A quantitative synthesis of the medicinal ethnobotany of the Malinké of Mali and the Asháninka of Peru, with a new theoretical framework

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Abstract

A quantitative synthesis of the medicinal ethnobotany of the Malinké of Mali and the Asháninka of Peru, with a new theoretical framework

by Nathaniel Bletter

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Although ethnomedically guided searches for new medicinal plants can improve the percentage of plants found containing active compounds when compared to random sampling, ethnobotany has fulfilled little of its promise in recent decades to deliver a bounty of new, laboratory-proven medicinal plants and compounds. It is difficult to test, isolate, and elucidate the structure and mechanism of compounds from the plethora of new medicinal plant uses described each year with limited laboratory time and resources and the high cost of clinical trials of new drug candidates.

A new, quantitative, theoretical framework of mathematical formulas called "relational efficacy" is proposed that should narrow down this search for plant-derived medicines based on the hypothesis that closely related plants used to treat closely related diseases in distantly related cultures have a higher probability of being effective because they are more likely to be independent discoveries of similar plant compounds and disease mechanisms. A prerequisite to this hypothesis, the idea that empirical testing in

traditional medicine will lead to choosing similar medicinal plants and therefore the medicinal flora of two distant cultures will prove to be more similar than their general flora, is tested using approximate randomization statistics on cross-cultural field data of the plants used by the Malian Malinké and the Peruvian Asháninka to treat malaria, African sleeping sickness, Chagas' disease, leishmaniasis, diabetes, eczema, asthma, and uterine fibroids.

In this case, the similarity of the medicinal floras is found to be significantly greater than the similarity of the general floras, but only when the diseases are grouped into the categories of parasitic and autoimmune diseases. This verifies the prerequisites for relational efficacy, but shows that subjective measures such as taxa groupings can confuse results and objective, phylogenetic measures of relations should be used, for which methods are detailed herein.

With the central theoretical framework of this hypothesis verified, the relational efficacy system will allow the synthesis of medicinal plant information from around the world to pinpoint the species with the highest potential efficacy to take into the laboratory and analyze further, ultimately saving much field and laboratory time and resources.

Spanish abstract

Las búsquedas que utilizan la etnomedicina y la taxonomía para descubrir nuevas plantas medicinales, pueden aumentar la probabilidad de éxito de encontrar compuestos químicos activos en plantas, en comparación con las búsquedas aleatorias. A pesar de lo anterior, en las últimas décadas, la etnobotánica no ha cumplido con las expectativas de proveer numerosas plantas medicinales y químicos nuevos una vez examinados en el laboratorio. Cada año se describen una plétora de plantas medicinales y sus usos, sin

embargo las limitaciones de tiempo y recursos en los laboratorios, unidos al alto coste de los ensayos clínicos de las drogas potenciales, hacen muy difícil probar, aislar, y elucidar la estructura y el mecanismo de los compuestos de estas plantas. Se propone un nuevo marco teórico cuantitativo cuyo fin es focalizar la búsqueda de nueva plantas medicinales. Este marco teórico está basado en la hipótesis que las plantas cercanamente relacionadas, usadas para tratar enfermedades cercanamente relacionadas en culturas distantemente relacionadas, tienen una eficacia potencial más alta, debido a que es más probable que estos hallazgos sean descubrimientos independientes de compuestos químicos similares. Parte de esta hipótesis, que las escogencias racionales se hacen para elegir plantas medicinales similares y que la flora medicinal de dos culturas distantes es más similar que su flora general, se probó usando métodos estadísticos de remuestreo con datos de campo de la comunidad Malinké de Malí y de la Asháninka de Perú, y las enfermedades de paludismo, enfermedad africana del sueño, enfermedad de Chagas, leishmania, diabetes, eczema, asma, y fibromas uterinos. Se encontró, en este caso, que la similitud de las floras medicinales es significativamente mayor a la similitud de las floras generales, solamente cuando las enfermedades analizadas se agruparon en las categorías de enfermedades parasitarias y enfermedades autoinmunes. Si se demostrara que las otras partes de esta hipótesis son ciertas, se podría sintetizar la información sobre plantas medicinales alrededor del mundo, para establecer así las plantas potencialmente más eficaces para llevarlas al laboratorio y analizarlas más profundamente.

