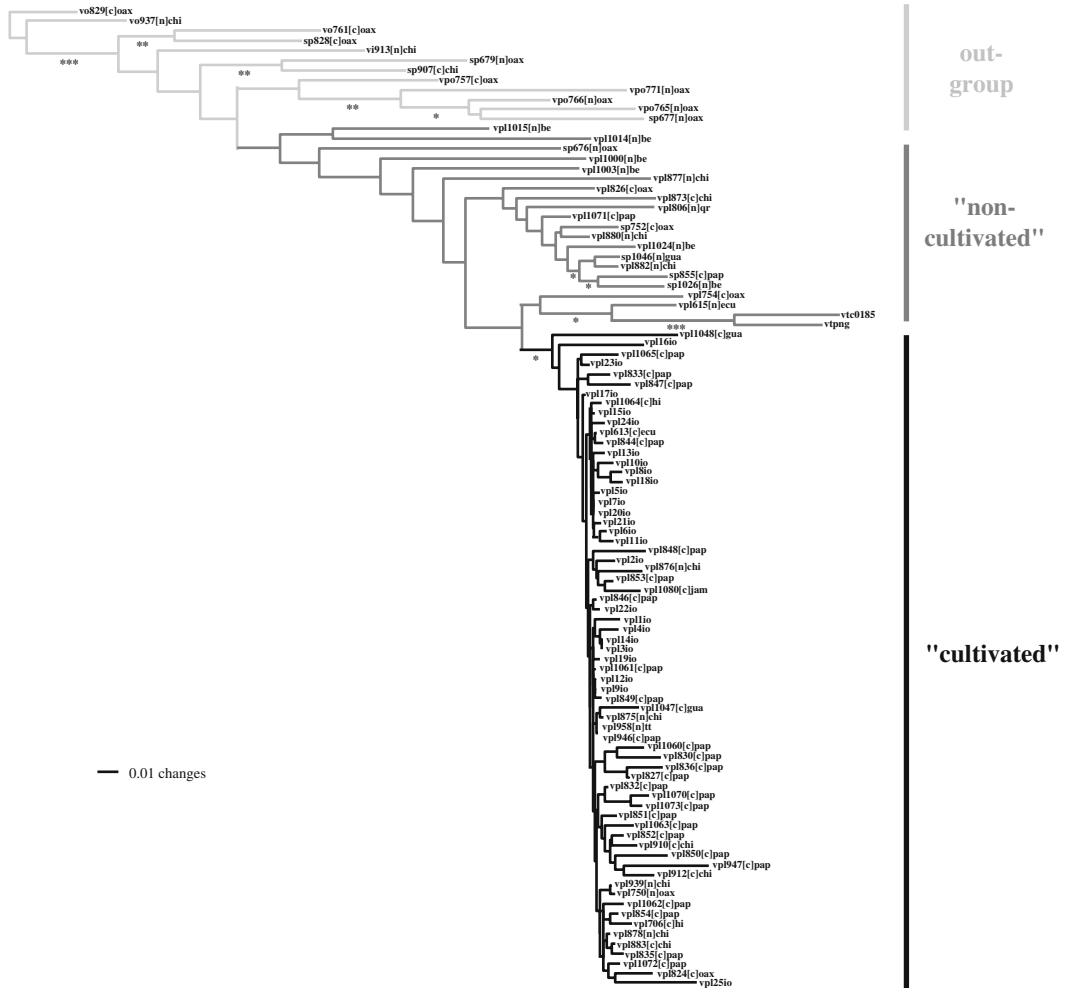


Fig. 2. Neighbor Joining (NJ) tree depicting genetic distances and relationships among non-cultivated and cultivated *Vanilla*. Asterisks under branches indicate relationships with at least moderate statistical support ([*] = >50%; [**] = >75%; [***] = >90%), as determined by bootstrap values based on 1,000 replicates of 50% majority rule in both NJ and UPGMA analyses. Letter codes (species, accession number, cultivation status, geographic location): [vpl = *V. planifolia*, vo = *V. odorata*, vi = *V. insignis*, vpo = *V. pompona*, vt = *V. tabitensis*, sp = *Vanilla* sp.], [c = cultivated, n = non-cultivated], [pap = Papantla, h = Hidalgo, oax = Oaxaca, chi = Chiapas, qr = Quintana Roo, be = Belize, gua = Guatemala, io = Indian Ocean, tt = Trinidad & Tobago, ecu = Ecuador, jam = Jamaica, png = Papua New Guinea]. [e.g., "vpl1064[c]h" = *V. planifolia*, PL1064, cultivated, Hidalgo].

