

Bryan A. Bailey · Lyndel W. Meinhardt  
*Editors*

# Cacao Diseases

A History of Old Enemies and New  
Encounters

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## Preface

Chocolate! My first memories of chocolate have nothing to do with science, but rather the simple pleasure of eating sweet milk chocolate. It seems chocolate and holidays in the United States have gone hand in hand for many years and I cannot remember a holiday passing in which chocolate was not included. Chocolate cake was a standard for my birthday, chocolate eggs and rabbits were a staple at Easter, chocolate combined with nuts and/or caramel were highly sought on Halloween, and chocolate in just about every form was fair game at Christmas. To be sure, it did not take a holiday to encourage my consumption of chocolate, as I was and still am willing to eat chocolate any day of the year. My love of milk chocolate has not diminished with age. Fortunately my wife, Susan, in whom I always depend, is almost always willing to aid this pseudo dependency by providing me chocolate in some form, chocolate pie, chocolate ice cream, and chocolate delight being my personal favorites.

Although the interaction between man and the cacao tree has continued for at least 3000 years, it is in relatively recent times that chocolate as we generally know it, a sweet/semisweet dessert, has become a global food phenomenon. The extensive global interest in chocolate has coincided with the establishment of organized farms, some large but mostly small, tended by the often overlooked but critically important cacao farmers. Having met some cacao farmers, yet never enough, I have found them to always be keenly interested in the crop they produce, primarily because, in most cases, they depend on it for basic necessities needed for supporting their families and improving their lives. The benefits of chocolate to farmers are not limited to cacao farmers only, since a multitude of other agricultural commodities are incorporated into chocolate dishes/products: peanuts, coconut, various other “nuts” like almonds and my personal favorite pecans, fruits, various flours, fats/oils, and of course sugar, not to mention more unique combinations involving meat products like bacon and spices like pepper. A surprise to me is the common occurrence of farmers having never tasted chocolate made from their own cacao trees. To me this is like a tomato farmer never tasting the tomatoes he grows. The fact is many cacao farmers seldom consume chocolate of any kind. Unfortunately, you cannot pick chocolate bars straight off the tree like a tomato from a vine,

although if that were the case I would probably live in a tropical climate. Fortunately, we have the chocolate industry, employing many people in its many forms, which assembles chocolate products, in their many forms, providing us with the endless pleasure of consuming old and new chocolate products whenever we can create/fabricate a good enough reason to celebrate. To me that means waking up and taking a deep breath or in other words being alive.

Now I realize my exposure to chocolate was not the same as for everyone else in the world. As I have traveled to different countries and learned about their cacao/cocoa cultures, I am still surprised when I find out consumption of chocolate in many countries falls far below that in the United States, not that the United States is the largest consumer. Growing up I had no idea of the history of chocolate, how chocolate was made, where cacao was grown, or of the many problems cacao farmers faced when growing the crop. It seems from the time (perhaps before) a cacao seed is planted in the soil until a mature cacao pod is harvested in the field, the crop is faced with continuing obstacles, some of the most important being the plant diseases we describe in the book. Chocolate may be the “food of the gods,” but cacao, the tree whose seeds (cacao beans) are used to make chocolate, is also the food of many pathogens which cause disease and limit its production. Plant diseases cause significant losses almost everywhere cacao is grown and have encouraged the global migration of cacao to the point where most production occurs outside the areas where the crop originated. In these new areas, new plant diseases have emerged and serious crop losses continue to limit supplies of that most precious commodity, the cacao bean. In South America, where cacao evolved, two major pathogens, *Moniliophthora roreri* (frosty pod rot) and *Moniliophthora perniciosa* (witches’ broom), severely limit production, each being capable under the right conditions of completely destroying the crop. In west Africa, *Phytophthora megakarya* (black pod rot) and *cacao swollen shoot virus* (CSSV) are serious and expanding threats to production, and in the island nations of southeast Asia, *Ceratobasidium theobromae* (vascular streak dieback) along with *Phytophthora palmivora* (causing the global threat of black pod rot) continually threaten production. This is not meant to underemphasize the many other recognized pathogens of cacao which cause serious losses locally or may have the potential for causing expanding losses in the future.

Globally, cacao researchers have not been standing by allowing these disease threats to continue unchallenged. Scientists are using new and traditional technologies to understand the processes leading to disease in cacao, developing new tools for managing cacao diseases, and selecting cacao trees that continue to yield well despite disease pressures. It should be comforting for the cacao farmer and chocolate consumer to know that, in locations all over the world, scientists are investing their time and efforts combating all of these diseases. Part of the impetus for assembling this book was the recognition that such excellent science is being carried out in these efforts and that assembling much of this information, past and current, into one place would be of value to those interested in cacao, cocoa, and chocolate. Cacao being produced all over the world complicates the sharing of

information between scientists and others interested in the crop. Researchers working in one part of the world are often literally half a world away from other areas where similar research is being carried out. Despite the availability of modern electronic technologies, much research remains out of reach to researchers due to barriers like publishing rights, perceived limitations to local interests, and in some cases a general lack of awareness to look and see what is going on in other areas. An obvious observation derived while editing this book was that a great deal of research is tied up in local institute/agency reports greatly reducing the impact of the research and, in many cases, leading to its loss from the institutional memory of global cacao researchers. This lack of communications of the most basic experimental results limits potential sharing of ideas and their resulting synergies, results in redundancy of effort, and prevents the recognitions of common themes that might accelerate the derivation of solutions to the problems being addressed, global losses to cacao diseases. The many excellent authors associated with this book have done their best to assemble and highlight the current state of knowledge of cacao diseases and their impact on production. Perhaps most importantly, authors have also been encouraged to provide direction, as they see it, to future work sharing their ideas where gaps in our understanding of cacao diseases occur and how the gaps might be filled leading to improved management of cacao disease. It is through these efforts that we hope to maintain and improve cacao production by limiting losses to disease and insure an ample supply in the future for all the world's chocolate fans.

