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LARGE GENETIC DIVERSITY FOR FINE-FLAVOR TRAITS UNVEILED IN CACAO (*Theobroma cacao* L.) WITH SPECIAL ATTENTION TO THE NATIVE CHUNCHO VARIETY IN CUSCO, PERU

Albertus Bernardus Eskes[1], C. A. C. Rodriguez[2] , David Cruz Condori[3], Ed Seguine[4], Luis Fernando Garcia Carrion[5], Philippe Lachenaud[6]*

[1]Ex-CIRAD/Bioversity International Cocoa Geneticist, Montpellier, France. albertuseskes@gmail.com.[2]Minagri-Senasa, Ministry of Agriculture and Irrigation, Quillabamba, Cusco, Peru. [3]Unidad de Investigacion de Productos Naturales, Universidad Peruana Cayetano Heredia, Lima, Peru. [4]Consultant to Guittard Chocolate / President of Seguine, Cocoa and Chocolate Advisors, Hanover PA, 17331, USA. [5]Universidad Nacional Agraria de la Selva (UNAS),Tingo Maria, Peru. [6]Ex-Cocoa Geneticist at CIRAD-BIOS, Montpellier, France.

The fine-flavor cocoa industry explores mainly six chocolate sensory traits from four traditional cocoa (*Theobroma cacao* L.) varieties. The importance of cocoa pulp flavors and aromas has been ignored until we recently showed that they migrate into beans and into chocolates. Pulp sensory traits are strongly genotype dependent and correlated to human preference. Growers of the native Chuncho variety from Cusco, Peru, which is the cocoa that the Incas consumed, make pulp juices from preferred trees (genotypes). Evaluations of 226 preferred trees evidenced presence of 64 unique mostly multi-trait sensory profiles. Twenty nine of the 40 flavors and aromas identified mimic those of known fruit and flower or spice species such as mandarin, soursop, custard apple, cranberry, peach, banana, inga, mango, nut, mint, cinnamon, jasmine, rose and lily. Such large sensory diversity and mimicry is unknown in other commercial fleshy fruit species. So far, 14 Chuncho-like pulp sensory traits have been identified among different cocoa varieties elsewhere suggesting that Chuncho is part of the "centre of origin" for cocoa flavors and aromas. Stable expression of multi-trait Chuncho sensory profiles suggest pleiotropic dominant inheritance, favoring selection for quality traits, which is contrasting with the complex sensory trait determination in other fleshy fruit species. It is inferred that the large sensory diversity of Chuncho cocoa can only be explained by highly specialized sensory trait selection pressure exerted by frugivores, during evolution, and by the indigenous "Matsigenkas", during domestication. Chuncho beans, still largely employed as a bulk cocoa source, deserve to become fully processed as an extra-fine cocoa variety. The valorization of the numerous *T. cacao* sensory profiles in chocolates, raw beans and juices should substantially diversify and boost the fine-flavor cocoa industry, this time based on the Matsigenka/Inca and not anymore on the Maya cocoa traditions.

Key words: cocoa fruit sensory traits, mimicking, pleiotropy, fine-flavor cocoa market, cocoa sensory identity.

Grande diversidade genética para características de sabor fino revelada no cacau (*Theobroma cacao* L.) com especial atenção para a variedade nativa Chuncho de Cusco, Peru. A indústria de cacau fino explora principalmente seis características sensoriais de quatro variedades tradicionais de cacau (*Theobroma cacao* L.). A importância dos sabores e aromas da fruta fresca de cacau tem sido ignorada até que mostramos recentemente que eles migram para as amêndoas e para os chocolates. As características sensoriais da polpa variam muito com o genótipo e são relacionadas com a preferência humana. Produtores da variedade nativa Chuncho de Cusco, Peru, que é o cacau que os Incas consumiam, prepararam sucos da polpa de árvores (genótipos) preferidas. Avaliações de 226 árvores preferidas mostraram a presença de 64 perfis sensoriais únicas baseados, na maioria, em mais de uma característica. Vinte e nove dos 40 sabores e aromas identificados imitam daqueles de espécies frutíferas, de flores ou de temperos conhecidos, como *mexerica, graviola, fruta do conde, oxicoco, pêssego, banana, ingá, manga, noz, hortelã, canela, jasmim, rosa e lírio*. Esta grande diversidade e imitação sensorial não é conhecida em outras espécies comerciais de frutas frescas. Até agora, 14 características sensoriais do Chuncho foram encontradas em diferentes variedades em outras localidades, o que sugere que Chuncho faz parte do "centro de origem" para sabores e aromas de fruta fresca do cacau. A expressão estável dos perfis multi-sensoriais de Chuncho sugere heritabilidade pleiotrópica dominante, facilitando seleção para características de qualidade, o que contrasta com a determinação geralmente complexa das características sensoriais em outras espécies frutíferas. Inferimos aqui que somente uma pressão de seleção muito especializada dos frugíveros, durante a evolução, e dos indígenas "Matsigengas", durante a domesticação, poderia explicar a grande diversidade sensorial do cacau Chuncho. As amêndoas de Chuncho, ainda amplamente utilizadas como fonte de cacau "bulk", merecem pleno aproveitamento para serem industrializadas como uma variedade extra-fino de cacau. A valorização dos numerosos perfis sensoriais do cacau identificados aqui em chocolates, amêndoas e em sucos deverá diversificar e impulsar substancialmente a indústria de cacau fino, desta vez baseada nas tradições dos Matsigengas e não mais nas tradições das Mayas.

Palavras-chave: características sensoriais do cacau, imitação, pleiotropia, mercado de cacau fino, identidade sensorial do cacau.

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Introduction

Fine and bulk cocoa

The cocoa (*Theobroma cacao* L.) market distinguishes between “bulk” and “fine-flavor” cocoa with bulk cocoa representing 95% of the world cocoa market. The fine-flavor cocoa corresponds currently to *ca.* 220,000 tons annually. The bonus paid to farmers for fine-flavor cocoa varies from +15% to +300 % (Pipitone, 2016). Fine-flavor chocolates have generally variable fruity flavor and/or flower/spicy aroma expressions, besides the typical “cocoa” flavor. Chocolates from bulk cocoa varieties do generally express just cocoa flavor (Pipitone, 2016). Fine-flavor cocoa has historically been provided by the Criollo, Trinitario and Nacional varieties and, more recently, by hybrids with the SCA6 genotype (TSH clones).

Pulp vs fine-flavor chocolate sensory trait relationship

Regular tasting of cocoa pulp by the first author in 11 cocoa producing countries led in 2006 to the assessment that “*countries that produce fine chocolates (e.g. Trinidad, Ecuador, Venezuela and Peru) grow varieties that have nice cocoa pulps*”. This reasoning lead to the hypothesis of a fine-flavor pulp *vs* fine-flavor chocolate relationship. Well-known pulp and chocolate (Presilla, 2009; Sukha and Butler, 2005; Afoakwa, 2008; Clapperton et al., 1994) sensory traits of commercial fine-flavor varieties are presented in Table 1. These are uniformly expressed in trees within uniform varieties (Criollo, Nacional and the Sca6 clone), demonstrating the genetic nature of such sensory traits. The chocolate sensory profiles “caramel”, “fresh fruit”, “floral” and “brown fruit/raisin/floral” are apparently related to the pulp sensory profiles “very sweet”, “lemon”, “jasmine” and “Muscat grape/lily”, respectively. Ancient Criollo pulps and chocolates both do not express fruity flavors nor aromas. These comparisons suggest the existence of a pulp flavor and aroma *vs* chocolate fine-flavor relationship, be it with certain modifications such as the grape flavor of SCA6 that is transformed into the related brown fruit/raisin flavor. The nutty flavor is not present in the pulp and is known to be formed only during post-harvest processing.