and genotypic diversity of cultivated vanilla in the regions near the Indian Ocean, and found little evidence for multi-clonal and/or sexually based variation (see *Discussion*).

The "cultivated" cluster also included two other types of cultivated accessions: (1) Meso-american cultivated accessions that are known to have originated from Papantla ([PL706, 1064, 1065; Hidalgo, Mexico], [PL1047; Guatemala]), and (2) geographically widespread, cultivated

accessions likely introduced from Papantla, consisting of a cultivated individual from Oaxaca (PL824, "colibri"), four accessions from the Maya area ([PL1048; Guatemala], [PL833, 910, 912; Chiapas]), and cultivated/feral *V. planifolia* from other countries ([PL613; Ecuador], [PL1080; Jamaica], [PL958; Trinidad & Tobago]). Papantla introductions into Oaxaca and the Maya area have been recently documented (Schlüter et al. 2007). The close genetic affinities of the South

American and Caribbean accessions are likely the result also of past Papantla introductions; otherwise, a geographically widespread wild clone (“genet,” or genetic individual) of *V. planifolia* would be required to explain the independent origin of cultivated vanilla in South America. The null prediction for clonally reproducing plants is for evolutionary differentiation to be observable both between and within populations (Ellstrand and Roose 1987), as was found in the other non-cultivated accessions of *V. planifolia* surveyed here. Likewise, *V. planifolia* does not occur naturally in Jamaica, but is known to have been introduced (Smith et al. 1992).

The remainder of the accessions found in the “cultivated” cluster consisted of five non-cultivated individuals: One from Oaxaca (PL750), and the others from the Maya area (PL875, 876, 878, 939; all from Chiapas). Since all these accessions were collected in secondary/disturbed forests along roadsides (Lubinsky 2007), it is more likely that they represent feral material from prior attempts at introductions of Papantla vanilla rather than truly “wild” individuals. In fact, three of the accessions (PL875, 876, 878) were collected at a single locality, a demographic abundance not known for wild *V. planifolia*.

Discussion

The telling of vanilla history conventionally begins with Papantla. This is understandable because Papantla was the first region to produce vanilla for export; hence, it is the first place outside of Mesoamerica to become conceptually bound to vanilla. Unfortunately, this narrative has proven to be susceptible to over-interpretation, to which vanilla’s proximal origins have indeed fallen victim. For instance, they have been reconfigured to meet wholly unsubstantiated claims that the Totonac have practiced vanilla cultivation for thousands of years (Kiple and Ornelas 2000).

Mesoamerican food historian Sophie Coe (1994) has remarked that “vanilla naturally follows chocolate.” This is an accurate historical characterization, both of the pre-Columbian evolution of vanilla use among the Maya and the post-Contact emergence in New Spain of an overseas vanilla trade that operated to provide Europe with the raw ingredients for chocolate. However, if the distinct Mesoamerican and European experiences with chocolate are conflated

so that they trace a single and uninterrupted trajectory, Papantla can speciously be re-imagined as a kind of static, unaffected vanilla supplier for whatever chocolate market there was throughout the period of Contact. This diminishes the knowledge that chocolate in Europe was triumphant only after the collapse of chocolate production in the lowland Maya area (Coe and Coe 1996; Macleod 1973). More specifically, i.e., in the interest of faithfully disposing on vanilla history, it confuses the fact that it was Spain and France—not Tenochtitlan—that initially drove demand for Papantla vanilla. In true cause-and-effect fashion, it was vanilla-based fortune that also gave impetus to the establishment of Papantla itself (Kourí 2004).

