Cacao, Chocolate and Conservation
A Kinship Garden for the Genus Theobroma
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Along the banks of the world's largest river and its tributaries grow the few living relics representative of another age in the biology of this earth. Before the forests were cut, reduced and eliminated, a well-defined yet raucous medley of living organisms had survived billions of years of uninterrupted growth and differentiation leading to an amazing and conceptually overwhelming abundance of kinds of living organisms. The density of the diversity in some ways reflects the success of evolution, in providing a maximum of variation given a changing environment and the continual reality of the unknown and the unexpected.

Shrubby many-stemmed trees 3-5 times the height of a person with fragrant flowers coming out of their stems and trunks with a peculiar fan-like branching pattern and elongated softball- sized pods filled with almond like seeds embedded in a sweet limey-white juicy pulp could be seen in some of the seasonally flooded banks along the river whose mouth is more than 300 miles across. Aeons of people came to the forest, some found out that the fermented seeds that came from the *cacao* trees, made a delicious drink when mixed with the sweet fruit juices. The mild euphoria conveyed by the drink became legendary. Seeds from the most productive, vigorous and tasty-seeded trees were carried to other areas in the tropics. As the trees spread, they found themselves neighbors to relatives with whom they were reproductively compatible. Cross-pollination by biting midges that propagate in the decaying fruit gave rise to new kinds. The best became popular, established in plantations worldwide in the warmer, wetter zones of the world. Intensive demand for the growth promoting substances in the seeds of these tropical trees led to over demands on production, giving rise to short- cuts in agricultural production which involved using synthetic fertilizers and chemically toxic pesticides leading to disease and productivity problems.

Meanwhile, the forests where the relatives of the Theobroma trees, the forests where the oldest of the original "food of the gods" trees lived are being cut down for cattle graze and timber. All our relations that had survived meteor crashes and cascades of volcanoes are disappearing in a several human generational blink of an eye.

Unless we decide to conserve diversity, provide enduring habitats and develop the cultural methods and traditions that make sustainable organic agriculture a goal of our society during the years to come, the Theobroma trees and their unique and special products will, like the forests that birthed them, become relics and memories.

The era of classic morphological taxonomy finds that the taxonomic systems of the leading botanical scientists of our times, including Cronquist, Takhtajan, Dahlgren, and Thorne, all put the Sterculiaceae in the Order Malvales (Brummitt 1992 pg 673, Cuerrier et al 1998). "Malvales are closely related to

Cistales, with many features in common with both Flacourtiaceae and the Elaeocarpaceae as well as with archaic members of the Theales." (Takhtajan 1997). In Dahlgren's final rendition of the dicots, he surrounds the Sterculiaceae with the Bixaceae, Dipterocarpaceae, Cistaceae, Tiliaceae and Plagiopteraceae (Dahlgren 1989). The era of plant taxonomy which emphasizes the flowers gained strength and recognition from the work of Linneaus in the 1700's. The pre-eminent botanists following Linneaus all used the flower morphology system as the basic organizing principle for deducing a tree of life for the plants.

Some of the traits used to define the Sterculiaceae include: anthers tetrasporangiate and 2-locular. Epicalyx only sometimes present. Style not gynobasic. Stamens connate in a tube around ovary, basically in 2-cycles. Flowers bisexual, some- times polygamous, actinomorphic. Leaves are alternate, from simple to palmately compound . With a group of 10-12 other traits Takhtajan defines the Sterculiaceae as a family. The Sterculiaceae is closely related to Tiliaceae, Bombacaceae, and the Malvaceae.

In the current era of molecular biological taxonomy, the Sterculiaceae, along with the Bombacaceae, Tiliaceae and Cistaceae all become subfamilies as part of the expanded concept of the Order Malvales (Bayer et al 1999). The Order Malvales is now seen to be part of the eudicots, specifically in the rosids, and in particular, part of the eurosid II alliance which includes the Brassicales, Sapindales and Myrtales. (Angiosperm Phylogeny Group 1998, Soltis et al. 1999, Qiu et al. 1999). Rearrangement of the relationships of genera, tribes, families and orders is taking place as new information based on amino acid sequences of proteins and DNA nucleotide sequences of plastid and nuclear genes becomes known. While some taxa are found to have truly novel kinship relationships, most of the new molecular science is consistent with the older, well-established patterns developed from plant and flower structure, small molecule biochemistry and ecology. However, some aspects of previously obscure relationships are being clarified by the deep taxonomy of informational macromolecules. It is this new information that is uncovering some of the lost biological history of Theobroma.

