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We All Love Chocolate: An Overview of Cacao in Latin America

Introduction

Chocolate is very common across the world and is associated with many different things. At least in the United States, I can think of a specific type of chocolate that is associated with many of the holidays we celebrate. For example on Halloween, thousands of tons of chocolate were sold ("Fun Facts About Halloween Candy"). Cacao, which is the tree from which chocolate is produced, has long been an important part of cultures it has been a part of. It has historically been significant across Mesoamerica and there is even evidence of cacao usage in the American South West (Crown et al. 2009) which is far north of the tropical climate cacao requires. The cultivation of cacao is a historically significant part of Mayan culture (Gómez-Pompa et al. 1990).

Originally, I was intending to write an essay largely focused on the medicinal uses of cacao since there is a brief description of some of the medicinal qualities of cacao in *Science of Latin America: A History*. However, I found it difficult to find sources that did more than very briefly mention cacao in a medicinal way. Instead, I found a plethora of sources that investigate the genetics and origins of cacao. So, I shifted my focus to the cultivation of cacao in Mesoamerica and some of the different hypotheses about how cacao ended up in Mesoamerica. A lot of the research that has been done about these topics was done in the 1990s and early 2000s. In order to incorporate more recent research, I included a section on the effects of climate

change on cacao. Part of this section is a contemporary paper that studied how the genetic diversity of cacao could be used to counteract the effect of climate change on cacao. I also wanted to include the chocolate drinking vessels that have been found across Mesoamerica since they are what inspired me to research cacao usage in Mesoamerica. The study of residue that is present in vessels that have been found in Mesoamerica is a key example of the importance of scientific processes to non-scientific fields such as art history and anthropology.

Cultivation of Cacao

Cacao (*Theobroma cacao*) is native to the tropical regions ranging from South America to southern Mexico. It is an understory tree found in rainforests (Whitkus et. el, 1997). It requires fairly specific conditions to flourish. It grows the best in rich, well-drained soils with plenty of organic material. Wild species typically grow in the shade of larger trees in evergreen tropical forests. Regions that are hot and humid with abundant rainfall in the summer and fall are most favorable, but cacao can be found in drier areas as long as the soil is humid enough. In rainfed cacao plantations, there is typically more than 2000 mm of rain annually (Gómez-Pompa et al. 1990).

There are two groups of wild cacao. Subspecies *sphaerocarpum* has rounded, melon-like fruit and is found only in South America. Subspecies *cacao* has long, ridged fruits and is found from northern South America to Central America. There are two main cultivars that have been bred. Forastero is cultivated from the *sphaerocarpum* subspecies and is found in South America. Criollo is found in Mesoamerica and it is thought to be the most delicious variety of cacao. The combination of Criollo and Forestario is known as Trinitario and was first found on the Island of Trinidad (Gómez-Pompa et al. 1990).

One of the interesting places that the Maya were able to successfully cultivate cacao was in sinkholes and cenotes in the Yucatan Peninsula. The sinkholes and cenotes create the perfect microclimate for cacao to grow so that cacao could be grown even in areas where the broader climate was too dry and the soil was too rocky. Water and silt collect in the base of sinkholes and cenotes which creates a hyper humid environment that can support cacao growth even through the dry season during which the Northern Yucatan receives little rainfall. Additionally, the sinkholes are lower than the surrounding ground which along with tall trees that cacao grows under, provides ample shade for cacao (Gómez-Pompa et al. 1990).

There are two hypotheses about the origin and distribution of domesticated cacao. The first is that wild cacao only existed in South America and cacao was transported to Mesoamerica by humans. There, it was domesticated by the Mayans. There are higher levels of genetic diversity among plants from the upper Amazonian region which suggests that this area is the center of origin for cacao. Additionally the long history of cacao cultivation in Mesoamerica supports the idea that it was domesticated by the Mayans from wild cacao (Whitkus et al 1998).

The second hypothesis is that cacao is naturally distributed from the Amazonian region to southern Mexico. In this hypothesis, the two subspecies, *sphaerocarpum* and *cacao* were produced through differentiation in the wild. Independent domestication created the two main cultivated varieties of cacao, forastero and criollo. This hypothesis is supported by the discovery of assumed-to-be-wild *Theobroma cacao* subspecies *cacao* plants in the Lacandona rainforest of Chiapas, Mexico, and a study of southern Mexico collections of the *cacao* subspecies shows that these plants are highly different from cultivated and South American plants (Whitkus et al 1998).