Table 1. Comparison of known chocolate and pulp sensory traits for traditional fine-flavor cocoa varieties and for SCA6

Variety	Pulp flavor/aroma	Chocolate flavor/aroma
Ancient Criollo	Very sweet pulp, no flavors or aromas	Caramel/honey, Nutty
Trinitario	Citrus flavor, balanced sweet/acid pulp	Fresh fruit
Nacional	Jasmine aroma and sweet pulp	Floral
SCA6 (clone)	Muscat grape flavor, lily aroma	Brown fruit or raisin, Floral

Neglect to cocoa pulp sensory trait studies

The mucilaginous cocoa pulp is composed of spongy parenchymatous cells containing cell sap rich in sugars (10 to 13%), pentosans (2 to 3%), citric acid (1 to 2%), and salts (8 to 10%). The main role attributed to cocoa pulp has been to provide the essential substrate for microorganisms to develop, during fermentation, the chocolate flavor precursors that react forming “cocoa” aroma components during the roasting process (Figueira et al., 1993). The effect of cocoa pulp sensory traits on fine-flavor chocolate qualities has only recently received attention. Visitors to cocoa plantations might be familiar with the broad variation in cocoa pulp flavor and aroma. The most conspicuous variation is for acidity and sweetness. While pulp flavor and aroma intensities appear to be low and unspecific for the majority of genotypes, a few clones such as SCA6 (*e.g.* Presilla 2009) and EET62 have been known since long to have very flavorful and aromatic pulp.

Selection of cocoa for fine-flavor traits

Objective breeding towards fine-flavor quality varieties over the last century has probably been restricted to the selection over the last 50 years of the TSH varieties in Trinidad that express high-quality multi-trait pulp and chocolate sensory profiles including that of SCA6. The selection for yield and resistance alone has occasionally resulted in lower than average bulk cocoa quality, as is the case with the CCN51 clone selected in Ecuador in the 1980’s. Lockwood and Eskes

(1995) concluded that selection for cocoa quality was hampered by differences in objectives among chocolate manufacturers and by lack of efficient individual tree screening methods. Our Chuncho survey represents the first systematic study in *T. cacao* demonstrating the feasibility of within-variety single-tree diversity evaluations and selection for pulp and raw bean flavors and aromas.

Volatile Organic Compounds (VOCs) in cocoa pulp and in beans

Pino et al. (2010) were the first to identify VOCs in fresh cocoa pulp from a farm in Colombia in 2010. The relevant compounds among 66 VOCs were esters (2-heptyl acetate and 2-pentyl acetate) and linalool that are related to the known fresh fruit flavor (esters) and floral (linalool) aroma from Trinitario and Nacional, respectively. Kadow et al. (2013) reported in 2012 on relative concentrations of VOCs in fresh fruit pulp and beans of three genotypes (EET62, SCA6 and CCN51) that were chosen for their well-known pulp and chocolate sensory qualities (Tables 1 and 2). VOC concentrations were very low in the low-flavor CCN51 clone and high in fine-flavor SCA6 and EET62 clones. The VOCs in SCA6 and EET62 derived from two different metabolic pathways: methylketones, secondary alcohols and esters in EET62 and monoterpenes in SCA6. VOC concentrations were substantially higher in the pulp tissue than in the beans, confirming empirical observations that sensory compounds are primarily metabolized in the pulp. Migration to the cotyledons appeared to start only upon fruit ripening and is likely to be completed during fermentation (Kadow et al., 2013). The authors confirmed that pulp sensory traits are the most important source for fine-flavor cocoa sensory trait variations in beans and in chocolates and that VOC profiles and concentrations are in full agreement with the assessments of contrasting pulp sensorial traits in these genotypes (Tables 1 and 2). Based on the study of Kadow et al. (2013), Hegmann (2015) analyzed the volatile profiles of the fruit pulp of five genotypes selected at CATIE, Costa Rica. Relative quantities of aroma-active substances differed strongly with genotypes, season and fruit ripeness. The VOC profiles were quite different from the SCA6 and EET62 control genotypes suggesting that large diversity exists among cocoa genotypes for pulp flavors and aromas.

Genetic determination of fleshy fruit sensory traits

Barry (2009) considers that aromas imparted by ripening fleshy fruits represent the most complex and species-specific aspect of ripening and one of the key attractants for frugivores. Species and varieties all have unique sensory profiles consisting of up to hundreds of individual compounds classified as alcohols, aldehydes, ketones, esters, terpenes, furans, phenolics and sulphur containing compounds (Barry, 2009). However, apparently only a fraction of the VOCs is most odor-active (*e.g.* Pino and Bent, 2013). The content and composition of VOCs show both genotypic variation and phenotypic plasticity (El Hadi et al., 2013). The number of flavor and aroma genes in fruits is generally large. Although transgenes altering many volatiles simultaneously are already available, fruits with improved flavor will likely still require coordinated regulation of multiple biosynthetic pathways (Klee, 2010).

The native Chuncho cocoa variety

"Chuncho" (="from the jungle") cocoa is native to the La Convención province (Urubamba valley) in the Cusco region in Peru. Reports exist on the trade of cocoa beans by the native Matsigenkas (=human beings) with the highlanders before and during the Inca Empire (Johnson and Johnson, 1996). Chuncho cocoa was cultivated already in the XVI century, but occurring also spontaneously in association with the Matsigenkas (Aparicio, 1999). This tribe has always had a special interest in consumption of Chuncho fruit pulp (Gade, 1975 ; Missioneros Dominicos, 2009) and also of slightly fermented and roasted raw beans. Rozas (1861) cited by Aparicio (2000) describes native cocoa in the valley of "high quality, noble and healthy" while Paz Soldan (1852), cited by Gade (1975), labels Chuncho cocoa from the Echerate Estate as "the best cocoa in the world". Chuncho cultivation increased between 1850 and 1890 with the arrival of immigrants from Cusco (Encinas, 2009). Chuncho acreage culminated in the 1980s with 14,000 ha and a production of 10.000 tons. The acreage of Chuncho has thereafter declined to ca 11,000 ha with production of 2600 tons currently, which is due to low productivity (150-250 kg/ha), ageing trees and competition from CCN51 and from other replacement crops. Chuncho is still mainly used as a low-valued bulk cocoa bean source for butter and powder extraction. However, traditional farmers consume pulp juices from selected Chuncho trees which is a practice not known to occur elsewhere in the world. Our pulp and bean survey was therefore focused on Chuncho trees used by farmers for juice production. "Common" Chuncho beans express already interesting unspecific flavors and aromas (Condori Cruz, 2015). Chuncho pulps are generally sweet with low acidity and astringency. Even when unfermented or little fermented, Chuncho beans are neither acid nor bitter nor astringent which is a rather unique feature within Forastero cocoas. However, they may become so with fermentation duration of more than four days (Condori Cruz, 2015). Among the 10 currently recognized major genetic groups of Theobroma cacao (Motamayor et al., 2008), the Chuncho variety is part of the "Contamana" group including SCA6. SNP marker studies showed however a closer genetic relationship of Chuncho with native accessions from the Madre de Dios and Beni river basins (Céspedes-Del Pozo et al., 2017) that are geographically related. The within Chuncho molecular diversity can be considered as moderate to high as compared to that of other native cocoa varieties (Zhang, 2014).