In recent surveys of wild and cultivated vanilla from Mexico and Central America (Cibrián Jaramillo 1999; Schlüter et al. 2007), Papantla vanilla, on the one hand, and wild and cultivated vanilla from Oaxaca and the Maya area, on the other, were found to constitute separate clusters of geographically structured genetic diversity. Our genetic distance analysis (Fig. 2), which also produced this division, serves to confirm the results from these earlier studies. Furthermore, our analysis supports the interpretation that the origins of cultivated vanilla outside of Mesoamerica are from dispersals of vanilla cuttings from Papantla. The close genetic affinity of vanilla from the Indian Ocean regions with Papantla vanilla inferred from the short branch-lengths we found here suggests that they collectively constitute a single clone, with only somatic mutations accounting for the observed differences among genotypes. This hypothesis has been upheld by another recent AFLP study focusing on diversity in over 300 Indian Ocean cultivars, where sexually based genetic differences were controlled for by the genotyping of selfed-progenies (Bory et al. *in press*). Together, these studies are evidence that the cultivation of *V. planifolia* worldwide depends almost entirely on the risk-inherent practice of clonally propagating a single genet.

On an international level, then, the area of origin of cultivated vanilla can most certainly be said to be Papantla. Such would not be the case had the genetic profiles of the “wild” vanilla from the Maya area we surveyed here matched those of cultivated vanilla from outside of Mesoamerica. Since they did not do so convincingly, we can conclude within the sampling limits of this study

that pre-Columbian Maya vanilla cultivars are essentially lost varieties. This historic genetic erosion of Maya vanilla has not reversed with the recent cultivation efforts of vanilla in the area, which are based on introductions over the last 20 years of material from Papantla rather than on wild/remnant material from the surrounding forests (Schlüter et al. 2007). The closest approximation of a pre-Columbian Maya vanilla cultivar may be *V. tabitensis* (Lubinsky 2007). Although completely unknown in a wild form, this cultivar may have been traded along the Pacific littoral of Mesoamerica after Contact, introduced over the Pacific to the Philippines as the “vanilla of Guatemala” via the Manila Galleon, and finally taken in the mid-nineteenth century from the Philippines to French Polynesia (Lubinsky 2007).

In contrast to both Papantla and the Maya area, the multi-species composition that typifies cultivation in Oaxaca highlights the primacy of these agroforests for *in situ* conservation efforts. Similar to what has been documented in the case of cassava cultivation in parts of Brazil (Elias et al. 2000), the cultivation in Oaxaca of mixed individuals of vanilla from the wild are influenced by inconsistent local taxonomies. For instance, the usage of the common name “colibri” is applied to individuals of both *V. planifolia* (PL754, 824, 826) and *V. odorata* (PL761). One individual called “oreja de burro” from Oaxaca was identified as *V. pompona* (PL757), a name otherwise restricted to aborting types of *V. planifolia*. This management of diverse indigenous crop varieties not only maintains diversity, but it also can be a mechanism for generating novel diversity through hybridization and introgression (Anderson 1967; Blanca et al. 2007; Casas et al. 2006; Elias et al. 2004; Emshwiller 2006; Hughes et al. 2007).

Still, vanilla—more than other crops—may need to rely on the scientific community for its conservation. The persistence of indigenous crop varieties in the face of urbanization, habitat loss, and development has been observed in landraces of Mexican maize (Chambers et al. 2007) and Andean potatoes (Brush 2004), but for reasons, such as ecological adaptation or the ceremonial or ritual value of certain varieties, that may not pertain to a cash crop like vanilla.