Although most of the members of the genus Theobroma are found in the Amazonian tropics, three species are well represented in Mexico and meso-America. These are *Theobroma bicol or, T. angustifolia* and *T.cacao*. In fact, the chocolate trees of the Maya, carefully cultivated for millennia in cenotes in the desert country of the Yucatan peninsula, are unique and distinct from all other cultivars. The architecture of the Mayan chocolate trees, the structure of the pods and of young seedlings makes it possible if it is likely that selection for chocolate trees occurred in at least two locales, southern Mexico and Amazonia (H.Y. Shapiro, personal communication. Gomez-Pompa et al 1990). In support of this concept is the absence of wild species of *T.cacao* in Amazonia and the discovery of cultivars of cacao in the Yucatan with small pods having 10 sutural groves. While commercial T. cacao cultivars have large pods with 5 sutural groves, the finding of potential wild ancestors in southern Mexico makes it likely that horticultural selection was done in this bioregion as well as Amazonia thousands of years ago.

Takhtajan's (1997) taxonomy for Theobroma, the genus that includes *Theobroma cacao,* the Chocolate tree is as follows:

Order Malvales 282-301genera / 4597- 4697+species (12 families): Families: Tiliaceae 46- 50/450+, Dirachmaceae 1/2, Monotaceae 4/40, Dipterocarpaceae 13/700, Sarcolaenaceae 10/62,
Plagiopteraceae 1/ 2, Huaceae 2/3, Sterculiaceae 62-69 I1500, Diegodendraceae 1/1, Sphaerosep- alaceae 2/17, Bombacaceae 30/320, Malvaceae 110-120/1500-1600.

Family Sterculiaceae 66/1575 (2 subfamilies):

Subfamily Byttnerioideae (9 tribes, 47/1112):

1. Lasiopetaleae 10/91+-Hannafordia 3, Leptonychiopsis 1+, Trichostephania 1, Thomasia 31, Seringia 1, Keraudrenia 9, Maxwellia 1, Lysiosepalum 2, Lasiopetalum 37, Guichenotia 5
2. Hermannieae 4/ 222-Hermannia 100, Melochia 54, Dicarpidium 1, Waltheria 67
3. Helmiosideae 3/ 21-Helmiopsiella 1, Helmiopsis 3, Nesogordonia 17
4. Byttnerieae 4/229-Rulingia 20, Commersonia 10, Byttneria 131, Ayenia 68
5. Theobromeae 7/109-Glossostemon 1, Scaphopetalum 15, Leptonychia 45, Abroma 2, Theobroma 22, Herrania 20, Guazuma 4
6. Helictereteae 6/ 71-72-Pterospermum 25, Helicteres 40, Neoregnellia 1, Kleinhovia 1, Reevesia 3-4, Ungeria 1
7. Fremontodendreae 2/3+- Fremontodendron 2, Chiranthodendron 1+
8. Eriolaeneae 1/17-Eriolaena 17
9. Dombeyeae 10/348-Ruizia 3,Astiria 1, Cheirolaena 7, Trochetia 6, Dombeya 200, Paradombeya 5, Harmsia 3, Melhania 60, Paramelhania 1, Pentapetes 1

Subfamily Sterculioideae (4 tribes, 13/454):

1. Sterculieae 9I408-Sterculia 200, Brachychiton 30, Firmiana 9, Scaphium 6, Pterocymbium 15,Pterygota 15,Acropogon 3, Octolobus 5, Cola 125
2. Tarrietieae 1I30-Heritiera 30 3. Mansonieae 2/13-Mansonia 5, Hildegardia 8
3. Triplochitoneae 1/3-Triplochiton 3 unassigned to tribe: Aethiocarpa l+, Cotylonychia 1,Franciscodendron 1+,Gilesia 1 =Hermannia, Megatritheca 2, Pimia 1, Raylea 1.

In the Bayer et. al. (1999) revision of the Malvales, the tribe Theobromeae includes the genera Theobroma, Herrania, Guazuma, Abroma, Scaphopetalum, Kleinhovia and Leptonychia based on cucullated petals. "The androecium includes staminodia which are usually conspicuous and alternisepalous bundles of two or more stamens that are produced through secondary increase of primordia." Of the seven genera, Theobroma, Guazuma and Herrania are neotropical. The others are paleotropical. Further science will probably separate the two groups into separate tribes.

Mabberley (1996) says of Theobroma "Trees with cauliflorous influorescences and usually large woody fruits; some cultivated for cocoa esp. *T. ca-* cao-cultivated since antiquity in the neotropics for seeds, the source of chocolate containing stimulating alkaloids including theobromine, the butter also used medicinally, the seeds currency in Yucatan until 1850 and still valued in 1923; probable origin is the upper Amazon ...Multi-million pound industry based on confectionery utilizing chocolate derived from numerous cultivars (sometimes polyembryonous) of *T. cacao* L. sometimes crossed with or the product mixed with that of *T. bicolor* Bonpl.(tiger cacao), *T. angustifolium* DC (monkey cacao), *T. grand ifloru m* (G . Don £.)Sch u mann (cupuacu)."