T. cacao frugivores and the Matsigenkas

Ripe cocoa pods do not drop off the tree and do not open (indehiscent fruit). Consequently, evolutionary dispersal of cocoa seeds must have depended on frugivorous animals. Together with color, specific odor is considered to be a key fruit trait for attracting frugivores (Barry, 2009; Dominy et al., 2001 and 2004; Nevo et al., 2015). Important *T. cacao* frugivory-related traits are (Van Roosmalen, 2008): 1. Ripe fruit adherence to the tree, 2. Thick fruit husks, 3. Strong seed adherence to the pulp, 4. Nutritious and sweet fruit pulp, and 5. Frugivore swallowing of the seeds. Six efficient cocoa frugivores were identified in the Amazon basin: four monkeys (spider, woolly, capuchin and saki), the kinkajou and the coati. These are also associated with eight other fleshy fruit families, including *Annonaceae* (e.g. soursop, custard apple and annona) and *Mimoseae* (e.g. inga). In La Convención Carlos Rodriguez identified five *T. cacao* frugivores (spider and squirrel monkeys, coatis, nocturnal rodents and squirrels) three of which are different from the Amazon frugivores. Olfactory cues for ripe fruit identification have been demonstrated in several primate species (Nevo et al., 2015). Spider monkeys inspect fruits by sniffing or biting (Van Roosmalen, 2008) and were able to discriminate odors from wild fruits of *Coumama crocarpa* and of *Leonia symosa* which are part of the *T. cacao* frugivory syndrome in the Amazon (Nevo et al., 2015). Laska et al. (2009) demonstrated an unexpectedly high olfactory sensitivity of the squirrel monkey, equaling that of the dog. No information was found on the role of fresh fruit flavors in establishing efficient seed dissemination by frugivores in general.

The Matsigenkas must have disseminated Chuncho seeds since historical times in La Convención which is related to their cocoa pulp and bean consumption traditions (Missioneros Dominicos, 2009). While they don't have names for fellow human beings they have names for cocoa and three cocoa products. They must have selected Chuncho trees for their pulp sensory traits and planted trees in their traditional home gardens.

Objectives and rationale of the paper

Fresh cocoa fruit pulp sensory traits include basic flavor traits (acidity, bitterness, astringency), sweetness, fruity flavors and floral/spicy aromas as well as bean bitterness. Our first objective was to identify variations in intensities for these traits in nine genetically different clones. Such allowed us to show the relationship between cocoa pulp sensory traits and human preference. Our second objective was to identify variations in flavor and aroma identities as found in diffferent cocoa populations with specific emphasis on Chuncho cocoa. It became rapidly clear that pure Chuncho trees have generally sweet pulp and non-bitter beans. When we started to discover the large variations for fruity flavor and aroma identities in this germplasm we decided to carry out a survey involving a 226 Chuncho trees that farmers use for pulp juice production. Therefore, in the second part of our results we only mention variations in Chuncho for identities of flavors and aromas that are compared with what is known to occur in cocoa growing areas outside the La Convención province.

Results are presented in chronological order:

- Genotype x pulp sensory traits' associations Tingo Maria, Peru, 2007;
- Identifications of 12 hitherto unknown cocoa varietal sensory traits in five different locations outside La Convención, 2007 to 2017;
- A survey on fresh fruit flavor and aroma variations among 226 Chuncho trees in La Convención, 2012 to 2017;
- Relationship between flavor and aroma identities with quantitative sensory traits (acidity, bitterness and astringency) flavor identities in a group of 64 preferred Chuncho bean samples .

In the discussion we provide hypotheses that try to answer the many questions raised by our unusual findings and to present the many opportunities opened by them. We infer the apparent absence of a clear resemblance of the genetic determinations of the Chuncho vs other fleshy fruit species sensory traits. We infer also that most of the Chuncho flavor and aroma traits are in fact mimics of those of other fleshy fruit species.

Materials and Methods

Sensory trait vocabulary and perception

By "sensory traits" we mean all expressions of flavors (= taste and odor, El Hadi 2013) such as acidity, bitterness, astringency, cocoa flavor, fruity flavors, nutty/nuts flavor, spicy flavors and aromas (flower, herbal, malt odors). "Flavor" is used in short to indicate flavors from fruits, dry fruits, nuts and malt detected in our sensory surveys. "Aroma" is used to identify smells or odors from "flowers", "herbs" or "spices" but not from fruits, nuts or malt. Pulp aromas have been perceived by the nose when opening cocoa pods. Pulp flavors and aromas were perceived by fresh pulp tasting. Flavors and aromas of dry 2-4 days fermented beans were perceived by tasting the cotyledons. The presence of cocoa or chocolate flavors that are formed during roasting is not considered in our study on pulp and raw bean sensory traits.

Genotype associations for cocoa pulp sensory traits

Six pulp sensory traits of nine unrelated cocoa genotypes from the cocoa genebank of the Universidad Nacional Agraria de la Selva (UNAS) in Tingo Maria, Peru, were assessed in September 2007 including two fine-flavor (EET 64 and ICS1) and one low-flavor (CCN51) genotypes. Five randomly chosen Ucayali (U43, U45 and U53) and Huallaga (H56 and H60) clones from collections held by UNAS, Tingo Maria, Peru were also included. Mixed pulp from two ripe pods of each genotype was placed in closed plastic boxes in the laboratory two hours before sensory evaluation. The use of two pods is justified by the low variation found in the maternal pulp flavor and aroma traits between equally ripe pods from the same tree or even from different trees from the same clone. The panel of six cocoa scientists shared experience on scoring of pulp sensory traits in the field on a mixture of genotypes during a few days before the blind laboratory evaluations. Traits were evaluated in the laboratory on a 0 to 5 point scale using seven descriptors: volatile aroma (odor) perceived by smelling after opening of the boxes followed by traits perceived after pulp tasting: flavor, acidity, sweetness, astringency, bean bitterness/astringency and finally "overall preference". The evaluations resulted in highly significant statistical differences among genotypes despite the relatively low number of panelists. Statistical analyses using linear models (ANOVA, linear correlation) were performed to calculate the treatment effects, and Principal Component Analysis (PCA, using the XLSTAT 2007 program version 8.01) to visualize the associations between traits and genotypes.

Cocoa pulp and bean sensory trait identifications

The cocoa pulp sensory traits evaluations outside the La Convención province were carried out between 2007 and 2017 by experienced individual cocoa scientists during occasional visits to cocoa fields in four different countries. The scientists identified flavors and aromas of randomly choosen genotypes within different cocoa populations. Because the Chuncho basic flavors are generally uniform and very mild among trees (see Table 6), our sensory trait survey aimed mainly at identification of the large variation for fine-cocoa flavors and aromas. The Chuncho pulp sensory trait survey, performed by two experienced cocoa scientists, included 100 trees from a 50 km long central stretch of the La Convención valley. The main criteria for tree selection were preference by the farmers for pulp juice consumption, typical Chuncho traits (morphological and sensory) and age of the trees. Confirmation of the Chuncho trees identity was obtained in 2014 with SNP markers (Zhang 2014). The assessment consisted of identification of aromas perceived upon pod opening and of fruity flavors during tasting of pulp of two equally ripe pods per tree.

For the bean survey wet beans were collected in early 2015 and fermented during 2-4 days followed by sun-drying (2-3 hrs per day). The bean survey included 126 Chuncho trees scattered over the 200 km long La Convención valley. The criteria for Chuncho tree selection were the same as for the pulp assessments. Evaluation was done in April 2015 by the first three authors. Two Peruvian fine-chocolate manufacturers Alain Schneider (Chocomuseo) and Pablo Morales (Amaz Food) participated afterwards in 50% of the assessments that confirmed identified traits. Bitterness, acidity, astringency, flavor and aroma intensities and general preference was scored on 0-5 point scales after deliberation and consensus of the panel members. Any observed flavor and aroma identities from known fruit, flower or spice species were recorded also by consensus. The logics behind flavor and aroma identification studies are presented in the Discussion. Associations between flavor and aroma traits with basic flavor traits were analyzed with the Kruskall/Wallis test. Repeatability of trait identifications was assessed by blind re-evaluation of pulp and bean sensory traits from 20 different genotypes during the 2016/17 harvest.