Beltsville, MD

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**Part I**  
**The Crop and Its Pathogens**

# Chapter 1

## Origin, Dispersal, and Current Global Distribution of Cacao Genetic Diversity

Dapeng Zhang and Lambert Motilal

**Abstract** Cacao (*Theobroma cacao* L.) is native to tropical South America, but as the unique source of cocoa butter and powder for the 200 billion USD global confectionery market, it is cultivated globally. Despite its economic importance, cocoa was, and continues to be, predominantly produced in low-input and low-output systems. Production constraints, including depletion of soil fertility on cacao farms, increasing damage due to diseases and pests, and expanding labor costs, limit cacao sustainability. Therefore, instead of increasing yields, the predominant contributing factor that keeps up with the rising demand for cocoa products has been expansion to new production regions. The future of the world's cocoa economy depends significantly upon using germplasm with a broad genetic base to breed new varieties with disease and pest resistance, desirable quality traits, and the ability to adapt to changing environments. Cacao differs from major field crops with regard to the untapped wild populations, which are still abundant in the Amazon region where they are coevolving with the pathogens. Moreover, in the absence of reproductive barriers, these wild populations could be readily crossed with cultivated crops. Yet only a very small fraction of the wild germplasm, mostly represented by a small number of clones in the so-called Pound collection, has been used for breeding since the 1940s. Contributions from this small set of clones have made tremendous impacts in disease resistance and adaptability. However, breeding efforts in the past 70 years have been reshuffling this small fraction of genetic diversity, with little addition of new variation. The on-farm genetic diversity in Southeast Asia and West Africa is low and cannot meet the challenge of the mounting pressure from diseases and pests. New breeding strategies are needed to combine more disease resistance genes/alleles from untapped wild germplasm and provide farmers with enhanced genetic diversity.

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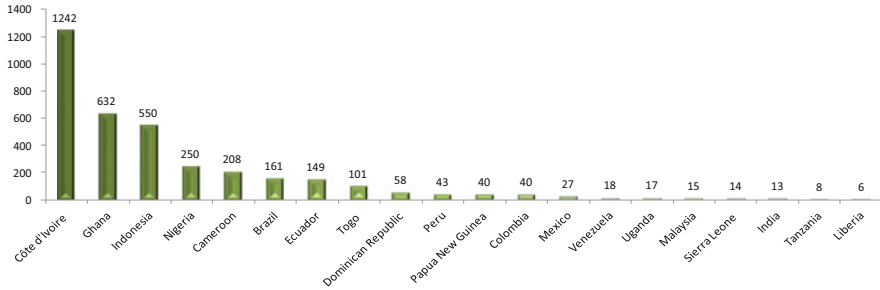


## 1.1 Introduction

*Theobroma cacao* L. is an important tropical rainforest tree, previously classified in the Sterculiaceae and presently recognized as a member of the family Malvaceae (Bayer and Kubitzki 2003) that originated in tropical South America (Bartley 2005; Cheesman 1944; Wood and Lass 2001). The tree is commonly known as cacao, while the term cocoa is reserved for the products made from the dried and fermented seeds. *T. cacao* encompasses many morphologically variable populations with a marked potential for inter- and intra-matings (Bartley 2005; Cheesman 1944). Although self-fertilization is possible in self-compatible plants, outcrossing is the predominant strategy.

Early use of the plant concentrated on the pulp and beans, and the former may have been the first factor that led to cacao farming. Archeological studies, in the Ulua Valley in Honduras, showed that the Olmecs fermented the sweet cacao pulp to make an alcoholic drink at least 3000 years ago, well before the practice of grinding the bitter seeds to produce a chocolate drink (Henderson et al. 2007; Powis et al. 2011). Since the cacao bean was additionally used for monetary, cultural, and political reasons, the tree was widely grown in Mesoamerica before the Spanish arrived (Bergmann 1969; Young 1994). However, the earliest people carried only a single strain of cacao out of the Amazon into Mesoamerica, where cacao was cultivated deliberately. Later on, the European colonists introduced a couple of other strains from the Amazon and transferred these traditional varieties to their newly won lands and colonies. Therefore, the cacao economy in the colonial time was based on a very narrow genetic background (Dias 2001a; Bartley 2005). On the other hand, little difference exists between cultigens and wild cacao in terms of their morphological characteristics and agronomic traits. It is still common today for the Amerindian to directly take native trees found in nature and adopt them as a crop, with little deliberate changes to their phenotypic features. Therefore, unlike many other domesticated field crops, cacao has the advantage that wild germplasm can be directly used in breeding or commercial production (Bartley 2005; Dias and Resende 2001; Eskes and Efron 2006).

The “wild relatives of cacao” include two types of germplasm. The first type is the large spectrum of wild populations that spontaneously grow in the Amazonian rainforest, from French Guiana to Bolivia. The second type of germplasm refers to the 22 related *Theobroma* species (Cuatrecasas 1964; Zhang et al. 2011), which have made negligible contribution to cacao improvement, due to interspecific crossing barriers. So far, conservation efforts have focused on the wild *T. cacao* populations. The main exception is *T. grandiflorum* (cupuassu), which is considered an important fruit crop in various Amazonian countries. Research studies on cupuassu have included breeding and germplasm collection (Alves, et al. 2007); germplasm characterization, interspecific hybridization, and product development (Silva et al. 2001); and phylogenetic studies (Silva et al. 2004; Silva and Figueira 2005).



**Fig. 1.1** Top 20 cacao-producing countries with a total output of 4,309,000 tons of cacao beans in 2010–2011 (ICCO, 2012). [http://www.icco.org/about-us/international-cocoa-agreements/cat\\_view/30-related-documents/45-statistics-other-statistics.html](http://www.icco.org/about-us/international-cocoa-agreements/cat_view/30-related-documents/45-statistics-other-statistics.html)

Today, cacao is cultivated extensively as the unique source of cocoa butter and powder for the confectionery industry. According to the World Cocoa Foundation (WCF), the production of cacao takes place mainly on small-scale farms in developing countries across Africa, Asia, and Latin America. The number of cacao farmers, worldwide, is 5–6 million, and the number of people who depend upon cacao for their livelihood is 40–50 million worldwide (World Cocoa Foundation 2012). The majority of cacao farmers employ a low-technology and low-finance approach, bordering on subsistence agriculture.