French abstract

Par rapport aux recherches menées de façon aléatoire, les recherches effectuées par des critères ethnobotaniques et taxonomiques ont de meilleures chances à découvrir de

nouvelles plantes médicinales à produit chimique actifs. Pendant les dernières décennies pourtant, l'ethnobotanique a réalisé peu de ces promesses à révéler un grand nombre de plantes médicinales et de nouveaux produits chimiques, testés au laboratoire. Avec les ressources limitées pour la recherche au laboratoire et le coût élevé des épreuves cliniques pour trouver de nouveaux candidats aux médicaments, il est difficile d'étudier, d'isoler et d'élucider la structure et le mécanisme des produits chimiques de chacune des nombreuses plantes médicinales (et les utilisations de ces plantes) décrites chaque année. Nous proposons une nouvelle technique théorique et quantitative pour préciser la recherche de nouvelles plantes médicinales ; elle est basée sur l'hypothèse que les plantes étroitement apparentées, employées pour traiter les maladies étroitement apparentées dans les cultures très éloignées les unes des autres, ont une potentialité d'efficacité supérieure parce qu'elles représentent la découverte indépendante des propriétés chimiques semblables des plantes. Une partie de cette hypothèse– qui démontre que la sélection des plantes médicinales semblables est un choix rationnel et qu'il y a davantage de ressemblance dans la flore médicinale de deux cultures éloignées que dans leur flore générale– est examinée par un re-échantillonnage des données de recherches effectuées parmi les Malinké au Mali et les Asháninka au Pérou, en particulier sur la malaria, la maladie africaine du sommeil, la maladie de Chagas, la leishmania, le diabète, l'eczéma, l'asthme et les fibromes utérins. Dans ces cas précis, la similitude de la flore médicinale s'avère sensiblement plus grande que la similitude de la flore générale, mais seulement quand les maladies en question sont regroupées ensemble comme maladies parasitaires et auto-immunitaires. Si cette hypothèse est prouvée, elle permettra la synthèse des informations recueillies sur les plantes médicinales du monde entier pour en sélectionner

de façon plus précise celles qui sont les plus efficaces et qui méritent analyse plus approfondie au laboratoire.

Asháninka abstract

Aayiantyarori iròpero aavintane, ontzimatye ancovacovatero ayotero ovaqueraripaye incashi iyoyetziri ashaninka, ayotzityaro aajatzi iyotane viracocha paitachari “quimica” ancantero aaca oshintsinka inchashipaye. Atziri yotacotzirori cametsa, ishtoriajacotzirori iyotane ashaninkapaye te iroñàrantero maaroni ocaratzi yamenacotaqueri laboratorioki. Aaviantyarori cametsa, ayotacotero aavintarontsiyetatsiri osamani antzimaventero ishtoriatacotaro, aajatzi osheki opinata ampinaventero aparopaye inchashi, acoviriqui ayotacotero, osaretsikipaye. Tzimatsi ovaquerari quenquishiriantsitatsiri ero opinata osheki ashitoriatacotero aparopaye inchashi, asampiyetatyrey pashinipaye atziri saicatsiri intaina puitarika inchasshi yavintari, ajatzirica oshiyaro ayotzi aaca, quemetachari atziri saikatsiri nampitsiki malinke aajatzi ishiyari ashaninka saicatsiri peruki, tzimatsi inchashi aajatzi yaavintari osheki okamètsatzi aririka anteri mantsiyarentsi icantaitziri ompetarentsi catsirentsi, pochokirentsi, patsarontsi(matatsi) ashipetate maaroni, ampochavathate, ancainikentsite, oncatsithakite tsinani. Aririka añaker aajatzi ahiyaro inchashi yaavintayetari pashinipaye atziri intainasatzi irdotake ahitoriatacoperoteri anàashityard aavintarontsi ovamairiri shithanentsi, onàshitaavintarontsi tzicaacoventairi ero antane mantsiyarentsi. Omanperotatyarica iròperotzi avintarontsi, oshitovake laboratorioki aritaque iyoitanaquero maaroni quipatsiki iroperori avintarontsi.

Keywords: antiplasmodial plants, autoimmune diseases, computational statistics, cross-cultural communication, herbal medicine, parasitic diseases, quantitative ethnobotany, traditional medicine, women's reproductive health

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It has been a long and difficult yet gratifying nine years working on this Ph.D. research, taking me from computer work, through organic chemistry, phytochemistry, plant taxonomy, collecting and preserving plants, field work, wild food foraging, politics, anthropology, chocolate research, biostatistics, and eventually back to computer programming, but I have enjoyed every minute of it, learning so much along the way, keeping my enthusiasm about the plants and the people who use them, and I thank everyone who has taught me and made this work possible. I and many others thought I was a bit crazy to leave a well-paying job in silicon valley to pursue ethnobotany as a student, but this has been an amazingly rewarding research project that I would keep the same if I did it over again. I hope have been able to make a valuable contribution to the field, to both indigenous communities, and to traditional herbal medicine along the way.