According to Cuatrecasas (1964) whose excellent and informative paper is the primary reference for the genus Theobroma, the basic features of the genus Theobroma are: bisexual flowers, 5- partite, valvate sepals; petals hooded (cucullate) in lower half concealing the anthers and twisted towards the top with 5 stamens opposite them and 5 evident, alternating showy staminodes united into a short basal tube; stamens shortly 2-3 branched with 2 celled anthers; superior ovary, 5 celled with many ovules, anatropous with 2 integuments; fruit subdrupaceous or subbacccate; seeds with pulpy envelope; cotyledons folded, corrugate; evergreen trees with dimorphic branching and dimorphic entire alternate leaves. A unique morphological aspect of the Theobroma cacao plant is the generation of a "jorquette" or "fan." "After reaching a height of a few feet, the vegetative end of the stem stops growing and by the way of a cluster of secondary buds it forks into 3-5 spreading branches arranged in a terminal whorl or jorquette.

The genera closest to Theobroma, 22 species, are Herrania, 20 species, and Guazuma, 4 species. Herrania and Theobroma have the same chromo- some numbers 2n=20. For Guazuma, 2n=l6. They form the core of the tribe Theobromeae which has been distinguished and separated from the tribe Byttnerieae with which it has sometimes been united. Cuatrecasas ventures that if Theobroma and Herrania are included in the Byttnerieae, then they are the most advanced of the 11 genera and 338 species that comprise this generic cluster...."because of the structure of the fruit, with thick and partly or totally carnose pericarp and delicate, short-lived seeds." These traits are adaptive to the hot and humid ecological conditions of the tropical American rainforest.

Addison and Tavares (cited by Cuatracasas) crossed *T. cacao* with all the Amazonian species of Theobroma, without success. In 1946 from 719 pollination of *T. speciosum x T. cacao,* they obtained 29 fruits and 979 seeds which were mostly abnormal and did not germinate. Similar results from crosses with *Herrania mariae*. *T. microcarpum x T. cacao* gave 11 fruits and 26 seeds generating 3 seedlings that died young. Similar negative results with *T. cacao x*

T. obovatum and *T. bicolor x T. cacao..The* cross of *T. cacao* with *T. angustifolium* gave small plants that did not mature. Some particular trees were more receptive to crossing than others. *T. cacao x T. microcarpum* gave 28% fruits but they failed to mature. *T. grandiflorum x T. obovatum* gave many hybrid seedlings and several grew into perfect trees in 1 1/2 years. *T. grand iflorum x T. subincanum, T. obovatum x T.*

subincanum, T.speciosum x T.sylvestre (=T. spruceanum) all gave well-developed hybrids.

The 22 species in the genus Theobroma have been divided into six sections separable into two clusters of three sections each as follows:

Pseudoterminal or pseudoapical growth where the new growth is produced from a bud above the whorl of plagiotropic branches. Germination is hypogeal, cryptocotylar in which the cotyledons remain underground enclosed in a testa.

Section Glossopetalum 12 species- T. *angustifolium, T. grandflorum, T. cirmolinae, T. stipulatum, T. simiarum, T. chocoense, T. obovatum, T. sinuosum, T.canumanense, T.subincanum, T.hylaeum, T. nemorale*

Section Andropetalum 1 species- T.*mammosum*

Section Telmatocarpus 2 species- T. *gileri, T. microcarpum*

Subterminal or subapical growth in which the new growth comes from below the whorl of plagiotropic branches and the seeds emerge epigeally with extended cotyledons. *Guazuma ulmifolia* has the same germination characteristics.

Section Oreanthes 5 species- T. *velutinum, T. speciousm, T. sylvestre, T. glaucum, T. bernouillii*

