Cacao Flavor Through Genetics — Anatomy of Fine Flavor

The goal of the Heirloom Cacao Preservation Initiative is to preserve the diversity of flavor for generations to come.

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Within the cocoa world, there is a great deal of concern about both the cocoa supply and the sustainability of cocoa farmers the world over. Initiatives such as CocoaAction within the World Cocoa Foundation, as well as large cocoa and chocolate company programs, focus on long-term solutions to sector problems. One of the critical elements is the need to extend resistance to the major diseases of cacao to all of the growing regions. At the same time, these initiatives are not able to focus on maintaining the suite of flavors that are part of the heritage of cacao as we know it today. Flavor, with all of its diversity, is extremely hard to include within a breeding program for cacao. It is for this reason, and the fear that not including flavor as a breeding criteria will cause the loss of previous chocolate flavor diversity, that the Fine Chocolate Industry Association (FCIA) has launched the Heirloom Cocoa Preservation Initiative.

We know that flavor in the finished chocolate comes from a multitude of sources: the genetics of the trees; terroir, including the land and soil conditions as well as climate during pod maturation (5 1/2 months long); fermentation and drying; the local microbiome where the fermentation is being carried out; and the factory processes such as roasting, conching, etc.

This article will look at the genetics of the tree itself. Some of what we're going to talk about is pretty obvious, and some nobody knows anything about, including us. There is much to learn. The issue that we face is answering the question, *Where do these unique and special flavors come from?* Frankly, we just don't know right now. This goes back to the question that biologists have faced for years on most crops and keep debating, *Is it nature or is it nurture?* In our case, nurture would be the kind of things that the farmers do to the cocoa on the farm—the postharvest practices as well as the climate and microbiome. Ultimately, the question of where

Fine Chocolate Industry Association (FCIA) is focused on supporting fine chocolate professionals, with the purpose of being an "unbiased collective voice of quality and innovation that promotes fine chocolate making practices from blossom to bonbon and bar." This couples farmer and environmental sustainability to the reason we are all here: the beautiful diversity of flavor presented by *Theobroma cacao*. Those who share a passion for the flavors of chocolate, farmer and environmental sustainability are encouraged to find information on joining the FCIA as a member or friend at their website. www.finechocolateindustry.org

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do these flavors come from—and how can we preserve them in the face of critically needed breeding for disease resistance and productivity—is of paramount importance to us, in particular, as fine chocolate makers and to consumers everywhere who enjoy and love all of the diversity of flavors in cacao.

While there are a multitude of factors that influence the flavor, within the FCIA Heirloom Cacao Preservation (HCP) program we standardize as much as possible so that we're treating these beans in an equivalent way in both recipes and processing. We do adjust the roasting process for the type of bean that we have, but, other than that, we are trying to standardize our processing so that we can then address what flavors are coming inherently from the beans.

The HCP is important in preserving the diversity of cocoa flavor. For the first time, we can understand and identify tree varieties that are really unique in flavor. It allows us to take information to the next level where we can analyze it to tease out what contributes to flavor. This is all being done at the USDA Agricultural Research Service laboratory in Beltsville, Maryland, with researcher and geneticist Dapeng Zhang, one of the world's leading experts in cacao genetic diversity.

The FCIA has a rigorous submission protocol for cocoa beans being entered into the HCP program. It is described in detail on the FCIA website (www.finechocolateindustry.org/hcp). Following conversion of the beans into liquor and 68.1 percent cacao chocolate using standardized procedures, the FCIA-HCP Sensory Panel renders an evaluation of the flavor. At this point, the sample is considered as HCP recommended but A site visit and genetic characterization are required before HCP status is awarded.

Standard DNA Population Map

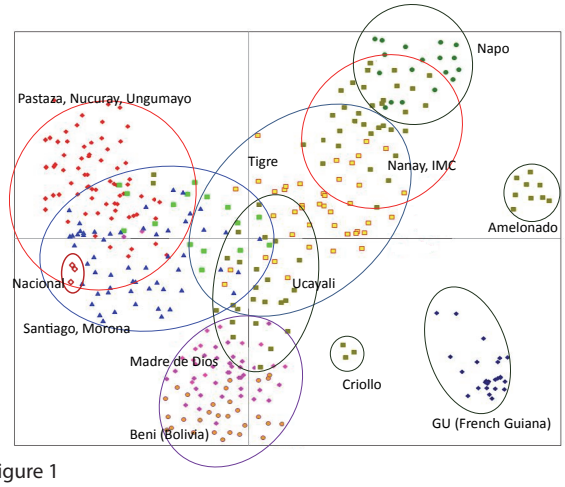

Figure 1

At the site we see if the fermentation process is a standard system, or if they're adding something to adulterate or make the flavor different. Then we look at their management practices. Are they doing something really great that we need to know about, that we can pass along in the future? Finally, we collect the genetic material. We do not use any living material—we take a small, dried piece of a leaf and analyze the DNA within the leaf.