Chocolate making

Chocolates were made on one occasion to compare cocoa pulp and chocolate flavors and aromas by the fine cocoa quality specialist Ed Seguine in the USA. Roasting was done at 125°C for 26 min according to the ECA/CAOBISCO/FCC Cocoa Quality Guide Appendix B protocol.

Results

Pulp sensory traits are discriminative varietal traits

In 2008, pulp sensory trait intensities in nine cocoa genotypes were evaluated in Tingo Maria, Peru. Discrimination level between varieties was high for all traits (P=0.05), with group means varying from a to d (Table 2). Discrimination between varieties was highest for pulp astringency (F=13.8) and lowest for pulp sweetness (F=3.5). Differences between panelists were not significant for overall preference, pulp acidity and pulp sweetness suggesting that these traits were evaluated in a uniform manner by the panelists. Overall preference scores were positively related to pulp odor, flavor and sweetness intensities and negatively, but not significant, to acidity and astringency (Table 2, Figure 1). Pulp acidity and astringency and bean bitterness/astringency were positively correlated with each other and negatively correlated with pulp sweetness. Highest average pulp preference scores were obtained for EET62 (sweet pulp, strong flavor and odor, high preference).

The second highest preference score was given to the unknown Pandora clone from Colombia that expressed the surprising soursop flavor. The third most preferred clone was the well-known ICS1 (Trinitario) with a strong fresh fruit/lemon flavor. The least preferred clone was CCN51 (acid and astringent pulp, bitter beans, no flavor and aroma). Sensory trait attributes of EET62 and CNN51 represent opposite extremes among the nine clones (Table 2, Figure 1).

Table 2 . Sensory preference and associated traits for pulps of nine cocoa genotypes evaluated in 2008 in UNAS, Tingo Maria, Peru by six panelists

Clone	Pulp description	Preference
EET 62	Aroma, fruity and sweet	4,3 a
Pandora 1	Sweet, soursop flavor	3,2 b
ICS 1	Sweet/acid, citric (lemon)	2,7 bcd
U 43, 45, 53	Sweet and intermediate flavor	2,6 bcd
H 56, 60	Astringent, acid, low fruity	2,1 bcd/d
CCN 51	Astringent, acid, low flavor, very bitter/astringent bean	2,0 cd

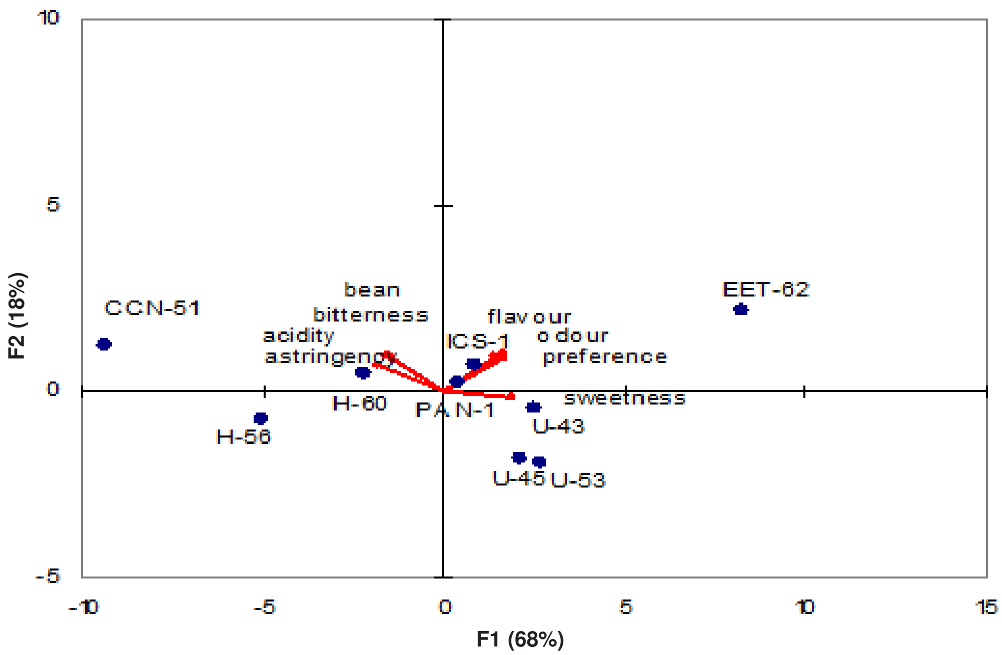

Figure 1. Principal Component Analysis for five sensory traits of the pulp and for fresh bean bitterness of nine cocoa genotypes.

New pulp sensory traits identified outside La Convencion

Between 2007 and 2017 twelve hitherto unknown cocoa pulp flavor and aroma traits (Table 3) were discovered as a result of limited pulp assessment exercises carried out by three cocoa scientists in seven locations in Trinidad, Peru, Ecuador and Brazil. The most common pulp flavor was soursop identified in all seven locations in widely different cocoa varieties. The mango/rose profile was found in Brazil in a clone that originated from the Ecuador Amazon and was introduced into Brazil for its witches' broom resistance. The banana flavor was discovered in Trinidad in 2008 in an old Trinitario plantation with predominating pulp sensory variation for sweetness and acidity. Five sensory traits (banana, soursop, jasmine, citrus and annona) were identified in Amazon genotypes, as verified with molecular markers (Zhang, 2014) in Satipo, Junin, Peru. Recently it was shown that the formerly widely grown Amelonado variety in Brazil harbors the jasmine aroma which is very surprising. Amelonado has generally been considered as a "bulk" cocoa variety, in contrast with the fine-flavor Ecuadorian Nacional variety that also displays the same jasmine aroma. Other Brazilian clonal varieties also showed

Table 3. Twelve new Chuncho-like sensory traits found in cocoa pulps in seven locations outside La Convencion between 2007 and 2017

Year	Flavor/aroma trait	Cocoa population	Country
2007	Soursop*	Amazon collection INIAP	Quevedo, Ecuador
2007	Soursop*	Pandora clone from Colombia	Tingo Maria, Peru
2008	Mango/Rose*	EET397 from Ecuador	Bahia, Brazil
2008	Banana*	Old Trinitario	Trinidad
2009	Soursop*	French Guiana collection	Bahia, Brazil
2010	Soursop**	Old Trinitario	Trinidad
2012	Mandarin, Soursop and Grape*	Piura "White" or "Criollo" variety	Piura, Peru
2012	Banana, Soursop, Jasmine, Citrus, Annona***	Amazon genotypes verified with genetic markers***	Satipo, Peru
2017	Soursop, Rose, Lily, Grape, Red Fruit, Citrus, Ingá and Jasmine*	15 clones plus Amelonado	Bahia and Espirito Santo, Brazil

*Albertus Eskes; **Maharaj K, 2010; ***Cruz and Zhang, 2014 fine flavors and aromas, *eg* the CEPEC 2008 clone with soursop flavor and rose aroma (Table 3).