Section Rhytidocarpus 1 species- T. *bicolor*

Section Theobroma 1 species- T. *cacao*

Theobroma cacao is a very variable species. It is likely that interspecies crosses and intraspecies selections have broadened the base of the original plants that give rise to what is now called the Chocolate Tree. Two major morphologies are the Criollo and the Forastero with CriolloxForastero = Trinitario. Criollo has elongated, ridged, warted and pointed fruits with white cotyledons and Forastero has short, roundish, almost smooth fruits with purplish cotyledons. The Criollo is wide- spread in Mexico and Central America, especially in older plantations, where it makes seeds that produce the best chocolate but give low yields. Forastero cultivars grow more vigorously, have better productivity and resist disease more effectively. Forastero cultivars are either Lower Amazon or Upper Amazon in origin (Laurent et al 1993). The Upper Amazon Forastero cultivars have been introduced into cultivation most recently because of the breadth of their genetic diversity, their vigor, precocity and resistance to disease. Analyses of the ribosomal genes has confirmed the hybrid origin of the Trinitario cultivars (Laurent et al 1993). The Trinitario hybrids are widely planted at the current time and are not known in the wild state. The in- creased vigor (heterosis) associated with the Criollo x Forastero = Trinitario hybrid grex, together with disease resistance are the primary reasons for their selection in new cacao plantings. New directions in breeding involve backcrossing the Upper Amazon Forastero's with Trinitario hybrids.A continuing goal is the breeding of stable, highly productive and disease resistant cultivars. The disap- pearance of species and cultivars, both wild and in cultivation, arising from the sensitivity of *Theobroma cacao* to drought and overexposure to direct sunlight must be countered by direct action of continuing the establishment of cacao germplasm centers in favorable ecologies. Since Theobromas live for several decades, the survival of chocolate must be a credo passed on from one human generation to another.

The treasure of chocolate tree germplasm still exists in the neotropical forest remnants of Meso and South America. In particular, rare and valuable cultivars still exist in the Lacandon forest where sacred groves keep living examples of cultivars selected hundreds to thousands of years ago (de la Cruz et al 1995). Other regions in Meso America, on the Caribbean Coast of Honduras and Guatemala also harbor relic trees whose relationship to a healthy world commerce in organic chocolate resides in a sustainable cultivation environment. Perennial forests with productivity balanced through the different levels of the forest makes Theobroma a choice economic and ecological crop.

References

Angiopenn Phylogeny Group 1998 "An Ordinal Classification for the Families of Flowering Plants" Ann. Missouri Bot.I Gard. 85:531-553.

Bayer, C., M. Fay,A. deBruijn, V.Savolainen, C. Morton, K Kubitzki, W. Alverson, M.Chase "Support for an expanded family concept of Malvaceae within a recircumscribed order Malvales; a combined analysis of plastid atpB and rbcL DNA sequences" Bot.J.Linnean Soc.129:267-303.

Cheesman, E. 1944 "Notes on the nomenclature, classification and possible relationships of cacao populations" Trop. Agr. 21:144-159.

Cuatrecasas, J. 1964 "Cacao and its Allies; A Taxonomic Revision of the Genus Theobroma" Contrib.U.S. Nat.Herbarium 35:379-614.

Cuerrier, A., L. Bourillet and D. Barabe 1998 "Numerical and Comparative Analyses of the Modern Systems of Classification of the Flowering Plants" Bot. Rev. 64:323-355.

Dahlgren, G. 1989 "The Last Dahlgrenogram System of Classification of the Dicotyledons" in The Davis and Hedge Festschri ft 237-248 Edingburgh Univ. Press.

de la Cruz, M., R. Whitkus, A. Gomez-Pompa, L Mota- Bravo 1995 "Origins of cacao cultivation" Nature 375:542-543.

Gomex-Pompa, A., J. Salvador Flores and M. Aliphat Fernandez 1990 "The Sacred Cacao Groves of the Maya" Latin American Antiquity 1(3):247-257.

La Duke, J. and J Doebley 1995 " A Chloroplast DNA Based Phylogeny of the Malvaceae" Syst. Bot. 20:259-271.

Laurent, V., A. Ristucci and C. Lanaud 1993 "Variability for nuclear ribosomal genes within Theobroma cacao" Heredity 71:96-103.

Mabberley, D.J. 1993 The Plant Book Cambridge Univ. Press, London.

Qiu, Y.,J. Lee, F. Bernasconi-Quadroni, D. Soltis, P. Soltis, M. Zanis, E. Zimmer, Z. Chen, V. Savolainen, M. Chase 1999 "The earliest angiosperms: evidence from mitochondrial, plastid and nuclear genomes" Nature 402:402-404.

Soltis, P., D. Soltis and M. Chase " Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology" Nature 402:402-404.

Schultes, R. 1958 "A synopsis of the Genus Herrania" J. Arnold Arb. 39:216-278.
Takhtajan, A. 1997 Diversity and Classification of Flowering Plants Columbia Univ. Press, NY.
Whitlock, D. and D. Baum 1999 "Phylogenetic Relationships of Theobroma and Herrania (Sterculiaceae) Based on Sequences of the Nuclear Gene Vicilin Syst. Bot.24:128-138.
Wilde, J., R. Waugh and W. Powell 1992 "Genetic fingerprinting of Theobroma clones using randomly amplified polymorphic clones using randomly amplified polymorphic DNA markers" Theor. Appl. Genet. 83:871-877.