On the DNA side of it, we're looking at something called *single nucleotide polymorphic markers* (SNP, pronounced "snip" for short). Every organism has changes in their DNA, even humans. It's as unique as your fingerprint. You've got hundreds of thousands of these base pair changes within your DNA which you received from your parents and that make you unique. The same is true for cacao. We use a high-throughput analysis system that can analyze thousands of these markers at the same time. It is a very complicated system and it takes some time to do. After our site visit it might take several months to get the samples to the lab, and a few more months after that to actually analyze it and run it through our process. Once we've identified that tree or group of trees, we then compare the results to what we know about other populations that we've already looked at. We're looking not only at the populations, but we're actually looking at their genetic makeup and how they're interrelated.

Figure 1 is our current DNA population map that we're using now. It is constantly evolving because every sample that comes in is added to our library of information. There are numerous populations. You can see that the *morona/santiago* population includes *nacional* now. We're trying to encompass everything and bring it all together so we can understand how all of these groups are interrelated.

HCP DESIGNATE SAMPLES

The first four HCP-designate samples were awarded HCP status in January 2014 and unveiled at an FCIA

public event at the Institute of Culinary Education in New York on February 11, 2014. The first two samples we'll discuss are from Bolivia and, as you will see, they differ genetically and they also differ by location.

Bolivia Alto Beni

The Bolivia Alto Beni cocoa is grown and produced by Volker Lehmann. This is a population of *trinitario* clones (Figure 2). It falls within the classic trinitario group (crosses between *criollo* and *amelonado*) or it has some bit of influence of the upper Amazon. These are trinitarios that were moved into Bolivia decades ago and they've been managed to such a point where they are recognized now as heirloom cacao.

Now, what's interesting about this is that you could look at this and say, "Oh trinitario, it's halfway between the amelonados and the criollos, and it's got dispersion going up into the *nanays* and *parananaris*; I would expect complexity out of this. It's covering a lot of genetic real estate here. I expect there to be things happening."

It's a little bit darker in color than the Tranquilidad samples, with slightly orangish hues. The aroma is chocolate and fruit with green stem-like character. The depth of the chocolate flavor that comes out in this sample is striking. Remember the genetic position being cut through between the trinitarios and the amelonados, which is the basic source we know of today of this rich cocoa flavor. There are fruit and floral overtones, slightly spicy, resinous with green notes and a trace of vanilla character. The fruit overtone has citrus with slight balsamic wood resin and aged fruit notes. The chocolate presents a complex mix of chocolate, fresh citrus and aged fruits. This symphony of flavor has a nice residual finish.

Bolivia Tranquilidad Estate

The second HCP-designated cocoa is also provided by Volker Lehmann but from the Tranquilidad Estate. This is a different location in Bolivia and represents a new population group. It's not too far away from the Alto Beni but it is run by the same group, so it's under the same degree of management; they're doing the same kind of things with the postharvest processing of the beans. Nevertheless, you will find a different flavor profile in the chocolate due to a combination of the genetics and the microenvironment, the terroir.

The Tranquilidad samples are all falling within a new population group from Bolivia and southern Peru (Figure 3). This cacao type represents another group of semi-domesticated cacao like criollos. It's inherently very homogeneous, unique and it's special from a flavor standpoint. All of the cocoas that we are tasting and we have qualified as HCP designates are very complex. They are not simple chocolates.