Table of Contents

List of Figures

List of Tables

Chapter 1. Introduction

Promoting ethnobotany by claiming it will find a cure for cancer, AIDS, or diabetes has done wonders for the image of the field in the popular conception, but little to win respect for the field in other areas of science or medicine because this claim has been largely hollow. Although ethnobotany was a large part of the foundation of modern medicine with the discovery of powerful medicinal compounds in *Digitalis purpurea* L., *Cinchona officinalis* L., and *Papaver somniferum* L. validating traditional uses, in the past 30 years there have been almost no new medicinal plant compounds discovered from traditional herbal pharmacopoeias of indigenous peoples, at a time when many new treatments are direly needed for global epidemics like malaria, HIV, diabetes, and cancer, as well as more minor but equally deadly or debilitating diseases that modern medicine has made little progress with like leishmaniasis, Chagas' disease, and asthma. A wealth of new techniques and information available have been adopted by other areas of biology aside from ethnobotany, such as large botanical databases, elucidations of plant and disease evolution, high-speed computational power, and bioinformatics, and it is the thesis of the present work that these can and should be harnessed to discover new, effective medicinal plants and compounds with reasonable cost and effort, especially in light of the suffering and economic loss these disease cause worldwide. By using these modern techniques and information, we can help reduce the amount of time and resources necessary to uncover these effective medicinal plants to help treat the millions of people in developing countries afflicted by these diseases. At the same time, we can use new, effective technologies to help preserve, protect, and compensate traditional medicinal knowledge, languages, cultures, and traditional healers. Without the effort of traditional

healers, modern medicine may never have existed, and this must be recognized and appreciated, so we learn from our history, mixing the ancient and the modern to treat some of the world's ills.

To this end, this work attempts to delineate a predictive quantitative ethnomedicine system, termed "relational efficacy," to synthesize medicinal plant knowledge from many cultures and the phylogenies and relations of plants, diseases, and cultures to create a list of medicinal plants prioritized by their potential medicinal efficacy, a sort of "top ten list" of medicinal plants for any disease that can be the first ones analyzed in the laboratory to verify their efficacy out of the thousands of described but untested medicinal plants. These plants need to be weeded out of the wealth of global information on medicinal plants. This can potentially save substantial time, resources, and money, while ensuring that these diseases are treated quickly and at the low cost of growing a plant in areas of the world that can barely afford food and housing, let alone expensive pharmaceuticals manufactured and transported from thousands of miles away. If a plant that can easily be grown in a backyard can treat a deadly disease like malaria effectively, there is no need to turn to expensive drugs.

In order to provide a consistent test case for the "relational efficacy" system, I conducted interviews and ethnobotanical field work in 2001 to 2004 with the Itza Maya, Qek'chi Maya, and mestizos of Northern Guatemala; the Asháninka of Paititi in Amazonian Peru; and the Malinké of Kita in Western Mali, collecting plants traditionally used to treat malaria, leishmaniasis, Chagas' disease, African sleeping sickness, asthma, diabetes, eczema, and uterine fibroids. The plants collected during this fieldwork were preserved, brought back to the NY herbarium for identification, to the CUNY Lehman

College phytochemistry lab for crude extraction, and the extracts were then tested for efficacy in several bioassays at Lehman; the Swiss Tropical Institute in Basel, Switzerland; and at the Rutgers University phytochemistry lab. The relationships among the plants collected, among the seven diseases, and among the ethnic groups being studied were determined from the literature and phylogenetic analysis of the plant groups involved. The relatedness values derived from this were fed into a computer program I wrote to synthesize all these values into a potential efficacy value for each species and disease using the equations discussed herein. These potential efficacy values were then compared to the efficacy values derived from the bioassays to determine if there was a correlation between the two measures, as a way of testing the predictive power of the "relational efficacy" system. This analysis showed there was a significant correlation between the calculated potential efficacy and the bioassay-derived efficacy for the particular way relatedness was measured, but only for one of the diseases, leishmaniasis. The hit rate was increased using relational efficacy over random screening for two of the diseases: by four times for leishmaniasis and by two times for malaria. The prerequisite to the relational efficacy hypothesis was also found to be true: the Asháninka and Malinké had significantly more similarity in their medicinal flora than in their overall flora. This implies that both cultures are focusing on similar taxa for medicinal uses. Though the potential efficacy did not correlation significantly to bioassay data for all the diseases, this may have been due to the small sample size, the relatedness measures used, or the structure of the modeling formulas, and these can be improved upon over time until it becomes a useful tool.

Several "spinoff" research results and applications ranging from field collection and interview methods to analytical techniques and ways of protecting and preserving indigenous knowledge were derived from the research discussed here:

- verification of the concept that it is better to describe disease symptoms than to name a disease in order to collect more accurate information on effective medicinal plants from collaborator interviews;
- programs to easily and quickly calculate species accumulation curves in the field to improve collection efficiency;
- methods of maintaining confidentiality of intellectual property information from ethnobotanical herbarium vouchers; and
- “talking books” for returning results to non-literate collaborators.