Beans from the Old Trinitario "banana" flavored tree (Table 3) were transformed into chocolates by Ed Seguine. Three cocoa quality specialists described the chocolate flavor as follows: "*The banana flavor was detectable from the mid-session*" (Maharaj, 2010), "*The chocolate comes across with banana skins/strings astringency*" (Ed Seguine) and "*The long lasting aftertaste remembers that of a banana jam* (Albertus Eskes). This suggests that naturally occurring pulp sensory traits in cocoa may be transformed into very original chocolate flavor traits.

Chuncho pulp sensory traits survey

Pulp flavors and aromas of 100 Chuncho trees were evaluated in 2012. Ninety seven of the 100 trees showed qualitative expression of pulp flavors and/or aromas (Table 4), representing 30 distinct pulp sensory profiles. These involve 17 fruit flavors and nine aromas or spices. The most frequently encountered sensory profiles are soursop/jasmine, floral and citrus/jasmine found in at least 13 trees each (Table 4).

Chuncho raw bean sensory traits survey

In April 2015 the sensory panel identified and evaluated intensities of sensory traits and assigned preferences to 126 slightly fermented raw bean samples. Table 5 shows the sensory profiles for 92 bean samples out of the 126 that received overall preference scores of 3 and above. A total of 39 different sensory profiles were identified involving 16 fruity flavors and eight aromas or spices (Table 5). The four most frequently encountered sensory profiles were mandarin/jasmine, soursop/floral, cranberry and malt found each in 6 or more trees.

Combined pulp and bean surveys

The combined pulp and bean evaluations resulted in a total of 64 unique and highly diverse sensory profiles identified among the 226 evaluated Chuncho genotypes (Tables 4 and 5). Only five identical sensory profiles (8%) and ten (16%) sensory traits were found in the pulp survey as well as in the bean

Table 4. Unique pulp sensory profiles found in 2012 in 100 farmers' preferred Chuncho trees in La Convención province, Cusco, Peru

No of Profiles	Sensory traits	No of trees
1	Soursop and Floral	16
1	Floral	14
1	Citrus and Jasmine	13
2	Mandarin and Jasmine / Jasmine	6
2	Grape and Jasmine / Tangelo and Jasmine	5
2	Fruity / Banana and Floral	4
3	Soursop / Lily / Annona	2
18	Grape* / Guava and Heavy Floral / Rose Apple and Floral/Banana (var "Cavendish")*/Banana (var. "Manzano") / Green Apple and Floral* / Soursop and Rose* / Rose and Vanilla* / Mint and Rose / Mango* / Custard Apple* / Inga / Inga and Floral / Citrus and Yeast* / Citrus and Banana (var. "Isla") / Citrus, Rose and Jasmine* / Basil / Banana (var. 'Chinito')	1*
Total 30	Fruit flavors: 17 Aromas/spices: 9	Total trees 97/100

* = profile confirmed on occasions outside the survey

Table 5. Sensory profiles of slightly fermented and dried beans collected in La Convención, Cusco, Peru, in 2015 from 126 Chuncho trees

No of Profiles	Sensory traits	No of trees
1	Mandarin and Jasmine	16
1	Citrus and Jasmine	14
2	Cranberry/Malt	13
2	Mint and Floral/Malt and Jasmine	6
2	Cranberry and Rose	5
	Mint and Jasmine/Nuts	4
3	Mint, Nuts and Lily/Peach and Jackfruit/ Dried Apple/Peach/Ripe Red Fruit / Nuts and Raisin/Nuts and Rose / Peach, Nuts and Rose /Ripe Plum, Raisin and Rose	2
18	Peach and Nuts/Peach and Raisin*/ Peach, Raisin and Rose/Peach and Rose /Malt and Lily*/Rose*/Lily*/Lily and Rose*/Fresh Red Fruit*/Ripe Red Fruit and Rose*/Ripe Yellow Fruit and Lily* / Nuts and Lily*/ Nuts, Honey and Cinnamon/Raisin and Jasmine*/ Soursop* /Soursop and Lily/ Jackfruit and Floral/Mint and Nuts/Custard Apple (Cherimoya)* /Mango and Spices / Malt, Nuts and Rose	1*
Total 39	Fruit flavors: 15 Aromas/spices: 8	Total trees 92/123

* = profile confirmed on occasions outside the current survey

survey. Out of the 64 profiles, 25 (39%) are made up by only one flavor or one aroma trait. The remaining 39 profiles (60%) are based on combinations of two or more sensory traits. Thirty-three (52%) contain exclusively fruit flavor and/or flower or spice aroma combinations. Six profiles (9%) are made up of three flavor and/or aroma combinations. Five of the 15 bean flavors are also present among the 17 identified pulp flavors (Table 4) resulting in a total of 28 Chuncho pulp and bean flavors. Five out of the eight bean aromas or spices are also part of the eight identified pulp aromas or spices (Table 4), resulting in a total of 12 identified Chuncho pulp and bean aromas or spices. Therefore, the total number of sensory traits identified so far in Chuncho is 28+12=40. Out of the 40 traits 29 (73%) are mimicking those of known fruit, flower and spice species. Chuncho mimics not only flavors of fruit species but also of varieties within species, such as observed for the four mimicked banana varieties (Tables 4 and 5). It also mimics flavors of related species within the citrus genus (citrus, mandarin and tangelo) all combined with the aroma of the citrus flower aroma (jasmine) (Tables 4 and 5).

The nine and two occurrences of nutty and raisin traits, respectively, were only found in the bean samples (Tables 4 and 5). On the other hand, the soursop flavor trait occurred 19 times (21%) in the assessment of pulp traits (Table 4) but only twice (5%) in the bean assessments. Furthermore, the malt trait was not found in the pulp survey while it was identified 12 times in the bean assessments.

Out of the 64 unique sensory profiles 39 (61 %) were identified in only one tree each. However, 22 of the existing single-tree profiles have been confirmed outside the survey to exist in at least one more tree (Tables 4 and 5). Therefore, only 18 profiles (28%) have so far been identified in only one tree.

Basic and fruit flavors and aromas in 64 bean samples

Scores on 0-5 point scales for bitterness, acidity, astringency, fruity flavor and aroma traits as well as for overall preference of 20 out of 64 most preferred bean samples, with average scores of 3 and above, are shown in Table 6. These represent 20 of the 39 unique bean sensory profiles identified (Table 5).

Table 6. Examples of 20 unique sensory profiles of 2-4 days fermented Chuncho cocoa beans evaluated on a 0 to 5 point scale in 2015 by a panel of three judges

Bitterness	Acidity	Astringency	Fruity	Floral	Flavor type	Aroma type	Preference
3	0	1	4	5	Mandarin	Jasmine	5
2	4	1	4	0	Cítrus	-	4
2	3	1	4	4	Cranberry	Rose	5
2	1	2	0	4	-	Rose	5
2	2	1	2	2	Peach	Rose	4
3	2	3	3	2	Peach/Raisin	Rose	3
0	1	1	4	2	Mint	Jasmine	5
2	1	0	3	0	Mint/Nutty	-	4
2	2	1	4	0	Nuts	-	5
2	1	2	4	0	Peach	-	5
1	1	1	3	0	Peach/Nuts	-	5
1	1	1	3	0	Peach, Banana and Jackfruit	-	4
2	2	3	5	0	Cherimoya	-	5
2	0	2	0	3	-	Lily	4
2	2	1	0	5	-	Lily/Rose	5
2	1	1	3	2	Ripe Yellow Fruit	Lily	4
0	0	1	2	2	Malt	Lily	3
3	2	4	4	0	FreshRed Fruit	-	3
3	1	3	3	2	Raisin	Jasmine	3
0	2	2	3	4	Nuts	Lily	4

Despite the low level of fermentation, scores were equal or below 2, on a five point scale, in 80% of the samples for astringency and bitterness (excluding mandarin profiles) and in 89% for acidity, showing the very mild basic flavors in raw Chuncho beans. Overall preference scores of 4 or 5 were given to 60% of the 64 bean samples, showing the appreciation of the panel for the perceived sensory traits.