We can note the rich, medium dark brown color and reddish hues in this sample. The aroma is a mildly rich chocolate base, with fruit and tart aromatics. These beans are on the more fermented side. That is what

Bolivia Alto Beni DNA Principal Coordinate Plot

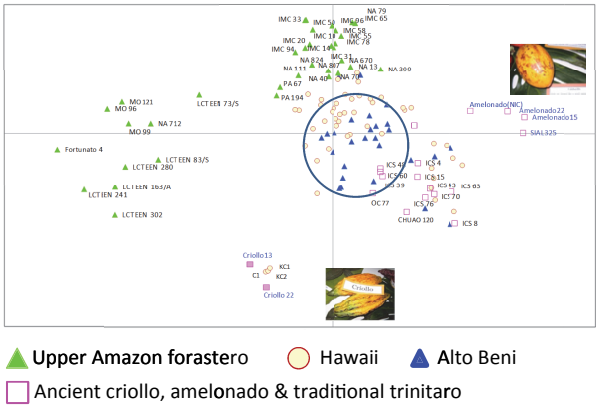

Figure 2

Bolivia Tranquilidad Estate DNA Principal Coordinate Plot

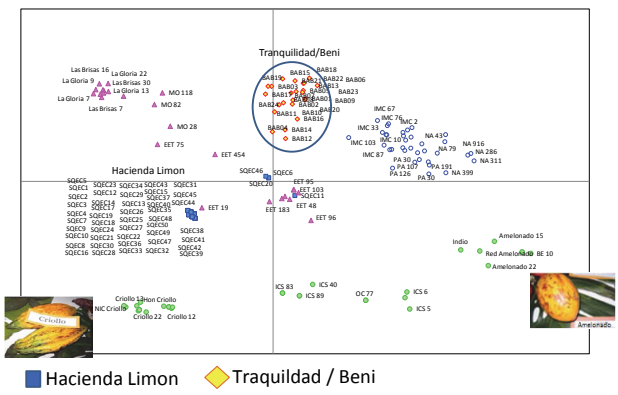

Figure 3

drives some more of these dark notes, but there's an underlying brightness as well. It's a very complex sample, with a very interesting fruit character aftertaste that seems to remain in the mouth. It has a long finish in that sense. There's an inherent sweetness associated with it. Sweetness is actually a part of the presentation of this bean and we don't know why.

We have these two Bolivian samples that are different from one another. We can make some sense out of the genetics. But they're also grown in a different location from one another physically. It could be the soil. It could be the climate, although these are probably a little closer together than many other places. The bottom line is, they're different from one another.

Ecuador Hacienda Limon

From Bolivia we go to Ecuador. This sample was sent by Samuel von Rutte, at Orecao in Ecuador. His estate is the Hacienda Limon and it is located down the hill from the Vulcan Cotopaxi in the flat cocoa lands.

The genetics associated with this sample are very different than the previous ones that we've talked about (Figure 4). This one lies within criollo and ancestral nacional or pure nacional. There is a very tight cluster representing a very limited diversity within what he has but it is very unique. Mostly, von Rutte's clones fall within a single group. Also, there are a few outside the cluster that are a little more associated with some of the other crosses. If we look at what he has, we see a modern variant of the classic, older nacional type. Around the 1920s, this material was prevalent in most of Ecuador and it was a trinitario crossed with nacional. He also has the amelonado/nacional cross. He has two basic types that are linked together which form the uniqueness of his chocolate.

The Hacienda Limon cluster is very tight compared to the spread of the Tranquilidad, Bolivia. One would expect there to be some tightness or pointedness to the character of the sample.

For an Ecuadorian this particular sample is surprisingly light in color, with reddish and distinct yellowish hues. Many people are familiar with the nacional arribas and know the depth of their color. The aroma is very mild, with chocolate, nut skin, nuts and mild red fruits. The initial flavor profile is mild but persistent. It has complex nutty and caramelic notes that dominate with a smooth background. This flavor was not produced as a byproduct of the processing. This is inherent in the bean and it happens every time we roast the beans and process them. What you see here is what came with the beans; it's what von Rutte did with his genetics.

Maunawili Experimental Station

The next sample we're going to discuss is from Hawaii, on the island of Oahu at the Maunawili experimental station. It is an experimental stand of trees that is owned by the Hawaii Agriculture Research Center. It's deliberately a collection. It never started out as a production planting. Daniel O'Doherty from Cacao Services went there, pulled them all together and processed them into the cacao.