1.1. Naming diseases vs. describing symptoms

There is an often-stated hypothesis among ethnobotanists that when one wants to collect more accurate diagnoses and plant remedies from collaborator interviews it is better to discuss symptoms in the local medical system than to name diseases (Alexiades 1996), e.g., asking about a condition during which ants are attracted to the area where someone urinates (because of the extra sugar in the urine) will mean that collaborators will give the researcher plants that better treat diabetes than if they simply ask, "what plants do you use to treat diabetes." By first asking collaborators if they knew the name of and plants used to treat a disease describe in locally applicable symptoms and only then naming the disease if they did not use the known local disease name and using the efficacy of both sets of these plants (described disease and named disease plants) derived from assays and the "relational efficacy," I was not able to verify this hypothesis, finding

no significant difference between the these two categories' efficacy. On the contrary, the named remedies for leishmaniasis had nearly significantly higher efficacy ($p < 0.07$) than remedies found by describing leishmaniasis symptoms.

1.2. Species Accumulation Curves

A consistent effort of biological field collecting does not give a constant rate of unique collections since at the beginning of any collection there are many unique species found, while as collections progress more and more species will not be unique. The curve plotting this return versus effort is called the collector's curve or species accumulation curve, and seeing and analyzing this graph can be a great aid to planning future collections most efficiently. This is most effective if the curve can be calculated in the midst of collections, but this is not always possible since the calculation requires specialized software and training. In an effort to simplify this calculation while in the field, I have written a short program that runs within the ubiquitous Microsoft Excel program that many people have on the laptops they bring into the field with them. This should improve the efficiency of ethnobotanical and other biological collections, which is becoming increasingly urgent with the rapid global disappearance of both species and cultures

1.3. Confidentiality of Herbarium Specimens

Once plants are collected, preserved, and analyzed, in order to keep intellectual property information derived or inferred from ethnobotanical voucher specimens confidential, scenarios and methods were developed with my advisor Doug Daly and the NY herbarium staff to control access to both physical and digital herbarium records. This

was done in an effort to honor the promises not to publish or reveal species names and uses, conditions specified in prior informed consent agreements with my collaborators in Mali and Peru. This is somewhat new ground for herbaria, as previously many have allowed access without much screening to their collections by botanists with academic affiliations. . Screening researchers, having them sign confidentiality agreements, keeping certain specimens in separate locked or closely monitored cabinets, leaving certain information off labels, and having special access controls on or blocking certain fields in herbaria databases are each options that can help stem the unintentional revelation of collaborators' intellectual property.

1.4. Talking Books

In order to further protect and preserve traditional plant knowledge, using the "relational efficacy" analysis and the original information from collaborator interviews I developed weather-resistant, solar-powered “talking books” with images of each plant discussed in interviews. When the button next to each image in the book is pressed, an audio message recorded in the community's native language explaining how to prepare and use that plant is played. These talking books as well as regular printed books with the same information were given to the communities as a way of helping maintain their traditional language and medicinal plant knowledge, in which the communities have shown great interest.

Each of these spinoff research topics and results is as important to and useful as the main hypothesis of this dissertation, which all aim to make the most efficient use of field and laboratory time. These are both limited and expensive resources that need to be used judiciously while speeding the discovery of new plant-based medicines.

1.5. Chapter outline

This dissertation describes the entire process of the research undertaken, from the theoretical background of the quantitative system to a brief ethnography of the collaborators' groups, field and lab work, literature search, and the final analysis and synthesis. The chapters are laid out as follows:

Chapter 2, Theoretical Background, explores previous quantitative ethnobotany and the phylogeny of plants, diseases, and cultures. Methods of measuring the relations among these three elements using the phylogenies allows the data from of the study to be synthesized into a potential measure of medicinal efficacy for each plant.

Chapter 3, Ethnologies, reviews the ethnography and ethnology of the groups involved in this study– the Asháninka of Peru, the Malinké of Mali, and the Itza-Maya of Guatemala– especially their views towards plant medicine, derived from the literature and my own relatively short experience with them. This chapter also describes how each group has explained to me how they discover new medicinal plants and the possibility of medicinal plant knowledge transfer between these cultures.

Chapter 4, Field Methods, explains the prior informed consent agreements with my collaborators, collection and export permits, structured collaborator interviews, collection methods, field identification, in country herbarium deposits, the development and use of the species accumulation curves.