Research done by JC Motamayor and a group of researchers from France and Mexico has supported the first hypothesis. They found that the low amounts of genetic diversity among

cacao in Mexico pointed to the idea that cacao originated in South America and was brought to Central America relatively more recently. In order to study the genetic diversity of cacao, they collected samples of cacao plants from across Central and South America. There were eight categories that the samples fell into: Ancient Criollo, Modern Criollo, Trinitario, Lower Amazon Forastero, Orinoco Forastero, French Guiana Forastero, Upper Amazon Forastero and hybrids with at least one Upper Amazon Forastero parent (Motamayor et al. 2008).

The Criollo categories were defined by a list of characteristics defined by EE Cheesman in 1944. The Ancient Criollo samples came from more remote areas and abandoned farms, where the introduction of Forastero and Trinitario varieties was unlikely. Samples of Ancient Criollo were also collected from the Lacandono rainforest of Mexico where wild cacao had been reported and in the sinkholes where the Maya had cultivated cacao. The Modern Criollo samples were taken from modern farms where introductions of Forastero or Trinitario were highly suspected. The other categories of samples were studied to compare the structure of genetic diversity to the Ancient and Modern Criollo (Motamayor et al. 2008).

There were two different processes of analysis that were performed on the DNA extracted from the samples, Restriction Fragment Length Polymorphism (RFLP) analysis and microsatellite analysis. RFLP is "a difference in homologous DNA sequences that can be detected by the presence of fragments of different lengths after digestion of the DNA samples in question with specific restriction endonucleases" ("Restriction...(RFLP)") Through the RFLP analysis, it was found that the Forastero group had higher genetic diversity than Ancient Criollo. The average number of alleles was higher in the Forastero and there were large numbers of homozygosity among the Ancient Criollo. An example of the lack of genetic diversity that supports the original hypothesis is that some of the trees found in the Lacandona rainforest had

identical RFLP profiles to the trees that were cultivated by the Maya found in sinkholes of Yucatan, on the Pacific Coast of Mexico, or in Belize. The statistics for genetic diversity of Modern Criollo were found to be very similar to those of Trinitario which to be expected given the differences based on morphological traits are subjective. The microsatellite analysis yielded equivalent results (Motamayor et al. 2008). The results demonstrate that there is a difference between Ancient Criollo and Modern Criollo. Ancient Criollo is the true Criollo whereas Modern Criollo is basically Trinitario because of the introduction of Forastero to its lineage.

Contrary to previous studies, this DNA analysis found that there was no significant difference between what was believed to be wild cacao found in the Lacandona rainforest and the cacao that was cultivated by Maya in the sinkholes. The samples from Lacandona also matched with plants from Venezuela and Colombia. This evidence suggests that the cacao plants in Lacandona are in fact not wild and do not originate from this region. Additional evidence that supports this idea is that there is no evidence of any *theobroma* species, cacao or otherwise, in Central America before human colonization of the area. Additionally, remnants of Maya civilization have frequently been found in the Lacandona rainforest (Motamayor et al. 2008). All of which suggests that cacao was in fact brought to Central America by some form of human manipulation.

Effects of Climate Change on Cacao

Cacao, like many agricultural products, is expected to be severely affected by climate change. It is a very delicate plant that is very susceptible to pests and fungal infestations. The hot and wet climate that is required to grow cacao is also an ideal climate for fungi, pests and viruses which have the potential to be severely harmful to cacao crops. In 1988, 80 percent of cacao growing in the Bahia region of Brazil was lost to a fungal disease called witches' broom. More

recently, another fungal disease, frosty pod rot, has spread throughout Latin America and further threatens the production of cacao. There are many other diseases and pests that have imperiled cacao yields across the globe. Climate change only threatens to heighten the risk of damage to crops from disease (Stamitz and Shapiro 2012).