Ecuador Hacienda Limon DNA Principal Coordinate Plot

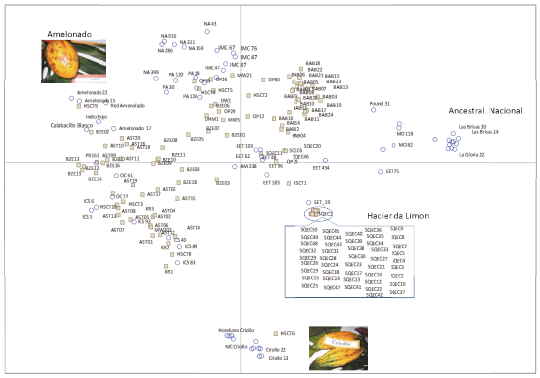

Figure 4

Maunawili DNA Principal Coordinate Plot

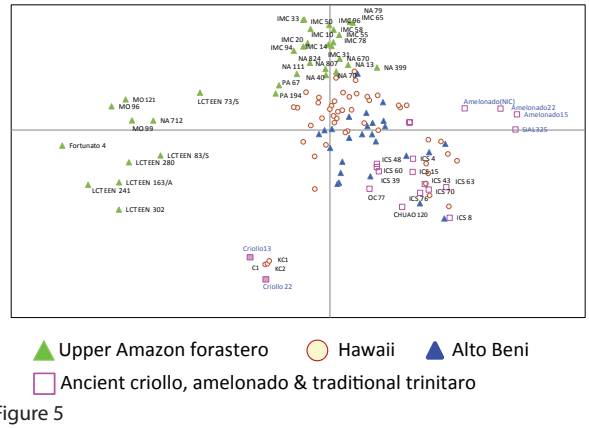

Figure 5

You can see a cluster with a number of the Hawaiian samples fitting really closely with the criollo (Figure 5). In this principal coordinate plot, the main take-away is that the closer together something is, the more related it is. The others fall between the mixture of amelonado, your classic trinitario or are trinitarios with the addition of upper Amazon forasteros. So it's a very broad mix. It almost covers the entire map. It probably has a very complex flavor.

The Hacienda Limon was a little light for an Ecuadorian. This Hawaiian sample is lighter yet, with slight reddish and yellowish hues. Think about the criollo blood in its genetics. It is very interesting that some of the trees are carrying that. There's also a dispersion and a little bit more aroma in this sample, a mild chocolate aroma with ripe and slightly overripe fruit notes. There's a lot of brightness in the flavor, with a mild, fresh-fruit acidity and an inherent mild spice note.

This sample is very soft and melts easily in the mouth as well. The beans also have a higher fat content. In the plant world, if you want something to be harder as a fat, meaning higher temperature melt point, go closer to the equator to get it. If you want something to be a softer fat, meaning lower melt point, go north or south. For example, Bahia is about 15° south and is nearing the lower southern limit of southern cocoa growing. Bahia cocoa butter was known, when it was available, as one of the softest cocoa butters that you could purchase. If you wanted a really hard butter you would look for West African or Malaysian butter, meaning higher-temperature melt point. It does not have anything to do with the heat. It is the daylight cycles that the plant experiences. The higher latitude in the northern hemisphere (or lower in the southern hemisphere) triggers in all plants the sequestration of the more unsaturated fats, which makes the fat softer. Hawaii is actually above the 20° mark north at 21.5° and therefore is getting a fair amount of day and night shifting over the course of the year. This influences the plant and the plant then generates a softer fat. So you've seen a difference created by the plant. This is clearly an environmental effect. To some extent, the observation that the sample seems more fatty in the mouth is also a manifestation of the softer, more unsaturated cocoa butter because when the fat melts so easily you see it more. You experience it longer in the mouth because it's there as liquid fat longer.

There is a lot of diversity here. One of the challenges when you have that level of diversity is how exactly does one choose to optimize the fermentation? If I have something like porcelana, criollo, merediño or guasare, those beans ferment very quickly. Ideally, most people say three days maximum. If you attempted to ferment an amelonado for three days, you won't get much chocolate flavor and it would be highly astringent. It's an interesting dilemma. So inherently, the manufacturer here had to play some compromise games because he has that diversity in the population. So there are some things that you can readily deduce. Knowing that population structure, even though we don't know the exact genes that cause everything, we can make some judgments or guesses on the connections with flavor as well as other plant traits. You can make judgments if you walk around the field and cut open a lot of beans, but it's nice knowing there's actual science behind it other than one's eye.