Chapter 5, Laboratory Methods, explains the extraction and preservation of the bulk samples, the antiparasitic assays that were performed on the extracts, and the analysis of the difference in efficacy of the Asháninka and Malinké plant remedies, as well as the efficacy of remedies derived from named vs. described diseases.

Chapter 6, Systematic Methods, delineates the identification methods used in the home herbarium at the New York Botanical Garden; how dated phylogenies from the literature were used to assign relatedness measures of different species, diseases, and cultures; how species were determined to be either native or introduced to the areas where they are used; methods used to maintain the confidentiality of species and plant uses; and the use of herbarium vouchers.

Chapter 7, Integration of Data and Analysis, explores how the statistical programs were developed to synthesize all the relatedness measures derived from the above phylogenetic methods in order to produce a list of medicinal plants ranked by their predicted efficacy, while allowing for uncertainty in species identifications and synergy of plants in mixed plant remedies.

Chapter 8, Conclusions, assesses the usefulness of the "relational efficacy" technique and the associated methods developed as part of this dissertation to ethnobiology. Future verification, extensions, and predictions of these methods are discussed as well.

Appendix A, Returning Results and Talking Books, discusses returning results to the communities, including the talking books for non-literate community members.

Appendix B.Sample Herbarium Confidentiality Agreement, delineates a potential agreement with a herbarium to ensure the confidentiality of herbarium specimens, mentioned in Chapter 6.

The hope of all of this work is to introduce new ethnobiological techniques to the search for new, effective medicinal plants that will increase hit rates, make the work more efficient and productive, preserve rapidly disappearing cultures and languages, and aid in improving health in developing countries, where this is sorely needed. This the main

impetus for focusing on diseases like malaria, leishmaniasis, Chagas, and asthma, is that they disproportionately afflict the world's poor and less money is given to researching treatments for these diseases in the U.S., as compared to cancer and heart disease. With the judicious use of ethnobotanical research and applicable technology, perhaps a small dent can be put in the effect of these deleterious diseases.

Chapter 2. Theoretical foundations of relational efficacy

Ethnobotanically guided searches have been touted as the best way to find new medicinal plants quickly as compared to random sampling of plants, yet there have been no wonder drugs found this way to reach FDA approval in the last 38 years since vinblastine was discovered from the Madagascar Rosy Periwinkle. This is most likely due to inaccuracies of the bioassays involved and the huge expense of taking a plant from its raw form, verifying its efficacy, elucidating the responsible compounds, and turning this into a synthesizable pharmaceutical. To cut down on the huge input of information to this screening system, we need a way to pinpoint the potentially most effective medicinal plants. What better way to do this than to take advantage of that actual flood of information of hundreds if not thousands of new medicinal plants that show some kind of positive indication from ethnobotanical reports every year that are waiting to be tested, by synthesizing all the existing relational data on these plants and the diseases they are used to treat?

Though it may seem overwhelming, this wealth of information on case reports of traditional uses of medicinal plants can actually be mined for the relationships of the medicinal plants, the diseases they treat, and the cultures that use them. Surprisingly, this information is usually discarded in merely picking the most popular plant to test next in the laboratory, but by integrating all this information we can cut down on the number of plants that need to be tested in the laboratory by pre-selecting the potentially most effective ones due to their relations with other plants, other diseases treated with the plants, and other cultures using these plants medicinally. To this end, a new mathematical

framework called "relational efficacy" is proposed here that attempts to predict the efficacy of medicinal plants based on the idea that if cultures that are not closely related, and therefore have most likely never communicated plant uses, happen to be using closely related plants to treat related diseases, it is more probable that they have independently discovered effective disease-treating compounds in the plants. It is these plants we should analyze first to save on limited laboratory time and resources. The ethnobotanical background and theory behind relational efficacy is described below.

2.1. Background

The field of ethnobotany is moving towards hypothesis-driven analytical research in recent years and away from simple inventories and descriptive work (McClatchey and Bridges 2002). As part of this movement, quantitative ethnobotany is an increasingly useful field that is necessary both for analyzing the huge (and growing) amounts of plant use data being generated (But, Hu et al. 1980; Trotter and Logan 1986; Moerman 1991; Lawrence, Phillips et al. 2005) and for improving the rigor and validity of ethnobotany as a science (Phillips and Gentry 1993). In general terms, Lewis et al. (1995) have declared the success of ethnomedically-directed searches for new medicines from plants, noting as an example that 30% of the plant species collected using anti-infective ethnobotanical leads were found to have anti-HIV in *in vitro* tests (the "hit rate"), vs. only 8.5% for random screenings where every plant seen is collected for testing. The hit rate went up to 71.4% when plants used traditionally as antivirals were tested vs. the more general anti-infective category. They stated the need for more of this type of research, including the search for more antimalarial plant compounds. The recent unique success with finding the as yet not-FDA-approved anti-HIV drug prostratin in a Samoan medicinal plant