Theobroma Cacao is very sensitive to environmental factors that would change with a warming planet. It requires a lot of water so high temperatures and low precipitation would have an adverse effect on quality and amount of crop yields. Climate change will exacerbate existing weather extremes of the tropics such as floods, droughts and windstorms. The intensification of these weather patterns could lead to worse pest and disease infestation and affect water supply. The changing climate will likely decrease the amount of terrain that is suitable to grow cacao and shift the areas that are viable for cacao farming to higher elevations. It is possible to move the production of cacao to higher altitudes in South America as it is relatively mountainous, but will cause issues in other cacao growing regions of the world such as West Africa as it is quite flat. Already, there is evidence that climate change is affecting cacao cultivation. In Indonesia, more intense monsoons have caused trouble for farmers as the ultra heavy rainfall has begun to knock flowers off the trees before they can develop into fruit (Stamitz and Shapiro 2012).

Breeding specialized varieties of cacao will be critical in ensuring that cacao is able to withstand disease and be grown even as climate conditions continue to worsen. There is a lot of genetic diversity in cacao, which is a key resource that has yet to be fully exploited by breeders to create hardier plants (Ceccarelli et al. 2021). In order to develop cacao that is more resistant to climate change and disease, breeders are looking to the genome of cacao to find traits that are desirable. For example, there is a cultivar of cacao that has been found to show resistance to frosty pod rot. By identifying exactly which gene sequence causes this resistance, breeders are

able to quickly determine whether or not new cultivars contain this trait. In addition to creating trees that are resistant to pests and disease and are more heat tolerant, breeders want to develop shorter cacao trees. Shorter trees that are still able to provide a high-quality, plentiful yield would require fewer resources to produce fruit that is easier to harvest (Stamitz and Shapiro 2012).

A 2021 study carried out by Peruvian scientists sought to assess the impact that climate change will have on Peru and identify areas where climate change-tolerant genotypes are present. They included in their study both wild and domesticated cacao. In order to study the genetic distribution they collected presence points of cacao. The points were divided into cultivated and wild varieties. There were 19,685 presence points for cultivated cacao and 1,182 presence points for wild cacao. For cultivated cacao, points were only counted within the borders of Peru. Presence points for wild cacao were counted within the geographical influence of Peru which included points in neighboring countries of Brazil, Colombia, and Ecuador. Cultivated cacao grown in regions with less than 600 millimeters of rainfall were omitted from the study because they were only able to be grown with irrigation (Ceccarelli et al. 2021).

The explanatory variable of the study was environmental differences. There were 34 climate, soil, and terrain variables that are commonly used in habitat modeling. These factors were selected because they are the main factors that influence species distributions. They generated models for both the cultivated and wild varieties that accessed how changing environmental factors impact cacao growth. The models were also used to determine where it would be possible to continue growing cacao in the future as the climate is changing (Ceccarelli et al. 2021).

By researching which genes of cacao can help to breed a more climate change tolerant variation of cacao, the findings of this study will be able to help protect the production of cacao

even as the globe is heating up. Since cacao is already a genetically diverse species so the genes that can survive climate change conditions already exist, it just comes down to finding them.

Eventually, even with more drought tolerant, efficient cacao trees, the way that cacao is farmed will need to change. Currently, rainfall is the primary source of water for much of the cacao that is farmed worldwide. With greater irregularity of weather patterns due to climate change, growers will have to turn to irrigation to ensure that their crops receive sufficient water. There are different solutions to this problem that are being introduced in various regions of the cacao growing world. In Brazil, there are two distinct strategies that are being developed. In the first, small growers are being encouraged to grow cacao in mixed agroforestry systems that improve water capacity by containing various root structures. These systems would include cacao that is planted among food crops, fodder and timber trees. The second solution is to grow large tracts of cacao trees at higher elevations which are out of the range of typical pests and diseases. In order to maximize yields, the trees are watered with fertilizer-enriched water (Stamitz and Shapiro 2012).

The preservation of cacao production is important both economically as many communities rely on income from cacao production (Stamitz and Shapiro 2012), but also culturally. As discussed previously, cacao is historically significant for many cultures so it is important to conserve the cultivation of cacao. Cacao is one of the most valuable cash crops in the tropics worldwide (Ceccarelli et al. 2021) so finding ways to alter cacao to be more climate change tolerant and reshaping farming practices will be critical. Additionally, chocolate is a product that is beloved around the world, so if its production were to falter because of climate change, I imagine that it would have an outstanding impact on individuals and the economy more broadly.