Bean flavor vs basic flavor associations

Statistical analyses were carried out on average intensities of acidity, bitterness and astringency for sensory profiles that contain specific fruit flavor traits in the 64 most preferred bean samples among the 126 evaluated samples. Three significant associations were identified between flavor and basic flavor scores. Firstly, the 13 bean samples with mandarin/jasmine flavored pulp also produced significantly (P=0.003) more bitter beans (score 2.9) than that of the 49 other bean samples (score 2.0). Secondly, the eight bean samples with citrus/jasmine flavor showed significantly higher (P=0.01) than average acidity scores (2.3) than that of the control samples (1.3). Thirdly, the average astringency score (3.3) for three red fruit flavored samples was significantly higher (P=0.007) than that for the control samples (1.3).

Discussion

Progress of cocoa pulp science

The ignition of pulp sensory trait studies came from the hypothesis launched in 2007 that pulp and chocolate sensory traits are correlated. Further proof came from the finding that during cocoa fermentations with foreign sensory substances transfer of such substances takes place from the fermentation mass into the cocoa beans and into chocolates. Cocoa pulp studies advanced between 2007 and 2012 by the identification in five locations of six hitherto unknown cocoa sensory traits: banana, mango, soursop, mandarin, grape and rose (Table 3). This finding was completely enigmatic until we learned that these traits are part of the genetic sensory trait diversity of Chuncho, hence of T. cacao. The first scientific pulp sensory study involved nine cocoa genotypes in Peru in 2008 (Table 2). Another scientific study concerning cocoa pulp VOCs was published in 2012 (Kadow et al., 2013). Results of both studies were in agreement with the descriptions (Tables 1 and 2) of pulp and chocolate sensory traits in three contrasting clones: EET62, SCA6 and CNN51. Cocoa pulp research is now expanding with the discovery of 64 unique pulp and bean sensory profiles in the Chuncho variety. The most responsible actors for this finding are the traditional Chuncho cocoa producers that use pulp of selected trees to make juices, a habit which is unknown to occur elsewhere. The large genetic sensory diversity found in pulps and beans from these trees suggests that Chuncho farmers select efficiently for diverse and attractive pulp sensory traits. This confirms twice the hypothesis on the existence of a large genetic flavor and aroma diversity in the Chuncho variety.

Cocoa pulp vs bean and chocolate sensory trait expression

Empirical evidence on pulp and chocolate sensory trait correlations for four fine-flavor cocoa varieties is shown in Table 1. However, it also shows modifications for grape and jasmine traits becoming brown fruit and floral traits, respectively, which however are still considered as fine-flavor traits in chocolates (Table 1). We found that 30 (79%) out of the 40 Chuncho sensory traits show high true-to-type expression of pulp flavors and aromas in the raw beans (Tables 4 and 5). However, we also detected modifications of pulp sensory traits in the fermented beans (Tables 5 and 7) of a few genotypes. The soursop flavor becomes a malt flavor in raw Chuncho beans if fermented too long (Table 5) while fresh grape, apple and banana pulp flavors were modified, respectively, into brown fruit/raisin, dry apple and dry banana in the raw beans (Table 7). The grape vs raisin modification resembles that of the SCA6 grape flavor modification (Table 1) in chocolates. Fresh banana flavor was expressed in chocolates as cooked banana/banana jam. These observations suggest that most of the fresh fruit flavors and aromas can be expressed in chocolates be it often with modifications related to processing conditions that will need to be adapted to each genotype in order to obtain a maximum expression of the interesting pulp flavors and aromas in the chocolates.

Table 7. Observed modifications in expression of five Chuncho fresh pulp flavors and two aroma traits in raw beans and in chocolates

Pulp	Raw Bean	Chocolate
Soursop	Malt*	?
Grape	Raisin	Raisin/Brown Fruit
Green apple	Dried apple	?
Mandarin	Mandarin	Citrus peel
Jasmine	Jasmine	Floral
Rose	Rose	Floral
Banana	Dry banana	Ripe Cooking Banana/ Banana Jam

*Only if fermented too long.

Significance of cocoa pulp sensory evaluations

The seven sensory traits evaluated with 9 clones in our 2008 study (Table 2, Figure 1) are considered to provide the basis of what can be evaluated statistically to identify a fine-flavor cocoa genotype. The most important positive traits are considered the preference of the pulp followed by aroma, flavor and sweetness. The most negative traits are acidity, astringency and fresh bean bitterness/astringency. For Chuncho we have however mainly concentrated on the qualitative aroma and flavor identifications because the variation for the quantitative quality traits is rather limited: mild basic flavors, sweet/low acid pulp and non-bitter fresh beans.

The well-known fine-flavor cocoa clones (EET62 and ICS1) had preferred quantitative pulp sensory traits in relation to the least preferred low-flavor CCN51 clone. The pulp sensory traits were correlated with the known chocolate sensory traits of these clones (Tables 1, 2 and 3). Kadow et al. (2013) showed high VOC concentrations in pulp of EET62 and SCA6 (Tables 1 and 2) while the low flavor clone CCN51 did not display any expressive VOCs in the pulp. EET62 and SCA6 displayed two different groups of VOCs in the pulp. This is in agreement with the different fine-flavor sensory profiles in the pulp of EET62 and SCA6. Kadow et al. (2013) concluded that the assessments of pulp sensory traits are in full agreement with the chemical results.

The significance of our qualitative sensory trait survey does mainly depend on the capacity of the panelists to identify flavors and aromas correctly, which would follow the logics of identifying common odors (*e.g.* Cain, 1979) that are part of food sensory traits (PubMed, 2012). Cain (1979) and Desor and Beauchamp (1974) found that trained panelists correctly identified 79% and 98%, respectively, of familiar whole odors. Correct recognition of sensory identities is also reflected in the repeatability. So far we obtained 100% repeatability for 20 bean samples in 2015 as compared to the same samples in 2017. The above results suggest that the level of correct identifications obtained by the observations carried out in our surveys is likely to be high.

Chunchos' vs other cocoa varieties' sensory traits

Fine-flavor cocoa varieties Traditional fine-flavor cocoa varieties present six flavor or aroma traits (Table 1) that are very similar to six of the 40 Chuncho pulp and bean sensory traits (Tables 1, 4 and 5). This indicates that 34 of the Chuncho sensory traits are completely new with regard to the traditional fine-flavor varieties. However, two trees identified in old Trinitario plantations in Trinidad expressed banana and soursop pulp flavors (Table 3). This suggests that more Chuncho-like flavors and/or aromas might be found within Trinitario and possibly also within other fine-flavor cocoa varieties.

Other cocoa varieties Between 2007 and 2017 twelve different fruit flavors and aromas that are part of the Chuncho trait diversity were detected in pulp of widely different varieties and locations (Table 3). These observations confirm that Chuncho-like flavors and aromas can also be found in different places inside and outside Peru and in different *T. cacao* varieties. It is likely that with systematic single-tree pulp assessments many of the known Chuncho flavors and aromas, and possibly others, will be detected in varieties that are not currently recognized for their fine flavor. An example is the jasmine trait identified in 2017 in the Brazilian Amelonado variety (Table 3) hitherto considered as a typical bulk cocoa variety.