Homalanthus nutans (G. Forst.) Guill. [Euphorbiaceae] (Balick and Cox 1996) and the antidiarrheal compound crofelemer from the Western Amazonian plant *Croton lechleri* Müll. Arg. [Euphorbiaceae] (Ubillas 1994) shows that there is hope for finding new medicines for epidemic diseases via ethnobotany while upholding indigenous intellectual property rights. McClatchey (2005), however, explains that despite successes like prostratin, modern bioprospecting from ethnomedical sources has largely failed and calls for better methods of analyzing and sharing of traditional medicinal plant knowledge. The goal of the "relational efficacy" quantitative technique described here is to raise the hit rate above even the 30% seen with ethnobotanically-directed medicinal plant searches, i.e. to find more effective medicinal plants per plant analyzed.

Several promising techniques and conclusions have already arisen from quantitative ethnobotany: targeting medicinal plants for drug development that are in families with above-average ratios of traditionally used medicinal species per total species in the family (Moerman 1991) by using residual values in a medicinal species vs. total species per family linear regression; showing how different cultures actually use rational (non-random and empirical) approaches in emphasizing certain taxa for their herbal remedies by focusing on plants with certain growth habits and ecology or in certain active families (Moerman 1991; Kapur, Shahi et al. 1992) ; and using informant consensus – the number of healers who agree on a particular plant use – to corroborate the usefulness of certain plants and remove some uncertainty from collaborator interviews (Friedman, Yaniv et al. 1986; Trotter and Logan 1986; Phillips and Gentry 1993). Albuquerque et al. (2006) have shown how two indices, use values (Trotter and Logan 1986) and relative importance values, correlate when applied to the same data set, but diverge in certain cases because

relative importance emphasizes the absolute number of use instances and the use value emphasizes informant consensus. The relational efficacy index proposed here tries to combine these two approaches and others into one coherent measure by integrating cross-cultural and intra-cultural informant consensus as well as the disease-treating and plant-phylogenetic consensus.

Andrade-Cetto et al. (2006) have introduced an interesting extension to informant consensus they call "disease consensus," which despite its name analyzes how multiple informants agree on and have knowledge of medicinal plants to treat one particular disease (not between several related diseases). This index tries to get around some of the inconsistencies of standard informant consensus techniques, so although it would be relevant to include as a weighting factor in the relational efficacy measure, it has yet to be definitively corroborated by other established indices or bioassays of disease treating efficacy. Reyes-García et al. (2006) have compared eight common indices of traditional ecological knowledge and found them to correlate fairly well. Some of these indices can be independently validated since they are simple counts of known species or interactions (e.g. ecological cultural knowledge), giving some external validity to the other indices.

Johns et al. (1990) have proposed a very interesting quantitative system for determining those plants in an ethnobotanical survey with the highest medical potential, based on a log-linear model that teases out what is called the “interaction effect,” which is what is left when the higher likelihood of finding a common plant treatment for a common disease is controlled for in a matrix of plants and their medicinal uses. In other words, Johns et al. claim that this residual amount, left over when the probability of encountering common plants and common diseases is subtracted out, explains the real

efficacy of the plant medicine, a bit like Moerman's (1991) residuals for medicinally speciose families. Although they call for verification by comparing the interaction-effect potential with efficacy determined by bioassays or current literature, they only attempt this qualitatively, not putting numbers on the medical efficacy of the plants found in the literature. Their work has been cited often (e.g. Begossi 1996; Bruni, Ballero et al. 1997; Galeano 2000), and the original authors have used this model in further studies (Johns, Mhoro et al. 1994; Johns, Mahunnah et al. 1999), although they seem to reject the model for lack of statistical significance (Johns, Faubert et al. 1995). One shortcoming is that Johns et al. never defend their choice of a log-linear model to describe people's choices of medicinal plants. They also perform a sort of cross-cultural analysis with their results, noting that the top ten potential plants they have found are used similarly in many diverse cultures, but again, the cross-cultural aspect of this analysis is not quantitative.