Science of Vessels Containing Cacao

Cacao usage has a rich history in Mesoamerica. Various civilizations from the Olmec to the Maya to the Aztec have all used cacao. One of the methods that has been used to put together a picture of how cacao was used is high-performance liquid chromatography-mass spectrometry (Powis et al. 2011, Powis et al. 2002). This tool has been used specifically to identify the presence of *theobromine* which is a compound found in cacao. It has been determined that cacao is the only plant in Mesoamerica that contains *theobromine* and is therefore an indicator of cacao presence (Powis et al. 2002). This testing has provided definitive evidence of what has been theorized in other fields.

Mayanists have identified text that commonly encircles the rim of cylindrical Mayan vessels as descriptions of the purpose of the vessels. Many of the vessels have hieroglyphs that identify that the vessels are used for drinking. Some of the vessels also contained glyphs that represent chocolate (Lebrun 2015). From these two factors, researchers were able to infer that the Mayan vessels were used for the consumption of chocolate, but the chocolate residue has confirmed this.

These same vessels have also given insight on the social significance of the cacao. Many of the vessels are elaborately painted with various scenes. The scenes depict many different aspects of Mayan life. Some are of scholars, some are of leaders, some depict various myths. And some include cacao trees. However, the real insight comes with the fact that these vessels are believed to be highly prized possessions. Many of the vessels include signatures from their creator, which suggests that certain artists were highly respected and sought after. The value of the chocolate drinking vessels tells us that cacao was a culturally significant product (Lebrun

2015). These vessels lend us insight into the overall place that cacao held in at least the Mayan slice of Mesoamerican society. This goes along with the effort that the Mayans put into cultivating cacao.

Mayan vessels discovered in Colha, Belize were analyzed using high-performance liquid chromatography-mass spectrometry in the Hershey Foods Technical Center at Hershey's Foods Corporation in Pennsylvania. Samples were taken from the bottom of 14 vessels that were found in structures in the Mayan site Colha. Since there was no visible residue, small amounts of clay had to be scraped off the bottom of the vessels. These samples were then sent to W. Jeffrey Hurst and Stanley M. Tarka at the Hershey laboratory. There, the study of the samples focused on finding *theobromine*. Two of the vessels were found to have definitive evidence of *theobromine* presence and one of the vessels had minor indicators that *theobromine* was present (Powis et al. 2002). This study showed that the Maya in Colha used vessels to prepare something that was cacao based. Later studies were done to investigate more of the details of how cacao was prepared and what other ingredients were added (Soleri et al. 2013), but this study proved only that there was cacao present in the vessels.

A similar investigation was carried out by a group from both American and Mexican universities. This study sampled 156 pottery shards found at sites from the Olmec civilization. Samples were collected from both the capital of the Olmec, San Lorenzo, as well as a secondary city, Loma del Zapote. Whereas the other study only looked at one type of vessel, this study examined a wide variety of types of pottery. Any vessel that reasonably could have been used to prepare cacao was included. The unique aspect of this study of Olmec pottery is that many of its samples were older than any previous study of its kind and it included pottery from every period of the Olmec civilization (Powis et al. 2011).

The residue was collected from the pottery by rubbing the interior surface with a fine sandpaper to remove any residue that was present. The dust from this process was collected on white paper and funneled in clean collection vials and sealed immediately and then sent to a lab at UC Davis for analysis. Fresh sandpaper and white paper was used for each collection to preserve the integrity of the samples. Like the previous study, *theobromine* was the compound that was being tested for. The samples were tested using liquid chromatography-tandem mass spectrometry (UPLC/MS-MS), which is a process in which the burr samples are heated in water to 80 degrees celsius for 30 minutes. Then they are filtered and filtrates are used for UPLC/MS-MS analysis. Two modern pottery samples were included as controls. The UPLC/MS-MS analyses showed that some of the samples clearly had a presence of *theobromine* whereas the modern samples did not have any *theobromine* present (Powis et al. 2011).

The was found to be a presence of theobromine in 17% of the pottery tested. Sample #11 confirms the early cacao use of the Olmec as it is dated between 1800-1600 BCE. Other samples are contemporaneous with pottery that has been tested from sites across Mesoamerica. This study did not determine the nature of the cacao being consumed, however it can be surmised that it was some sort of liquid because of the form of the vessels theobromine was found in. There are nine open bowls and one cup, which would have been appropriate for personal beverage consumption, and two bottles which would be most suitable to store liquids (Powis et al. 2011). Like the previous study, this study only supports that cacao was used in preclassic Mesoamerica, but it is unable to provide more definitive details on which form it was consumed in.