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Literature Cited

- Anderson, E. 1967. *Plants, Man and Life*. University of California Press, Berkeley, California.
- Bergmann, J. F. 1969. The Distribution of Cacao Cultivation in Pre-Columbian America. *Annals of the Association of American Geographers* 59:85–96.
- Blanca, J. M., J. Prohens, G. J. Anderson, E. Zuriaga, J. Cañizares, and F. Nuez. 2007. AFLP and DNA Sequence Variation in an Andean Domesticated, Pepino (*Solanum muricatum*, Solanaceae): Implications for Evolution and Domestication. *American Journal of Botany* 94:1219–1229.
- Bory S., P. Lubinsky, A.-M. Risterucci, J. L. Noyer, M. Grisoni, M.-F. Duval, and P. Besse. In press. Patterns of Introduction and Diversification of *Vanilla planifolia* (Orchidaceae) in Reunion Island (Indian Ocean). *American Journal of Botany*.
- Bruman, H. 1948. The Culture History of Mexican Vanilla. *The Hispanic American Historical Review* 28:360–376.
- Brush, S. B. 2004. *Farmer's Bounty: Locating Crop Diversity in the Contemporary World*. Yale University Press, New Haven, Connecticut.
- Cameron, K., M. W. Chase, W. M. Whitten, P. J. Kores, D. C. Jarrell, V. A. Albert, T. Yukawa, H. G. Hills, and D. H. Goldman. 1999. A Phylogenetic Analysis of the Orchidaceae: Evidence from *rbcL* Nucleotide Sequences. *American Journal of Botany* 86:208–224.
- and M. A. Soto Arenas. 2003. Vanilloideae. Pages 281–334 in A. M. Pridgeon, Phillip J. Cribb, Mark W. Chase, Finn Rasmussen, eds., *Genera Orchidacearum*. Volume 3: Orchidoideae (Part 2), Vanilloideae. Oxford University Press, Oxford, United Kingdom.