Chuncho vs *T. cacao* sensory trait specificity

When adding the sensory traits in the traditional fine-flavor cocoa varieties (Table 1) to the traits found in other varieties outside La Convenci n (Table 3) we arrive at a total of 14 different sensory traits detected so far outside La Convenci n that are all included in the 40 Chuncho sensory traits panoply (Tables 2 and 3). This suggests that all sensory traits identified should be considered as *T. cacao* traits rather than as individual cocoa variety traits. It seems likely that many more Chuncho-like sensory traits can be found in other cocoa varieties that might increase the market value of such cocoa varieties. However, Chuncho has the great advantage of presenting very mild basic flavor traits (Table 6). This reduces the need for harsh post-harvest and processing procedures required for most cocoa varieties worldwide to reduce acidity, bitterness and astringency but that at the same time reduce flavor and aroma intensities.

Chuncho vs other fleshy fruit genetic sensory trait diversity

The Chuncho mimicking of 21 flavors and six aromas from known fleshy fruit species seems to be unique when compared to reported variations in sensory trait mimicking in other fleshy fruit species. For example, among 52 descriptors used to analyze diversity of 16 apple varieties (Ulrich et al., 2009), reportedly being the species displaying the "greatest" sensory diversity among commercial fruit species (Baietto and Wilson, 2015), only two refer to other species (pears and almonds). Furthermore, the descriptor lists used for sensory evaluation of nine mango varieties (Vasquez-Caicedo, 2002) only contained non-specific flavors and aromas such as "sweet" or "acid" and "sweet, sour, juiciness and mango impact", respectively, something that is commonly found also for other fruit species. Presence of great diversity for fruity flavors and flower aromas in "primary wine aromas" is well-documented (*e.g.* Marais, 1983; Ulrich and Fischer, 2007). However, these are mainly generated by specific combinations of varietal precursors during the fermentation process. Diversity for pre-existing grape varietal aromas, which can be compared to fresh cocoa pulp flavors and aromas, is apparently restricted to a few exceptional grape varieties like Muscat and Shiraz. Grape juices from different grape varieties reportedly show little sensory variation (Ulrich and Fischer, 2007). These comparisons seem to exalt the Chuncho sensory capacity to mimic flavors and aromas from 29 known fruit and flower or spice species apparently with high precision. Chemical studies will probably be needed to analyze how perfect the mimicked traits of cocoa are.

Chuncho trait associations vs evolutionary forces

Three surprising Chuncho trait associations were identified as follows.

1. Citrus x jasmine combinations. Out of the 11 Chuncho profiles that present jasmine aromas (Tables 4 and 5), all three of the citrus fruit profiles (citrus, mandarin and tangelo) show the same association with the citrus flower aroma (jasmine). The chance that this is due to pure random events would be 0.001 (11/64x11/64x11/64).

2. Mandarin flavor vs pulp and bean bitterness. The sensory evaluations revealed a significant association between mandarin flavor with increased pulp and bean bitterness. Carlos Rodríguez discovered the location of a bitterness production site in the endocarp tissue of the basal part of pods with mandarin-flavored pulp. This bitterness site does not exist in pods of other Chuncho genotypes. It shows that increased bitterness of the mandarin-flavored beans cannot be an artifact of tasting. Could it be that frugivore animals prefer bittersweet rather than just sweet mandarin pulp?

3. Soursop cocoa flavor vs soursop pulp texture. Another intriguing Chuncho sensory trait association is that of soursop flavor and soursop pulp appearance observed in all Chuncho trees with soursop flavor. This was also the case with five trees with soursop flavored pulps discovered in Ecuador, in Piura (Peru) and in Brazil (Table 3) showing great stability of this association. It might be inferred that this association has been selected by simultaneous frugivore preferences for flavor and appearance, both part of "taste" (Schwab et al., 2008), of one of their sympatric occurring preferred fruits (soursop).

The above associations exalt the very specific and directive selection pressures that cocoa must have experienced during evolution and domestication.

Frugivore and human pulp preferences vs Chuncho

flavor and aroma diversification

T. cacao depended exclusively on frugivores that effectively disseminated its seeds during evolution. Five legitimate frugivore animal families or genus are associated with *T. cacao* in La Convención as observed by Carlos Rodriguez. The large sensory diversity of Chuncho suggests that evolutionary conditions must have been favorable for intensive and specialized frugivore x *T. cacao* interactions in La Convención.

Besides animal associations, the Matsigenkas have been directly associated with Chuncho cocoa even before the Inca Empire (Johnson and Johnson, 1996) and reportedly always consumed cocoa as fresh fruit pulp (Missioneros Dominicos, 2009) and as slightly roasted beans something that is currently still the case.

Color, smell and texture are reportedly the most important traits of fruit species to attract frugivores (*e.g.* Dominy et al., 2001; Rodríguez et al., 2013 and Van Roosmalen, 2008). The role of sensory compounds that accumulate inside ripening fleshy fruits in the interactions with legitimate vertebrate dispersers is still badly understood (Rodríguez et al., 2013). It has been suggested that once a frugivore identifies the pulp of a fruit as sweet and nutritious, that would be enough to ensure pulp eating and seed dissemination (*e.g.* Van Roosmalen, 2008). Chuncho pulp flavor traits are present in 53 (83%) out of the 64 analyzed sensory profiles while 36 pulp aroma traits (56%) were identified in the same 64 profiles. Thirty-three sensory profiles (52%) are made up of specific combinations of one flavor trait (taste) with one aroma or spice trait, which is unlikely to be just a result of chance. It is therefore inferred that ripening T. cacao fruits firstly offer an aroma to attract frugivores from a certain distance and then a flavorful pulp to stimulate consumption and effective seed dispersal. However, it is also possible that during domestication the Matsigenkas as well as nowadays the Chuncho growers may have selected trees more for their pulp flavors than for odors.

Chuncho pulps and beans mimic sensory traits associated with four fruit species that are sympatric with *T. cacao* in South American tropical forests: three *Annonaceae* species (soursop, annona and custard apple) and the inga species (Tables 4 and 5). Frugivores of *T. cacao* are equally associated with the *Annonaceae* and inga species (Van Roosmalen, 2008). It is understandable that these frugivores prefer, and therefore select, *T. cacao* fruit pulp that mimics sensory traits from familiar fruits to which they are also associated as frugivores.

An apparently more difficult question to explain is why *T. cacao* mimics as many as 16 flavors and aromas from allopatric fruit and flower or spice species. The T. cacao frugivores might have imposed extremely high evolutionary selection pressures on T. cacao for it to be able to metabolize cocktails of compounds that mimic the unique sensory identity of the allopatric fleshy fruit and flower species listed in Tables 4 and 5.

Genetic determination of Chuncho sensory profiles

The hundred Chuncho trees evaluated in the pulp sensory trait survey showed to belong to the Chuncho molecular genetic diversity cloud (Zhang, 2014) with very little duplication of genotypes. Trees with similar flavor and/or aroma traits were generally different molecular genotypes, suggesting that similar sensory phenotypes can be found in different Chuncho genotypes. However, this aspect deserves to be studied in more detail.

The intensity of the Chuncho lily aroma varied largely in our raw beans' evaluations (scores of 1 to 4) as well as in pulps of different trees in the field. This suggests quantitative expression and polygenic inheritance of this trait.

Several Chuncho hybrid trees, identified as such by molecular markers (Zhang, 2014), have shown high expression of Chuncho pulp sensory traits. The same is known from breeding trials including hybrids of SCA6 and EET62 with unrelated cocoa genotypes. The above is suggestive of dominant gene action of *T. cacao* pulp sensory profiles.