A study published in 2013 sought to determine more specifically how cacao was prepared in Mesoamerica, specifically the Valley of Oaxaca in Mexico during the Postclassic era (900 CE-Spanish conquest). In present day Oaxaca and across Mexico, there are a number of beverages,

such as tejate, that are made from some variation of maize and cacao. Tejate is made from cacao, other plant ingredients and nixtamalized maize. Similar beverages were described as being consumed in Spanish records from the 16th century in Mexico. Even though the Valley of Oaxaca is out of the tropical range that supports cacao growth through trade and extensive irrigation, cacao was available. Based on this information, the researchers hypothesized that some sort of beverage made of cacao and maize was consumed in the Valley of Oaxaca during the Postclassic period (Soleri et al. 2013).

Eight vessels from the Postclassic era were selected for this study plus one contemporary vessel that had been used for tejate preparation. The Postclassic era specifically was chosen because it is unknown when the Tejate consumption began so it is most likely that it was consumed during the Postclassic era since it is closer to known consumption of the beverage. Using high-performance liquid chromatography-mass spectrometry, they tested the vessels to identify a greater variety of organic compounds, not just theobromine like the previous studies (Soleri et al. 2013).

The two categories of compounds tested were methylxanthines and phytoliths.

Methylxanthines include theobromine, caffeine, and theophylline, all of which are present in
Theobroma cacao. Phytoliths are a result of the silicification of plant cells. Since the shape of
phytoliths vary across plant species, they can be used to identify which plant residue is from.
Theobromine and caffeine were present in three of the nine vessels tested. Phytoliths were well
preserved in seven of the nine vessels, one of the vessels that had no evidence of phytoliths was
the contemporary vessel. In fact, the interior surface of the contemporary vessel was found to be
too slick to have any evidence of either phytoliths or methylxanthines. Most of the phytoliths
found were from grass species that likely found their way into the vessels while they were in the

ground. However, three of the vessels contained phytoliths that are diagnostic of maize. These phytoliths indicated that there was repeated presence or preparation of maize kernels in the vessels. Two of the vessels contained evidence of both maize and *Theobroma* species (Soleri et al. 2013).

From the small sample size of the study, it is not possible to discover any greater patterns of cacao usage in the Valley of Oaxaca. The results, nonetheless, support the authors' hypothesis that a beverage similar to tejate was consumed in the Postclassic Valley of Oaxaca. While the previous studies only confirmed that vessels were used in the preparation, consumption, or storage of a cacao product, this study provides further details on how cacao might have been used. And although the presence of maize and cacao alone cannot prove that a beverage that resembles modern tejate was being produced, with the support of evidence from literature from before and after the conquest, it can be assumed that at least something that loosely resembles tejate was being made (Soleri et al. 2013).

The science that was done in these studies has discovered evidence that is useful across fields, from anthropology to art history. Specifically, I came across the science that proved the presence of cacao in Mesoamerican vessels in the art history class that I am taking this semester. This is an example of the importance of science in non-scientific fields as it can provide important evidence for analysis. As in the case of the vessels, researchers were able to theorize what some of their purposes were with non-scientific methods by interpreting the text that appears on them. Testing the vessels using a process that has been developed by scientists and is used across scientific disciplines (Thomas et al. 2022), gives further verification of what researchers had discovered using other methods. Scientific testing for the presence of cacao has

given those studying Mesoamerica another piece in the puzzle of what things were present in the lives of those living in pre colonial Mesoamerica.

Conclusion

Cacao is a small tree that is native to the rainforests in South America and likely made its way to Central America with human influence. In Central America, cacao was domesticated by the Mayans. From DNA analysis we can begin to understand how cacao trees gained their current range. Like many crops around the world, modern cacao production is threatened by global warming. Action in the form of developing climate change resistant varieties of cacao and changing farming practices must be taken to ensure that cacao is able to be grown for years to come. Analyzing the residue of pottery that was created across Mesoamerica has led to insights on the use of cacao, but also its cultural significance which can be ascertained from the vessels that cacao was found in.