- Casas, A., J. Cruse-Sanders, E. Morales, A. Otero-Arnaiz, and A. Valiente-Banuet. 2006. Maintenance of Phenotypic and Genotypic Diversity in Managed Populations of *Stenocereus stellatus* (Cactaceae) by Indigenous Peoples in Central Mexico. *Biodiversity and Conservation* 153:879–898.
- Caso Barrera, L. and M. A. Fernández. 2006. Cacao, Vanilla and Annatto: Three Production and Exchange Systems in the Southern Maya Lowlands, XVI–XVII Centuries. *Journal of Latin American Geography* 52:29–52.
- Castillo, R. and E. M. Engleman. 1993. Caracterización de dos tipos de *Vanilla planifolia*. *Acta Botánica Mexicana* 25:49–59.
- Chambers, K. J., S. B. Brush, M. N. Grote, and P. Gepts. 2007. Describing Maize (*Zea mays* L.) Landrace Persistence in the Bajío of Mexico: A Survey of 1940s and 1950s Collection Locations. *Economic Botany* 611:60–72.
- Childers, N. F., H. R. Cibes, and E. Hernández-Medina. 1959. Vanilla-The Orchid of Commerce. Pages 477–510 in C. L. Withner, ed., *The Orchids. A Scientific Survey*. Ronald Press Company, New York.
- Cibrián Jaramillo, A. 1999. Variación Genética de *Vanilla planifolia* en México. Master's thesis, Facultad de Biología, Universidad Nacional Autónoma de México (UNAM), Mexico City, D.F., Mexico.
- Coe, S. D. 1994. *America's First Cuisines*. University of Texas Press, Austin, Texas.
- , and M. D. Coe. 1996. *The True History of Chocolate*. Thames and Hudson, New York.
- Correll, D. S. 1953. Vanilla—Its Botany, History, Cultivation and Economic Import. *Economic Botany* 7:291–358.
- Ecott, T. 2004. *Vanilla: Travels in Search of the Luscious Substance*. Penguin, London.
- Elias, M., O. Panaud, and T. Robert. 2000. Assessment of Genetic Variability in a Traditional Cassava (*Manihot esculenta* Crantz) Farming System, Using AFLP Markers. *Heredity* 85:219–230.
- , G. S. Mühlen, D. McKey, A. C. Roa, and J. Tohme. 2004. Genetic Diversity of Traditional South American Landraces of Cassava (*Manihot Esculenta* Crantz): An Analysis Using Microsatellites. *Economic Botany* 58(2):242–256.
- Ellstrand, N. C., and M. L. Roose. 1987. Patterns of Genotypic Diversity in Clonal Plant Species. *American Journal of Botany* 74:123–131.
- Emshwiller, E. 2006. Evolution and Conservation of Clonally Propagated Crops: Insights from AFLP Data and Folk Taxonomy of the Andean Tuber Oca (*Oxalis tuberosa*). Pages 308–333 in T. J. Motley, Nyree Zerega, and Hugh Cross, eds., *Darwin's Harvest. New Approaches to the Origins, Evolution and Conservation of Crops*. Columbia University Press, New York.
- FAOSTAT (Food and Agriculture Organization of the United Nations). <http://faostat.fao.org/> April 30, 2008.
- Gentry, H. S. 1982. *Agaves of Continental North America*. University of Arizona Press, Tucson, Arizona.
- Hágsater, E., M. A. Soto-Arenas, G. A. Salazar Chávez, R. L. Jiménez Machorro, M. A. López Rosas, and R. L. Dressler. 2005. *Orchids of Mexico. Productos Farmacéuticos*, Mexico City, Mexico.
- Henderson, J. S., R. A. Joyce, G. R. Hall, W. J. Hurst, and P. E. McGovern. 2007. Chemical and Archaeological Evidence for the Earliest Cacao Beverages. *Proceedings of the National Academy of Sciences of the USA* 10448:18937–18940.
- Hernández Apolinar, M. 1997. Crecimiento y Reproducción de *Vanilla planifolia* (Orchidaceae) en Usila, Oaxaca. Master's thesis, Facultad de Biología, Universidad Nacional Autónoma de México (UNAM), Mexico City, D.F., Mexico.
- Hughes, C. E., R. Govindarajulu, A. Robertson, D. L. Filer, S. A. Harris, and C. D. Bailey. 2007. Serendipitous Backyard Hybridization and the Origin of Crops. *Proceedings of the National Academy of Sciences of the USA* 10436:14389–14394.
- Hurst, W. J., S. M. Tarka, Jr., T. G. Powis, F. Valdez, Jr., and T. R. Hester. 2002. Cacao Usage by the Earliest Maya civilization. *Nature* 418:289.
- Jones, G. D. 1983. The Last Maya Frontiers of Colonial Yucatan. Pages 64–91 in M. L. Murdo and R. Wasserstrom, eds., *Spaniards and Indians in Southeastern Mesoamerica. Essays on the History of Ethnic Relations*.