The annual worldwide production of cacao is estimated at 4.3 million tons for 2010–2011 (International Cocoa Organization 2012). During the past 100 years, there has been an average increase in demand of 3 % per year. The last 10 years have witnessed an increasing geographical concentration in cacao growing, with the African region now firmly established as the top supplier (Fig. 1.1). Increased demand has been met by expansion in production, mainly in the major West African cacao-producing countries. The demand for cacao is estimated to exceed supply with cocoa consumption increasing in emerging middle-income countries, including Brazil, China, Eastern Europe, India, Mexico, and Russia. Revenue derived from the sale and export of cacao provides crucial support to livelihoods of farmers and landholders throughout the tropics.

## 1.2 Agronomy of Cacao

### *Cultivation*

Cacao is cultivated within 20° of the equator (Toxopeus 1985) with the major producers having easy access to supplies of low-cost labor and forest land (Woods 2003). Irrigation is rarely applied, but may be undertaken in countries with a dry season (rainfall less than 100 mm/month) to prevent drought stress. Cacao can be

propagated from seeds, rooted cuttings, or grafted or budded plants. Many cacao farmers have seedling trees on their farms. Seeds can be obtained from open-pollinated or biparental crosses (sometimes called “hybrid” crosses) carried out in seed gardens or from their own farm or local community. Vegetative (or clone) propagation, by budding or grafting onto rootstocks or by cuttings, is increasingly practiced in Asia and Latin America (Maximova et al. 2005; Wood and Lass 2008). Large-scale vegetative propagation of planting materials by somatic embryogenesis is being undertaken in Indonesia and Côte d’Ivoire. Clones propagated by orthotropic rooted cuttings of somatic embryo-derived plants are not different from clones propagated by grafting in performance and bean quality traits (Goenaga et al. 2015).

In most countries, the average planting density is 1000–1200 trees ha<sup>-1</sup> with a minimum of 600 trees ha<sup>-1</sup>. In the high-yielding and high-input system in Malaysia, the planting density can reach 3333 trees ha<sup>-1</sup> and optimal planting densities are highly variable for different clones (Lockwood and Pang 1996).

Pruning increases the productivity of a tree because it can optimize the structure of tree canopy, improve photosynthesis activity, facilitate pollination of the flowers and strengthen the formation of new leaves and growth of the pods. Pruning is also an effective measure to control cacao diseases and insect pests. All dead branches and chupons (new branches that grow upward out of the trunk) need to be removed. As they grow, trees should be pruned to control both height (3–4 m is ideal) and shape of the tree, which expedites maintenance and harvesting (Wood and Lass 2008).

Phytosanitation is one of the most cost-effective method for reducing pests and disease for small-holder farmers. It refers to the removal and burial of diseased cacao pods, branches, leaves, and weeds. Field trials in Peru found that weekly removal of pods infected with black pod reduced incidence of the disease by 35–66 % and improved yield by 26–36 % (Soberanis et al. 1999).

Cacao farms can become significantly depleted of nutrients, due to the many years of low or no fertilization input (Baligar and Fageria 2014; Wood and Lass 2008). Soil nutrition deficits are a critical hindrance to cacao productivity in most areas. The current level of soil fertility on cacao farms in West Africa averages less than 10 % of what is necessary for productive crops and soil (Cocoa Fertilizer Initiative, <http://www.idhsustainabletrade.com/Fertilizer>). Cacao responds well to fertilization, especially on farms where harvested or pruned plant material is not left in the field to decompose (Baligar and Fageria 2014). For each 1000 kg of dry beans harvested, about 20 kg N, 4 kg P, and 10 kg K is removed from the soil. If pod husks are also removed from the field, the amount of K removed increases to about 50 kg (Puentes-Páramo et al. 2014). About 200 kg N, 25 kg P, 300 kg K, and 140 kg Ca are needed per hectare to grow the trees prior to pod production (Moriarty et al. 2014). Fertilization is also believed to extend the productive life of trees. It is estimated that fertilizer alone may be sufficient to increase yield by 500 kg to 1 ton/ha (Moriarty et al. 2014). Nonetheless, few smallholders use agrochemicals because they lack the funds to purchase them at the time they need to be applied (Cocoa Fertilizer Initiative, <http://www.idhsustainabletrade.com/Fertilizer>).

## ***Fruit and Harvest***

Cacao trees require approximately 3–5 years to bear their first fruits, commonly known as “pods,” and can remain productive for several decades. The cacao flowers develop as compact inflorescences, directly on the woody tissue of suitable physiological age, throughout the trunk and canopy of the tree, and are pollinated by small flying insects. The pods take approximately 5–6 months to develop and mature, and once ripe, each consists of a thick husk enclosing some 40–50 seeds that are surrounded by a semisweet acidic pulp. The average yield is 450 kg ha<sup>-1</sup> (Food and Agricultural Organization 2014), but yields of up to 3000 kg ha<sup>-1</sup> are possible under good agricultural practices that combine management practices, pest control, improved plant material, and appropriate fertilizer application (Maharaj et al. 2005; Pang 2006). Low farm gate prices, lack of access to farming inputs/fertilizers, and finance are the main barriers to high-yielding cacao production. In many cases, farmers have limited knowledge of improved production techniques and farm management skills. The participation of new or traditional farmers who use suboptimal farming practices also contributes to poor production and a low-grade product.