In this paper I mostly focused on the cultivation and use of cacao in Mesoamerica and took a broader view when looking at the effects of climate change on cacao. However, there is so much more to learn. Some topics that I think would be particularly interesting to delve more deeply into are how chocolate became so widespread across the world and the development of the modern style of chocolate which is likely vastly different from how ancient Mesoamericans enjoyed chocolate. I also think it would be fascinating to find out the place that cacao has in the mythological realms of Mesoamerica.

Works Cited:

- Ceccarelli, Viviana, Tobias Fremout, Diego Zavaleta, Sphyros Lastra, Sixto Imán Correa, Enrique Arévalo-Gardini, Carlos Armando Rodriguez, Wilbert Cruz Hilacondo, and Evert Thomas. 2021. "Climate Change Impact on Cultivated and Wild Cacao in Peru and the Search of Climate Change-Tolerant Genotypes." *Diversity and Distributions* 27, no. 8: 1462–76. https://www.jstor.org/stable/27040541.
- Crown, Patricia L., Hurst, W. Jeffrey and Smith, Bruce D. 2009. "Evidence of Cacao Use in the Prehispanic American Southwest." *Proceedings of the National Academy of Sciences of the United States of America* 106, no. 7: 2110–13. http://www.jstor.org/stable/40272633.
- "Fun Facts about Halloween Candy." Kansas Farm Food Connection. October 6, 2022. https://kansasfarmfoodconnection.org/spotlights/fun-facts-about-halloween-candy.
- Gómez-Pompa, Arturo, José Salvador Flores, and Mario Aliphat Fernández. 1990. "The Sacred Cacao Groves of the Maya." *Latin American Antiquity* 1, no. 3: 247–57. https://doi.org/10.2307/972163.
- Lebrun, David. 2015. Out of Maya Tombs. United States: Documentary Educational Resources.
- Motamayor, Juan C & Risterucci, A & Lopez, PA & Ortiz, Carlos & Moreno, A & Lanaud, C. 2002. Cacao domestication I: The origin of the cacao cultivated by the Mayas. Heredity. 89. 380-6. 10.1038/sj.hdy.6800156.
- Powis, Terry G., Ann Cyphers, Nilesh W. Gaikwad, Louis Grivetti, and Kong Cheong. 2011.
 "Cacao Use and the San Lorenzo Olmec." *Proceedings of the National Academy of Sciences of the United States of America* 108, no. 21: 8595–8600.

 http://www.jstor.org/stable/25830997.

- Powis, Terry G., Fred Valdez, Thomas R. Hester, W. Jeffrey Hurst, and Stanley M. Tarka. 2002. "Spouted Vessels and Cacao Use among the Preclassic Maya." *Latin American Antiquity* 13, no. 1: 85–106. https://doi.org/10.2307/971742.
- "Restriction Fragment Length Polymorphism (RFLP)." n.d. Nih.gov. Accessed November 4, 2024. https://www.ncbi.nlm.nih.gov/probe/docs/techrflp/.
- Schmitz, Harold and Howard-Yana Shapiro. 2012. "THE FUTURE OF CHOCOLATE." *Scientific American* 306, no. 2: 60–65. http://www.jstor.org/stable/26014203.
- Soleri, Daniela, Marcus Winter, Steven R. Bozarth, and W. Jeffrey Hurst. 2013.

 "ARCHAEOLOGICAL RESIDUES AND RECIPES: EXPLORATORY TESTING FOR
 EVIDENCE OF MAIZE AND CACAO BEVERAGES IN POSTCLASSIC VESSELS
 FROM THE VALLEY OF OAXACA, MEXICO." *Latin American Antiquity* 24, no. 3:
 345–62. http://www.jstor.org/stable/23645680.
- Thomas, S.N., French, D., Jannetto, P.J. *et al.* 2022. Liquid chromatography–tandem mass spectrometry for clinical diagnostics. *Nat Rev Methods Primers* 2, 96. https://doi.org/10.1038/s43586-022-00175-x.
- Whitkus, R. & De la Cruz, Marlene & Mota-Bravo, L. & Gómez-Pompa, A. 1998. Genetic diversity and relationships of cacao (Theobroma cacao L.) in southern Mexico.Theoretical and Applied Genetics. 96. 621-627. 10.1007/s001220050780.