Ecuador Coopertiva Nueva Esperanza

At this stage we'd like to unveil to you the fifth designate for HCP status. It comes from the Coopertiva Nueva Esperanza in Ecuador. It's downhill from Quito in the state of Pichincha which goes almost directly towards the Pacific Ocean. It was provided by Jose Meza and Barbara Williams, the husband-and-wife

Ecuador Coopertiva Nueva Esperanza DNA Principal Coordinate Plot

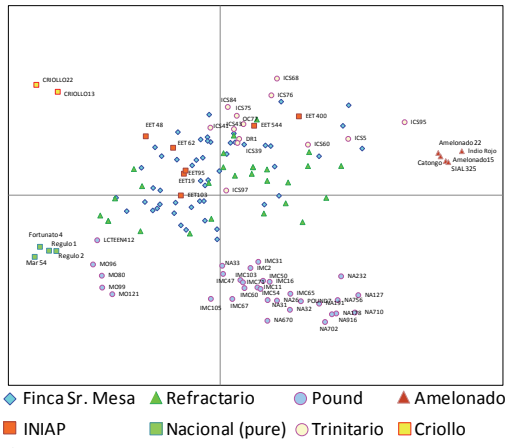

Figure 6

team from Mindo Chocolate Makers in Michigan.

We are very fortunate that we have worked with Barbara almost two years ago. We had received some of her samples and they fall in large patterns. You'll notice where the old nacionals are, the criollos are and the amelonados are. Now let's look at the structure map of it (Figure 6).

You'll see that they all fall within this same group. There's nacional influence, criollo influence and amelonado influence. It's in every single one of them. Now, there's another group that matches up very closely with them. These are called the *refractario* clones. The very old nacional from the 1920s were collected by F. J. Pound when he went there looking for witches' broom-resistant nacional clones. A lot of this material is sitting in Trinidad and we've looked at it numerous times as part of our reference collection. We can see that a lot of these match up with what Mindo has at the site. It's very nice, very old, classic nacional types.

Every single one of these vertical bars is an individual tree (Figure 7). So the diversity within this population in terms of the parentage (that is, the family contribution structure) is very similar even though genetically with the SNP markers it spreads across a fairly wide range. The reality of it is that the parentage represents a very specific, defined base. Yet each individual in this population is unique and genetically different, just like every single human is unique.

Our fifth HCP designate is also a medium brown

Ecuador Coopertiva Nueva Esperanza Genetic Markers

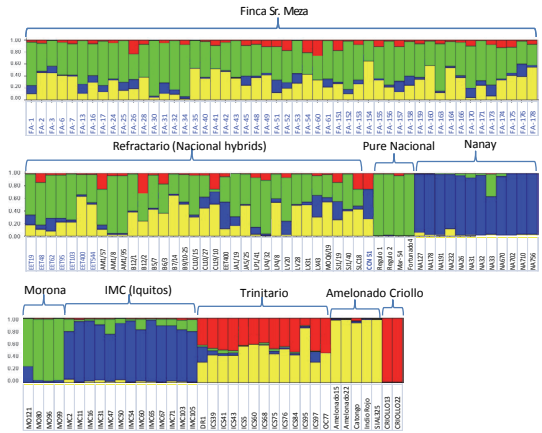

Figure 7

with reddish and yellowish hues, but the color is darker compared to the color of the Hacienda Limon, also from Ecuador. It has a rich chocolate aroma with a hint of wood. There is a little bit of the caramel nut notes that we found in the Hacienda Limon, but nowhere near the level. There is more chocolate flavor. We're getting some of the more classic, *arriba*-like notes, although not quite as floral as you would expect to see in say, *manabi*, *vinces* or *esmeraldas* beans at times. Mild fruit acidity and astringent notes continue to drive the nut/nut skins character along with a plum/dark fruit/complex fruit note with a trace of tobacco spice and very mild green floral notes. This complexity differentiates this sample from the classic arriba cocoas. We suspect that some of this complexity is due to the diversity and range of those genetic markers.

We learned from the site visit, too, that these trees originated from seeds. When you go back to that structural chart, you'll see that there's a lot of genetic diversity that's very similar to children. You have shifts in the crossing between the parents.

Here is a good example. Looking again at Figure 7, an individual bar that has a lot more of the green color, which represents the nacional, is going to be a different individual in its character and nature from a sample that has a much smaller level of that particular contribution. Genes that influence the nature and characteristics of a particular cacao are derived from their parentage.

CONCLUSION AND FUTURE DIRECTION

That broad map showing the entire world of cacao as we know it today is continuing to be expanded. We expect that the HCP samples will continue to drive some of that expansion. Ultimately, our objective is to understand what about the genome leads to the kind of functional changes that occur in fermentation and in drying and that then yield flavor when we process it. We do not know how all of these variables are interconnected today but we are adding to the body of knowledge that will let us do that at some point in time. At the end of the day, the mission of the HCP is to ensure that the diversity of flavor that exists within the world called *Theobroma cacao* is preserved for generations to come. □

Presented at the FCIA summer event