Harvesting pods from cacao trees is labor-intensive and occurs within a short season. Harvesting varies by area and climatic conditions with the first harvest typically falling between April and June and a second harvest around October. Harvested pods are cracked open by hand, and the pulp and seeds are manually separated from the husks. The bulked cacao seed mass is generally fermented in simple heaps covered by banana leaves, resulting in variable cacao bean quality. However, a better practice is fermentation in wooden boxes fitted with drainage holes. This is increasingly present in central facilities, cooperatives, or on large farms. The fermentation period is variable and depends primarily on the type of cacao, generally taking 3–8 days. Cacao with high Criollo ancestry typically has a shorter fermentation period than Forastero or Trinitario cacao. A critical mass of fermenting beans is required to achieve the temperatures necessary for ideal fermentation. After fermentation, the beans are commonly sun-dried to reduce the moisture content, ideally to 7.5 %. The fermentation process initiates the formation of flavor precursors which are only fully developed following drying and roasting. The dried cacao beans are usually bagged on farm and transported to the ports for export or local processors. Before making cocoa and chocolate products, the beans are roasted, usually by the manufacturer, to develop the final chocolate flavor. Then the shells are removed from the roasted beans and the cocoa nibs are treated with alkalizing agent (usually potassium carbonate), to modify the flavor and color. The nibs are then further milled to create cocoa liquor, which is used to make chocolate paste, cocoa, cocoa butter, and chocolate (International Cocoa Organization 2013).

### 1.3 History of Cacao

#### *Ancient Cacao Agriculture and Traditional Variety Names*

Cultivation of cacao started in Mesoamerica, where cultural elaboration and use of cacao can be traced back more than 3000 years (Gómez-Pompa et al. 1990; Henderson et al. 2007; Powis et al. 2011). The ancient cultigens that were deliberately planted and utilized by Amerindian civilizations including the Mayas (300–900 AD) and the Olmecs (400–1200 BC) (Henderson et al. 2007) became known as Criollo cacao (“Creole” in Spanish). Cacao depictions in Mayan artifacts provide supporting evidence for the deliberate planting by early peoples in Costa Rica, Belize, El Salvador, Guatemala, Honduras, Mexico, and Nicaragua (Wood 1985a; Coe and Coe 1996; Dias 2001a).

“Criollo” is frequently used in contrast to the later introductions called “Forastero” in the literature. Preuss (1901) and Bartley (2005) indicated that the word Criollo means native or first variety cultivated outside the indigenous range of the species, thus distinguishing it from later introductions, whereas Forastero or Forestero means “of the forest” or foreign, i.e., not among the first cultivated or indigenous variety of a region. The names applied to local variations in the Criollo group are often of a descriptive nature, usually referring to fruit characters, including Porcelana, Pentagona, Angoleta, and Cundeamor. In spite of the morphological variations, Criollo cacao is self-compatible and is nearly fully homozygous as revealed by SSR and SNP markers (Motilal et al. 2010; Ji et al. 2013).

In the Amazonian rainforest, the evidence of cacao cultivation by different indigenous groups has been minimal (Bartley 2005; Dias 2001a; Sánchez et al. 1989). It was suggested that Amazonian tribes might not have had the need to formally cultivate a tree that occurred in abundance (Bartley 2005; Dias 2001a). Clement (1999), based on the ease with which cacao survives in abandoned humid forest ecosystems, classified cacao as a crop with semidomesticated populations. Furthermore, cacao was probably not grown in the Caribbean islands during pre-Columbian times (Wood 1985a).

In addition to Criollo, the Nacional and Amelonado groups were classed in the category “traditional cultivars,” which was interpreted to represent some degree of domestication (Clement et al. 2010). These authors indicated that the results of Motamayor et al. (2008) show that Criollo and Nacional cacaos group together as an Ecuadorian assemblage of western Amazonia, whereas Amelonado groups with French Guiana cacao, indicating a possible eastern Amazonian origin. Bartley (2005) opined that it was likely that the Nacional cacao of Ecuador existed for several centuries prior to the arrival of the Europeans. However, Loor Solorzano et al. (2012) suggested that *T. cacao* and its products were part of the pre-Columbian culture around 2000 BC, a controversy that was not resolved, although the center of origin of Nacional cacao was suggested, based on microsatellite evidence, to be in the southern Amazonian area of Ecuador. Patiño (2002 as cited in Clement et al. 2010) argued that the Amelonado cacao in eastern Pará of Brazil was from ancient cultivation.

Similarly, Barrau (1979 as cited in Clement et al. 2010) suggested, based on ethnographic observations, that cacao had long been cultivated in French Guiana by the native peoples. Drawing on the opposite extremes of cacao distribution in the Americas for Criollo and Amelonado, and the low number of private alleles, Clement et al. (2010) reasoned that Amelonado cacao should be considered to be at least incipiently domesticated in eastern Amazonia.

Amelonado cacao, so called because of the fruit shape (Spanish for “melon shaped”), is another ancient variety. Based on the pod characteristics, this variety has been called Indio, Amelonado, Calabacillo, Matina, Común, Catongo, and Pará (Bartley 2005; van Hall 1932). The Amelonado cacao is more widespread than Criollo in Mesoamerica and the Caribbean, has better adaptability than the ancient Criollo cacao, and, thus, is either replaced or hybridized with Criollo in many places. The earliest time period for cultivating Amelonado (Lower Amazon Forastero cacao) in Mesoamerica and the Caribbean is not clear.

## *Colonial History*

The catastrophes (Table 1.1) of cacao production in the last four centuries, often caused by diseases and pests, were the main force driving cacao dispersal and the shift of production centers. Severe cacao disasters, generally known as blasts or blights, occurred in the early colonial plantations in Latin America and the Caribbean (Motilal and Sreenivasan 2012 and references therein). Historical records have shown that the “blasts” that occurred in several Caribbean countries had different origins (Motilal and Sreenivasan 2012). Cacao production in Martinique experienced severe disasters in 1671 and 1727 (Quesnel 1967), which decimated entire plantations and almost ruined the cacao industry of the island (Kimber 1988). However, the Amelonado cacao that survived was likely transferred to surrounding islands, which had undergone similar devastation events. Direct introduction from South America into cacao plantations of the Caribbean islands also occurred (Preuss 1901; van Hall 1932; Bartley 2005).