Browner et al. (1988) have designed a system that allows quantitative cross-cultural comparisons of medicinal plant treatments by determining through biomedical literature searches which of the plants used by several cultures for a particular disease have been shown to have some biochemical effect on the symptoms or causes of that disease. This is an enticing approach, combining a scientific and a cultural viewpoint while analyzing both a local cultural disorder, *susto*, and more physical female reproductive disorders, but their reliance on existing biomedical and biochemical literature for verification of all their medicinal plants means that rating and comparing plants that have not been studied in the laboratory is quite difficult. Juan et al. (1991) have devised a quantitative method of finding similarities in traditional herbal medicine systems of Asia using statistical clustering algorithms on the plants used by each system to treat a set of diseases, but have

stated that more innovative and broad methods are needed. Mace and Pagel (1994) have formalized cross-cultural comparisons using methods borrowed from systematics, mapping out cultural traits such as plant use on language-based cultural phylogenies to determine if these traits are basal or derived. Ostraff (1995) uses fuzzy clustering algorithms to look at how *tapa* cloth knowledge moves among several Polynesian islands. Weiss (1998) shows how clustering algorithms can be used to find similarities in disease etiology and medicinal plants between the divergent traditional medicine of China and the Chatino of Mexico, elucidating some similarities in their concepts of disease causation.

Bennett and Prance (2000) discuss related disease systems in deriving their species importance values from the number of body systems on which a medicinal plant species works and the number of pharmacological actions attributed to it, but this does not incorporate how these disease systems or actions are related. Moreover, the mainly non-quantitative techniques described mainly allow only comparing and describing differences between cultures and their remedies, not the synthesis of several cultures' knowledge to pinpoint the potentially most-effective herbal remedies. Additionally, no one yet seems to have combined these methods of plant, disease, and cultural relatedness into one analytical system as proposed here.

2.2. Plant knowledge communication

The ultimate goal of this research is to develop a set of formulas that will give us an estimate of the disease-treating potential of each plant species studied. Those plants with the highest potential would be the best candidates for undertaking the lengthy and expensive process of exploring their efficacy, phytochemistry and mechanisms of activity

in the human body in the lab and in clinical trials, increasing the hit rate and lowering the cost of finding and testing new botanical medicines. This measure should be reproducible among different investigators and therefore objective and even useful in predicting the potential that a certain species for which medicinal use data has not been collected may have for treating a certain uninvestigated disease.

One assumption of this technique is that the *less* related the cultures in the study are, such as Mali and Peru vs. Guatemala and Peru, the *less* chance those two cultures have had of communicating medicinal plant knowledge. If several unrelated cultures use closely related plants to treat the same disease, these discoveries of the effectiveness of the plants are *more* likely to be independent, and these plants should therefore be considered to have a *higher* potential than other plants that may be used for that disease in only one culture. To assess this assumption, the processes by which knowledge of medicinal plants is disseminated among cultures when different cultures interact and possibly intermingle needs to be well understood. Does the culture to which another culture migrates pick up a significant portion of the medicinal plant knowledge of the other culture? Johnson (2006), Palmer (2004; 2004), Campos et al. (2003), and Cox (1991) have discussed these mechanisms of medicinal plant knowledge transfer, but this needs to be quantified on a more global basis. Lenaerts (2006) confirms the concept for closely related cultures that medicinal plants are not merely selected by how effective other groups believe them to be by showing that Peruvian Amazon indigenous groups like the Asháninka do not borrow medicinal plants based on the plants' efficacy from nearby groups such as the Shipibo, but rather based on each group's relationships with and respect for their neighboring groups and their medicinal plant knowledge (i.e. the

esteem they hold for their neighbors), with the caveat that the biomedical efficacies of the medicinal plants were not tested in the laboratory as part of this research. This intercultural exchange of medicinal plants that do not undergo long-term experimentation in the culture that adopts these plants can confound the effects of experimentation that leads to acceptance of the most effective medicinal species.

The ratio of medicinal species to total species in each plant family has been used in the past to make cross-cultural comparisons of medicinal plants, contrasting the medicinal flora of Jammu and Kashmir, India with that of the North American Indians (Kapur, Shahi et al. 1992) by comparing Moerman's (1991) plant family residual values. Heinrich et al. (1998) made some simple cross-cultural comparisons of Mexican indigenous groups and said that selection of plants in traditional medicine is definitely not random. Rather, a rational process of experimentation and exchanges between cultures goes on, sometimes up to a 70% exchange of medicinal plants, as in the example of the Gitksan of Western Canada and their neighboring groups (Johnson 2006).

Many of these studies have been ad hoc, asking only whether the two cultures are connected or not, instead of how connected they are, thereby losing some of the information in their analysis of the measure of relatedness of cultures. There is an important quantitative difference between two neighboring groups in Peru using similar plants to treat a disease, and groups in Peru and Mali using similar plants to treat the same disease. The latter case is much more suggestive that the two cultures independently discovered similar plant uses, and that this was not communicated plant knowledge as in the example of the Gitksan (2006). Campos and Ehringhaus (2003) have found that a quarter to a third of species-specific plant uses of two indigenous groups in the Brazilian