- University of Nebraska Press, Lincoln, Nebraska.
- Kelly, I. and A. Palerm. 1952. The Tajin Totonac: Part I. History, Subsistence, Shelter and Technology. Institute of Social Anthropology Publication No. 13. Smithsonian Institution. United States Printing Office, Washington, D.C.
- Kim, S.-C., C. Lee, and A. Santos-Guerra. 2005. Genetic Analysis and Conservation of the Endangered Canary Island Woody Sow-Thistle, *Sonchus gandogerii* (Asteraceae). *Journal of Plant Research* 118:147–153.
- Kiple, K. F. and K. C. Ornelas. 2000. The Cambridge World History of Food. Vol. 2. Cambridge University Press, Cambridge, United Kingdom.
- Kourí, E. 2004. A Pueblo Divided: Business, Property, and Community in Papantla, Mexico. Stanford University Press, Stanford, California.
- Lubinsky, P. 2007. Historical and Evolutionary Origins of Cultivated Vanilla. Ph.D. thesis, Botany and Plant Sciences, University of California, Riverside, California.
- , M. Van Dam, and A. Van Dam. 2006. Pollination of *Vanilla* and Evolution in Orchidaceae. *Lindleyana* 75(12):926–929.
- Macleod, M. 1973. Spanish Central America: A Socioeconomic History, 1520–1720. University of California Press, Berkeley, California.
- McNeil, C. L., ed. 2006. Chocolate in Mesoamerica: A Cultural History of Cacao. University of Florida Press, Gainesville, Florida.
- Medellín-Morales, S. G. 1988. Arboricultura y Silvicultura Tradicional en una Comunidad Totonaca de la Costa. Master's thesis, Ecología y Recursos Bioticos, Instituto Nacional de Investigaciones Sobre Recursos Bioticos (INIREB, Xalapa, Veracruz, Mexico).
- Millon, R. F. 1955. Trade, Tree Cultivation, and the Development of Private Property in Land. *American Anthropologist* 57:698–712.
- Nei, M. and W.-H. Li. 1979. Mathematical Model for Studying Genetic Variation in Terms of Restriction Endonucleases. *Proceedings of the National Academy of Sciences of the USA* 76:5269–5273.
- Nuttall, Z. 1925. The Gardens of Ancient Mexico. Annual Report of the Board of Regents of the Smithsonian Institution, Publication 2758. United States Printing Office, Washington, D.C., pp. 453–464.
- Portères, R. 1954. Le genre *Vanilla* et ses espèces. Pages 94–290 in G. Bouriquet, ed., *Le Vanillier et la Vanille dans le Monde*. Éditions Paul Lechevalier, Paris.
- Purseglove, J. W., E. G. Brown, C. L. Green, and S. R. J. Robbins. 1981. *Spices*. Volume 2. Longman, London.
- Rain, P. 2004. *Vanilla: The Cultural History of the World's Most Popular Flavor and Fragrance*. Jeremy P. Tarcher/Penguin, New York.
- Ramírez, S. R., B. Gravendeel, R. B. Singer, C. R. Marshall, and N. E. Pierce. 2007. Dating the Origin of the Orchidaceae from a Fossil Orchid with Its Pollinator. *Nature* 448:1042–1045.
- Sauer, J. D. 1993. *Historical Geography of Crop Plants: A Select Roster*. CRC Press, Boca Raton, Florida.
- Schlüter, P. M., M. A. Soto Arenas, and S. A. Harris. 2007. Genetic Variation in *Vanilla planifolia* (Orchidaceae). *Economic Botany* 614:328–336.
- Schultes, R. E. 1941. Economic Aspects of the Flora of Northeastern Oaxaca. Ph.D. thesis, Botany Department, Harvard University, Cambridge, Massachusetts.
- Smith, N. J. H., J. T. Williams, D. L. Plucknett, and J. P. Talbot. 1992. *Tropical Forests and Their Crops*. Cornell University Press, Ithaca, New York.
- Soto Arenas, M. A. 1999. Filogeografía y Recursos Genéticos de las Vainillas de México. Project J101, CONABIO, Mexico.
- Spooner, D. M., K. McLean, G. Ramsay, R. Waugh, and G. L. Bryan. 2005. A Single Domestication for Potato Based on Multilocus Amplified Fragment Length Polymorphism Genotyping. *Proceedings of the National Academy of Sciences of the USA* 10241:14694–14699.
- Swofford, D. L. 2002. *PAUP 4.0: Phylogenetic Analysis Using Parsimony*. Sinauer, Sunderland, Massachusetts.
- Tanaka, R. and H. Kamemoto. 1984. Chromosomes in Orchids: Counting and Numbers. Pages 323–410 in J. Arditti, ed., *Orchid Biology. Reviews and Perspectives*. Comstock Publishing Associates, Ithaca, New York.
- Thompson, J. E. S. 1958. *Thomas Gage's Travels in the New World*. University of Oklahoma Press, Norman, Oklahoma.

- Toledo, V. M., B. Ortiz, and S. Medellín-Morales. 2001. Biodiversity Management in a Sea of Pasturelands: Indigenous Resource Management in the Humid Tropics of Mexico. *Etnoecológica* 2(3).
- Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Homes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: A New Technique for DNA Fingerprinting. *Nucleic Acids Research* 23:4407–4414.
- Zerega, N. Y. C., D. Ragone, and T. Motley. 2004. Complex Origins of Breadfruit (*Artocarpus altilis*, Moraceae): Implications for Human Migrations in Oceania. *American Journal of Botany* 91:760–766.