During the Spanish colonial rule, Trinidad cacao planters grew mostly Criollo cacao. Prior to, and even more so after, the 1725 destruction of the majority of the cacao crop by a trifecta of climatic, agronomic, and genetic causes (Motilal and Sreenivasan 2012), Forastero material was introduced, most likely from Brazil (Shephard 1932; Joseph [1838] 1970), Hispaniola (Bartley 2005), and Venezuela (Bartley 2005). Several resultant natural hybridization events (Motilal et al. 2010; Motilal and Sreenivasan 2012; Yang et al. 2013) led to the Trinitario germplasm, which is noted for its fine flavor (Toxopeus 1985). Heterosis (hybrid vigor) resulted in vigorous planting material, which was then reintroduced to Venezuela. The term Trinitario probably accompanied the transferred germplasm and has since been used to describe these cacao types, arising as products of hybridization and recombination through various generations, which are now known in the trade for their floral/fruity flavors. Much later, at the then Imperial College of Tropical Agriculture

**Table 1.1** Catastrophic cacao events caused by diseases and pests

Country	Period	Attributed cause	Decline	Reference
Venezuela	Mid-1630s	Blight "alhorra"	>50 % trees lost	Ferry (1989)
Cuba, Hispaniola, Jamaica	1660s–1670s	Drought, "blasts of unknown origin," "blasting," heat, and drought	General failure—all trees	Joseph (1838 1970), Historicus (1896), Johnson (1912)
Santo Domingo in Hispaniola	1715	Malicious destruction by residents of Martinique	100 % trees lost	Shepherd (1831 1971), Southey (1827 1968)
Martinique	1718	Wind, hurricane	100 % trees lost	Shepherd (1831 1971)
Trinidad	1725	Trifecta of climatic, agronomic, and genetic causes	General total failure	Motilal and Sreenivasan (2012)
West Indian islands from Trinidad to Jamaica	1727	"Blasts" of unknown origin [these were probably due to the same trifecta of causes given above and reported in Motilal and Sreenivasan (2012)]	General total failure	Bartley (2005), Quesnel (1967)
Martinique	1727–1732	Two "plagues": root fungus, leaf-eating caterpillar	~95 % trees lost	Revert (1949) cited in Kimber (1988)
Ecuador	1920s	Witches' broom disease and frosty pod	60–70 % drop in production; reduced numbers of Nacional trees	Wood (1985b)
East Java	1936	Cocoa pod borer	All trees removed	Wood (1985b)
Ghana	1930s–1980s	Cocoa swollen shoot disease	185.5 million trees removed; 31 million trees still affected	Lass (1985) and references therein
Valle, Cauca (Colombia)	1950s	Ceratocystis wilt	>50 % trees destroyed	Barros (1981)
East coast of Peninsular Malaysia	Late 1950s–1970s	Vascular-streak dieback	Badly affected; 30–50 % disease	Lass (1985), Chan and Syed (1976)
Togo	1955–1977	Cocoa swollen shoot disease	3.5 million trees destroyed	Lass (1985) and references therein
Fiji	1969	Phytophthora canker	45 % of trees in one estate	Firman and Vernon (1970)
Bahia (Brazil)	1970	Phytophthora canker	Significant loss of trees	Rocha and Ram (1971)
Bahia, Espirito Santo (Brazil)	1979	Phytophthora canker	Eight million trees affected	Pereira et al. (1980)
Brazil	1989–1999	Witches' broom disease	70 % drop in production	

in Trinidad, an extensive survey of the Trinitario population was conducted, resulting in the selection of approximately 100 Imperial College Selections (ICS) clones, which were selected principally for yield characteristics (Pound 1932, 1934).

### ***Ancestry of Trinitario***

The genetic composition of Trinitario cacao has been further dissected to clarify whether their ancestry includes only Criollo and Amelonado (Johnson et al. 2009; Motilal et al. 2010; Yang et al. 2013). Recent SSR analysis showed that the genesis of Trinitario cacao was when a limited population of Criollo × Forastero hybrids emanated from the introduced Forastero population of Trinidad (Motilal et al. 2010). The multi-lineage origin of modern Trinitario is also supported by analysis based on plastidic single nucleotide polymorphisms (cpSNPs) and polymorphic simple sequence repeats in plastids (cpSSRs) (Yang et al. 2013). Three cpSNP haplotypes were revealed in the Trinitario cultivars sampled in Trinidad, each highly distinctive and corresponding to reference genotypes for the Criollo, Upper Amazon Forastero, and Lower Amazon Forastero varietal groups. These three cpSNP haplotypes likely represent the founding lineages of cacao in Trinidad and Tobago. The cpSSRs were more variable with eight haplotypes, but these clustered into three groups corresponding to the three cpSNP haplotypes. The most common haplotype found in farms of Trinidad and Tobago was Amelonado, followed by Upper Amazon Forastero and then Criollo. The authors concluded that the Trinitario cultivar group was of complex hybrid origin and was derived from at least three original introduction events.

### ***Out of America: Dispersal of Cacao to the Old World***

In spite of the complex requirement of planting materials, environmental factors, and management practices, cacao was, and continues to be, dominantly produced in low-input, low-output, and high-risk systems. Sustainability is limited by many factors, which constrain production, including the depletion of soil fertility on farms and the legions of pests causing damage and diseases. Cacao agriculture provides a prime example of the continued confrontation of crop production with new and recurrent epidemics (Table 1.1). As the center of cacao production shifted from place to place, a small fraction of the cultigens were transported to the new production sites. Genetic diversity represented in these cultigens is actually a tiny fraction of available genetic diversity in the primary gene pool of cacao in South America. The low level of genetic diversity in cacao farms could not meet the challenge of mounting pressure of diseases and pests, and expansion to new



production regions has been essential to keep up with the world demand for cocoa products.

The first contact that Europeans had with the crop was attributed [by Oviedo y Valdez (1855) as cited in Bartley (2005)] to Alonso Pinzón in 1510 in southern Yucatan. In the sixteenth century, the Europeans started to cultivate cacao in Asia and Africa where the Criollo, Amelonado, and the Trinitario hybrids started their route of dispersal from the Americas to the old world.