The multi-trait Chuncho sensory profiles represent 60% of the 64 Chuncho sensory profiles. Chuncho multitrait sensory profiles that were found in more than one tree (Tables 4, 5 and 6) are mainly expressed as stable qualitative traits with rather similar intensities among trees and locations. One example is the mandarin/jasmine sensory profile that always displays strong mandarin fruit flavor and medium to strong jasmine odor combined with higher than average bean bitterness. The soursop flavor occurring alone or with other traits is always associated with a translucent pulp (see also above). Other stable multi-trait sensory profiles are soursop/jasmine, citrus/jasmine, mint/floral, cranberry/rose, mint/jasmine, nuts/rose and peach/nuts/rose. These stable multiple trait associations, including in hybrid genotypes, are suggestive of pleiotropic dominant gene action such as Gregor Mendel identified in pea trait number "three" (Mendel, 1866) responsible for simultaneous expression of "brown seed coat", "violet flowers" and "axial spots". The molecular age proved that single genes can produce multiple products with pervasive effects on the phenotype (Stearns, 2010), including for mutants affecting ripening (Kovacs et al., 2009). *T. cacao* may have benefitted from pleiotropy as an evolutionary favorable mechanism associating several attractive traits for frugivores simultaneously. Genetic correlations between flavor and aroma traits may have exemplified *T. cacao* pleiotropy (Stearns, 2010). Such is contrasting with the commonly found quantitative and polygenic expression of sensory traits in other fleshy fruit species (*e.g.* Klee, 2010).

The possible common dominant pleiotropic inheritance of flavors and aromas in sensory profiles of *T. cacao* would facilitate largely the selection and breeding for specific qualitative sensory traits in cocoa. The above inferences on the genetic determination of flavors and aromas in cocoa would merit confirmation by carrying out more formal genetic studies.

Unique Chuncho sensory profiles vs the fine-flavor cocoa market

The commercial value of a fine-flavor cocoa variety is dependent on the full spectrum of flavor and aroma traits including basic flavor traits. Chuncho has the advantage of combining a multitude of very interesting sensory traits with favorable basic flavor traits (Table 6). While commercial fine cocoas usually have a narrow sensory profile, Chuncho offers a remarkable choice of 64 interesting flavor and/or aroma combinations. Thirty-three (52%) of the 64 unique sensory profiles are made up by fruity flavor x floral or spice aroma combinations. Six profiles (9%) are made up of a combination of three flavors and/or aromas. The 25 Chuncho profiles with just one sensory trait mimic 16 known flavors and aromas: cranberry, basil, dried apple, peach, rose, jasmine, lily, inga, mango, three banana varieties, soursop, grape, annona and custard apple. Fifteen of these traits are attractive already just because they are completely new to the current fine-flavor cocoa market. Complex Chuncho sensory profiles might be even be more attractive commercially as seems to be the case for the multiple sensory traits found in TSH varieties selected in Trinidad. A few examples of apparently interesting complex Chuncho sensory profiles (Tables 4 and 5) are: mandarin/jasmine, soursop/jasmine, rose/vanilla, mint/jasmine, citrus/jasmine, tangelo/jasmine, cranberry/rose, nuts/raisin, peach/nuts, peach/rose, lily/rose, mint/nuts/lily, ripe plum/raisin/rose and nuts/honey /cinnamon. The expected commercial use of such unique varieties should lead to a significantly refined diversification and enlargement of the fine-flavor cocoa market. We propose here that multiple-trait sensory profiles are baptized as "extra-fine" cocoa sensory profiles.

Potential for Chuncho fresh juice and roasted whole bean consumption markets

Matsigenkas and Chuncho growers consume selected cocoa pulps and juices as well as slightly roasted whole beans. These two ways of consumption may potentially become two new segments in the fine cocoa market. The fresh juices produced from selected Chuncho trees would be as diverse as the 64 unique sensory profiles, eg soursop", cranberry", mango/rose", peach/jasmine and tangelo/jasmine". Our sensory trait assessments have convinced us that eating the Chuncho dry raw bean from selected genotypes is a superior tasting experience. The flavors and aromas are fully expressed in raw Chuncho beans while several Chuncho sensory traits are modified or become weaker in chocolates (Table 7). Basic flavors of Chuncho beans are very mild (Table 6) favoring direct consumption of slightly roasted de-hulled beans or nibs. Such a new market might favor cocoa producers who would possibly receive a higher percentage of the final selling price of the cocoa beans that do need only minimal processing and no transformation of the Chuncho cocoa beans. Last but not least, consumption of de-hulled slightly roasted cocoa beans is expected to bring along health benefits through higher polyphenol concentrations than in chocolates combined with high tasting pleasure benefits.

Cocoa's sensory identity and evolutionary survival

Any fruit or flower species has built up its own sensory identity over millions of years during evolution and domestication (Barry, 2009). This sensory identity is very precious to the fruit and flower species as it serves as the main recognition factor for frugivores and human beings to be efficient seed dispersers, thus guaranteeing survival during evolution and domestication. Each of such sensory identities is based on a very delicate quantitative and qualitative balance among large numbers of VOCs (Barry, 2009; El Hadi et al., 2013; Klee, 2010). With Chuncho possibly being the "mother" of all *T. cacao* flavor and aroma traits we might consider the possibility that it is also the "mother" of the cocoa sensory identity. With our findings that Chuncho mimicks sensory traits of 29 fruit, flower and spice species one might wonder if T. cacao has at all built up its own specific sensory identity. We hypothesize that in the absence of a specific *T. cacao* pulp sensory identity, Chuncho and possibly other cocoa varieties may have developed a large number of mimicked flavors and aromas to attract specialized frugivores.

Identification, selection, breeding and multiplication of fine-flavor genotypes in Chuncho and in other varieties

As shown by our results, selection for fine-flavor cocoa genotypes can be done rapidly and efficiently by pulp evaluation for fine-flavor sensory traits. We expect that besides Chuncho, pulp flavor and aroma selection can be successful also in other cultivated and native cocoa populations or in landraces in Peru and elsewhere. Populations that are genetically narrowly related to Chuncho *eg* from Madre de Dios and Beni deserve extra attention. Farmer associations and research institutes could promote large scale competitions for farmers to identify trees, within any diverse cocoa variety, with the most delicious pulp combined with non-bitter beans that should thereafter be characterized for flavor and aroma identity and intensity, following the logics of our approach in Chuncho. Once trees with favorable pulp and bean traits have been identified and which have also interesting yield components then the next step is multiplication and/or breeding. Firstly, individually selected trees need to be multiplied as clones for conservation purposes. Secondly, if the selected tree has also interesting yield components and is self-compatible it could be used directly for commercial mono-clonal multiplication.

Breeding should aim at high yield, resistance and fine-flavor quality. An interesting option is the creation of hybrid varieties by crossing Chuncho selections with parental genotypes (*e.g.* PA121 and PA169, that are available in La Convenci n and elsewhere) that are known to produce high yielding and resistant hybrids and which neutral pulp does not interfere with the fine-flavor Chuncho quality expected to dominate in the hybrids.

Thirdly, the establishment of small-scale commercial plantations can be achieved by using open-pollinated seed from selected Chuncho trees which, even if self-incompatible, as expected from the pleiotropic dominant gene action should reproduce largely the sensory traits of the parental trees. Such seed progenies could be selected within 3-5 years for flavor/aroma traits and for yield components. The then selected trees should provide enough budwood to ensure establishment of larger commercial polyclonal plantations with uniform sensory traits. Finally, the definite answer to the need of rapid commercial multiplication of Chuncho trees with high quality and average or high yield potential is probably best provided by large-scale somatic embryogenesis.

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