The first shipment of cacao germplasm to Southeast Asia was recorded in 1560, when the Dutch introduced cacao that was believed to be the fine flavor variety “Venezuelan Criollo” into Celebes, Indonesia (van Hall 1932). Cacao production started in northern Sulawesi where cacao was processed and consumed only locally (van Hall 1932). Another introduction to this region in 1670, believed to be a Criollo variety from Mexico, was via the Acapulco-Manila galleons (Bartley 2005). Around 1770, the Dutch introduced cacao to Peninsular Malaysia (Thong et al. 1992), and fruiting cacao was subsequently found in Malacca [Koenig (1894) as cited in Thong et al. (1992)]. In 1798, the British took cacao to Madras, India, from the island of Amboina, and it was introduced into Ceylon (now Sri Lanka) from Trinidad at about the same time (Ratnam 1961; Wood 1991). From Ceylon, cacao was subsequently transferred to Singapore and Fiji (1880), Samoa (1883), Queensland (1886), and Bombay and Zanzibar (1887). Cacao was also grown in Malaysia as early as in 1778 and in Hawaii by 1831 (Bartley 2005). Remnants of the ancient Criollo, Amelonado, and Trinitario populations can still be found in Asia and Pacific regions, such as Indonesia (Susilo et al. 2011), South Pacific (Fiji and Samoa), and Madagascar (Zhang et al., unpublished data).

With the establishment of chocolate manufacturing in Europe in the second half of the eighteenth century and the increase in chocolate consumption in North America, there was an explosion in demand, requiring yet more cacao to be produced. Commercial cultivation started in Africa after the Portuguese introduced Amelonado cacao into Principe in 1822. By the 1850s, cultivation of cacao spread to the main island of Sao Tome, where the Amelonado cacao (“Común” in Bahia, Brazil) became known as Sao Tome “Creoulo.” This self-compatible variety was then brought by Spaniards into the island of Fernando Po (now Bioko), Equatorial Guinea, and repeatedly introduced into the mainland West Africa (Bartley 2005). The limited genetic diversity within the initial foundation of West Africa Amelonado was also mentioned by van Hall (1932). During the late nineteenth century, the colonial administration also introduced some red-pod cacao materials from British West Indies into botanical gardens established in Aburi (Ghana) and Lagos (Nigeria) (Toxopeus 1964). Consequently, the bulk of cacao grown on farmers’ plantation must have consisted of a mixture of these earlier varieties, with the self-compatible “West African Amelonado” type dominating the production at the beginning of the twentieth century.

The shift in the world’s center of cacao production followed a boom-and-bust pattern, from Mesoamerica to Venezuela, from Venezuela to Ecuador, from Ecuador to Brazil, and from Brazil to West Africa (Ruf and Schroth 2004). As new

countries/regions adopted the crop, the previous production centers collapsed. Production shifts from one country to the next were reproduced by similar cycles on a subnational scale (Ruf and Schroth 2004). Among the many factors contributing to this boom-and-bust cycle, the impact of biotic constraints, due to the limited on-farm genetic diversity, apparently played a key role. Subsequently, it was only a matter of time before coevolved fungal pathogens moved—naturally or human assisted—from their forest hosts into the cacao plantations. The various catastrophic “blasts” that occurred in the last 400–500 years suggest that disease was the main force that drove cacao dispersal and the shifting of production centers. Therefore, a brief review of the cacao primary gene pool is essential for improving our understanding about future sustainability of cacao production.

## 1.4 Upper Amazon: Cacao’s Primary Gene Pool

The term “Upper Amazon” has been used to describe the location of most of the known wild cacao populations from the “Alto Amazonas,” a region from the start of the Marañón River in Peru to the frontier of Brazil. In this region, a series of major river systems in Peru, Ecuador, Colombia, and Brazil flow into the Marañón and Amazon rivers. Wild cacao populations are found in these river basins in both spontaneous (without human interference) and subspontaneous forms (wild cacao trees exploited by man) prior to European occupation (Almeida 2001; Bartley 2005). Wild cacao germplasm samples from the expeditions in the Amazon were predominantly collected along the banks of navigable rivers (Pound 1938; Lachenaud and Sallée 1993; Lachenaud et al. 1997; Almeida 2001). Each natural cacao population has a narrow genetic base and is thought to have been founded by a limited amount of reproductive materials (Pound 1945; Bartley 2005).

Genetic diversity of natural cacao populations is generally stratified by the major river systems in the Amazon (Pound 1938; Almeida 2001; Bartley 2005). Within each river basin, wild cacao is usually grouped in patches and separated by large spatial distances between patches. It is hypothesized that gene flow in cacao is limited and mating is likely confined within patches (Chapman and Soria 1983), due to the short distance of seed dispersal by rodents and monkeys and short-distance pollen dispersal by insects, including midge species (*Forcipomyia* spp.) as well as other insect vectors.

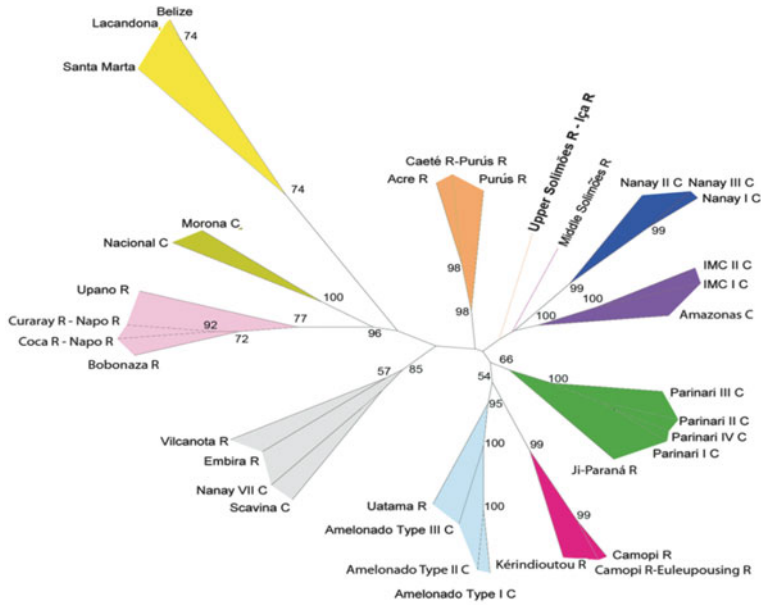
A significant departure from the Hardy-Weinberg equilibrium (HWE) was detected in the French Guiana wild populations (Lachenaud and Zhang 2008). In addition to the likely short-distance gene dispersal, cacao has a gameto-sporophytic self-incompatibility system (Cope 1962), which works in a quantitative manner (Lanaud et al. 1987). Self-compatibility in some genotypes is partially responsible for the high fixation index in several natural populations. Indeed, fully homozygous

genotypes were frequently found in the populations from French Guiana (Lachenaud and Zhang 2008). Some wild cacao trees are found in the form of single plants, but the majority will form a “clump” (several trunks at different development stages and overlapping generations at one growing site). This apparent generation overlap within a patch is another likely factor contributing to mating between relatives, thus increasing the level of inbreeding. The multiple trunks can also come from self-propagation by chupon production, which increases the chance of inbreeding by self-mating as opposed to inbreeding by mating between family members.

Despite the commonly perceived short-distance gene flow and limitation in effective population size in wild cacao, isolation by distance was detected only over a long geographical range (e.g., a few hundred kilometers) and not in a local basin or short distance (Zhang, et al. 2006). Sereno et al. (2006) reported that in the natural or seminatural populations sampled in four regions of Brazil (Acre, Rondonia, Lower Amazon, and Upper Amazon), most of the genetic diversity was allocated within populations rather than between populations, indicating a typically high gene flow. Therefore, some of the apparently isolated populations may actually belong to the same metapopulation in terms of gene dispersal, which impacted their genetic differentiation. A study on the cacao mating system at the hierarchical levels of fruits and individuals showed that the cacao population was spatially aggregated, with significant spatial genetic structure up to 15 m. Mating was correlated within, rather than among, the fruits, suggesting that a small number of pollen donors fertilized each fruit (Silva et al. 2011). A similar study in the northeast lowlands of Bolivia revealed 7–14 % self-pollination in wild cacao populations. Cacao pollen was transported up to 3 km, with an average of 922 m, suggesting pollination distances larger than those typically reported in tropical understory tree species (de Schawe et al. 2013).

Using SSR markers, Motamayor et al. (2008) genotyped 1241 cacao accessions existing in most of the *ex situ* germplasm collections in Latin America. The result led to the identification of 10 genetic clusters in the Forastero cacao, which was proposed as a new classification of the cacao germplasm (Fig. 1.2).

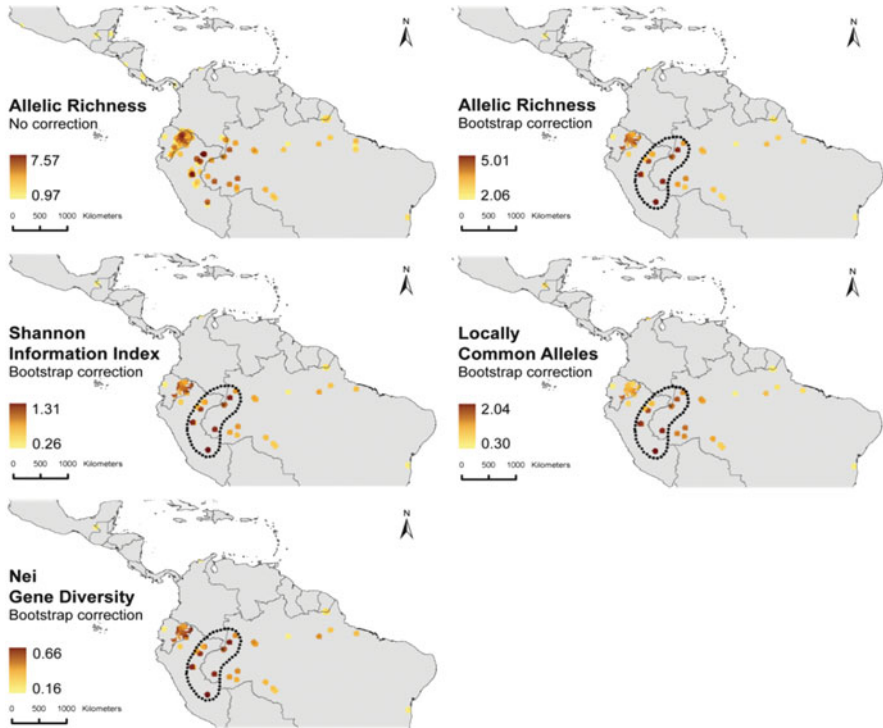
Thomas et al. (2012), using the same SSR data set, reanalyzed the spatial pattern of intraspecific diversity of cacao in Latin America. Grid-based calculations of allelic richness, Shannon diversity, and Nei’s gene diversity and spatial cluster analysis suggested the highest levels of genetic diversity were observed in the Upper Amazon areas from southern Peru to the Ecuadorian Amazon and the border areas between Colombia, Peru, and Brazil (Fig. 1.3). Simulation modeling suggests that cacao was already widely distributed in the western Amazon before the onset of glaciations. During glaciations, cacao populations were likely to have been restricted to several refugia where they presumably underwent genetic differentiation, resulting in a number of genetic clusters which are representative of, or closely related to, the original wild cacao populations. The analyses also suggested that genetic differentiation and geographical distribution of a number of other clusters seem to have been significantly affected by processes of human management and accompanying genetic bottlenecks.



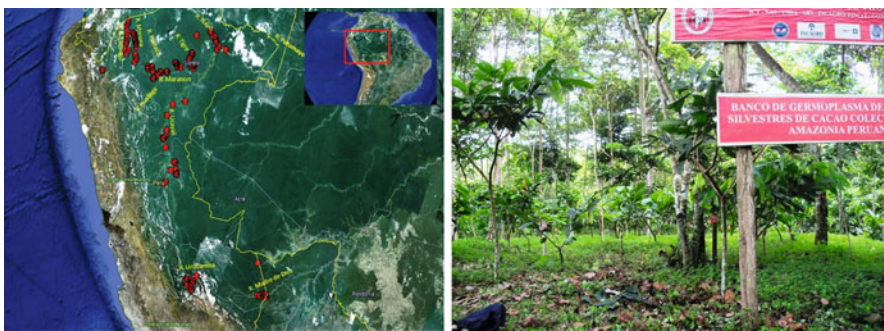
Colors indicate the inferred genetic cluster to which the subcluster belongs: Marañon (●), Guiana (●), Contanama (●), Curaray (●), Nanay (●), Iquitos (●), Nacional (●), Purús (●), Criollo (●), and Amelonado (●), (C=Clones; R=River).

**Fig. 1.2** Neighbor joining tree from Cavalli-Sforza and Edwards genetic distance [16] matrix among the 36 subclusters identified using structure (559 clones). Motamayor, et al. (2008) Geographic and Genetic Population Differentiation of the Amazonian Chocolate Tree (*Theobroma cacao* L). PLoS One 3(10): e3311. doi:10.1371/journal.pone.0003311

Since 2008, a series of collecting expeditions have been launched to survey the full cacao genetic diversity in the Peruvian Amazon (Fig. 1.4a). The expeditions were aimed at areas lacking representation in the ex situ cacao germplasm collections. The first geographical focus was the major tributaries of Rio Marañon, including Rio Santiago, Rio Pastaza, Rio Nucuray, Rio Urituyacu, Rio Tigre, Rio Ucayali, Rio Madre de Dios, and Rio Putumayo. Within each subbasin, the identification of collecting sites was assisted by GPS mapping tools. Habitat descriptions were examined and the target area was chosen based on the potential for complementary diversity. The expeditions were supported by the Peruvian government and by the USDA. To date, a total of 520 wild trees have been collected, representing 19 river basins. Preliminary characterization using SNP markers showed a significant amount of diversity complementing the existing national and international ex situ collections. The living trees were propagated in the facilities of Tropical Crop Institute (ICT—Spanish acronym) in Tarapoto, Peru (Fig. 1.4b). These trees are currently being evaluated for agronomic traits with the emphasis on resistance to diseases and bean quality and flavor. Next-generation sequencing (NGS) genotyping of these trees is being planned, together with all the wild trees



**Fig. 1.3** Spatial variation of different genetic parameters, represented at a resolution of ten-minute grid cells and a circular neighborhood of 1 degree. Highest values are consistently observed in the extensive bean-shaped Amazonian area covering both the Peruvian-Brazilian border and the southern part of the Colombian-Brazilian border, as well as Amazonian Ecuador. Thomas et al. (2012). doi:[10.1371/journal.pone.0047676.g001](https://doi.org/10.1371/journal.pone.0047676.g001)



**Fig. 1.4** (a) Ongoing new collection expeditions in Peruvian Amazon since 2008. Each red dot represents a collecting site. (b) Field genebank of the ICT wild cacao collection at Tarapoto, Peru

from the International Cocoa Genebank, Trinidad (ICG, T), and other major ex situ collections, to provide a comprehensive overview of the diversity distribution in the cacao primary gene pool.

## 1.5 The “Pound Collection”

### *Collection from Wild Cacao Germplasm*

The outbreak of witches’ broom disease (WBD) in Trinidad in the late 1920s led to the search for genetic resistance in the Upper Amazonian region. During the 1930s–1940s, wild germplasm was collected from the Upper Amazon basin of Ecuador and Peru (Pound 1938, 1945; Wood and Lass 2008). To date, the germplasm that has made the most fundamental contribution to the modern cacao breeding programs is, by far, the Pound collection (named after the collector F. J. Pound). This collection results from the first cacao germplasm collecting expedition into the Upper Amazon, and the collecting sites included part of the tributaries of Rio Ucayali, Rio Morona, and Rio Marañón (Pound 1938, 1945; Bartley 2005; Zhang et al. 2009; Fig. 1.5).

This led to the establishment of the “Pound collection” in Iquitos, Peru. Pound’s expeditions were aimed at searching for genotypes resistant to WBD, caused by the fungus *Moniliophthora perniciosa* (Stahel) (Aime and Phillips-Mora 2005), after the outbreak of witches’ broom disease (WBD) in Trinidad in the late 1920s. These germplasm accessions have henceforth served as the foundation for breeding programs around the world for resistance to WBD. The Pound collection was primarily comprised of five germplasm groups: “Iquitos Mixed Calabacillo” (IMC), “Morona” (MO), “Nanay” (NA), “Parinari” (PA), and “Scavina” (SCA) (Pound 1938, 1943, 1945). An unknown number of pods (fruits) were collected from trees without any symptoms of WBD. The 25 half-sib families yielded 250 fruits (Lockwood and End 1993; Motilal and Butler 2003). The seeds were then bulked and sent to Barbados where approximately 2500 seedlings were raised (Toxopeus and Kennedy 1984). These germplasms were transferred to Trinidad in the form of bud wood and vegetatively propagated onto rootstock at Marper Estate in Plum Mitan, Manzanilla, Trinidad, as 486 accessions (Motilal and Butler 2003). In addition to the 25 half-sib families from the five accessions groups, Pound’s collection also includes 32 clones, which Pound collected in 1943 when he revisited the same sites where the NA, IMC, and SCA were previously collected. These accessions were collected as bud woods and were referred as “Pound clones” or “P clones.”

In addition to these five groups collected from Peru from 1938 to 1943, Pound also collected 80 half-sib families from western Ecuador. The 80 half-sib families yielded 1185 Ecuadorian Refractario that are in the ICG, T (Fig. 1.6a, b) and were the result of the first expedition (Lockwood and End 1993).

Among the 80 or so different germplasm groups held in the ICG, T, those in the Pound collection are among the most widely distributed germplasm, due to their valuable agronomic traits and their potential for resistance to WBD (Lockwood and End 1993; International Cacao Germplasm Database, <http://www.icgd.reading.ac.uk/>) (Table 1.2). In many cacao-producing regions around the world, the Pound selections of Upper Amazonian cacao are either adopted directly as clones or used