Amazon, the Kaxinawá and Yawanawá, have been acquired from neighboring non-indigenous *seringueiros* (rubber tappers) or *ribeirinhos* (river dwelling people), so there is clearly influence of non-native cultures in addition to non-native plants. Cox (1991) claims that much of Polynesian herbal medicine is an indigenous tradition, although there are some introductions, and that 66% of medicinal plants used in Polynesia are not used elsewhere, and are therefore unlikely to be plants introduced by Europeans, while 34% have some use outside of Polynesia. In various studies analyzed between 1838 and 2002, Palmer (2004) found that anywhere from 14-53% of medicinal plants used in Hawai'i were species introduced from Polynesia, although this is different from introduced *uses*. These figures contrast Johnson's much higher 70% shared medicinal plant use figure, perhaps because of the greater cultural and geographic proximity of the Gitksan and their neighbors. If this degree of relations of the cultures being studies can be quantified as I am proposing, it can give us much more information about how much medicinal plant knowledge the cultures would naturally share.

The possible explanations for two different cultures using similar plants to treat related diseases are:

1. The two cultures have independently discovered that these two related plants treat the diseases effectively through experimentation and have not communicated these uses to each other. This explanation best fits the stated theory.
2. The two cultures have independently decided to use these two related plants to treat the diseases, but one or both of the cultures has used the plants only for a short time, without much experimentation, and therefore there is less evidence that these plants are medicinally effective.

3. The two cultures have independently decided to use these two related plants to treat the diseases through the doctrine of signatures, which is a common method of medicinal plant discovery around the world (Bennett 2007), and the related diseases are likely to effect the same organ system and the related plants are likely to look the same.
4. The two cultures have communicated to each other this medicinal plant use through immigration, literature, or other media moving from one culture to another.

The reason that it is important to look at less related cultures is that with increasing distance between cultures, the probability of option 4 goes down and the probabilities of options 1, 2, and 3 increase, with less possibility of communication. The ratio of options 1, 2, and 3 to each other is unclear, but asking questions such as how long a medicinal plant has been used during interviews helps to increase the probability of option 1 vs. options 2 and 3, as there has been more time for experimentation and verification with a particular plant remedy. Using informant consensus techniques during interviews about a medicinal plant (Trotter and Logan 1986) can act as a stand-in for the length of use of the plant remedy as a higher informant consensus value indicates that the plant has been better tested by the community, again increasing the probability of option 1.

Giving many clear examples, Bennett (2007) has proposed that the doctrine of signatures is a mnemonic method for remembering many medicinal plants, rather than a method of choosing medicinal plants merely based on their signatures. This implies that plants to which the doctrine of signature applies are actually quite well tested and known to be effective, rather than being chosen merely because they resemble the disease or

affected organ. Accepting this conclusion would lead to option 3 being less of a confounding factor, as the plants would be well tested as in option 1.

2.3. Mathematical background

The hypothesis of this dissertation is that in a database with N_s species, N_d diseases, and N_c cultures, the potential of a certain species *s*, from one culture *c*, to treat a certain disease *d*, ($P_{s,d,c}$), should increase with greater phylogenetic proximity of other plants *s'* used to treat related diseases ($R_{s,s'}$), increase with greater etiological proximity of the disease *d'* treated by related plants ($R_{d,d'}$), and increase with less phylogenetic proximity of cultures *c'* using related plants to treat related diseases ($R_{c,c'}$), but it should not increase solely by increasing the size of the dataset. These relatedness factors, discussed further below, would have a value of 1 for two plants, diseases, or cultures that are exactly the same, and would decrease towards 0 as they became less related, e.g., 1/time to their most recent branch point on a phylogenetic tree. Thus we assume that the less related or connected two cultures are, the more likely their discovery of related plants to treat related diseases is an independent event and therefore should increase the plants' medical potential.

The basic formula for the potential $P_{s,d,c}$ of species *s* to treat disease *d* in culture *c* proposed to meet these conditions is:

$$P_{s,d,c} = \frac{1}{N_s N_d N_c} \sum_{s',d',c'} \frac{R_{s,s'} R_{d,d'}}{R_{c,c'}} \qquad \text{Eq. 1}$$

where the relatedness factors are summed over all species, diseases, and cultures where species *s* is used to treat disease *d* in culture *c* and species *s'* is used to treat disease *d'* in culture *c'*. N_s is the number of species, N_d is the number of diseases, and N_c is the

number of cultures. If a species is not used to treat a disease it does not add to the potential, nor however does it subtract, as it is difficult to make the negative assertion that a particular plant is never used to treat a disease; more interviews may reveal that use. The numbers of species N_s, diseases N_d, and cultures N_c are divided out to normalize the equation and ensure that the potential of a plant does not increase solely by increasing the sample size. The plant species and disease relatedness values are in the numerator so that the plant's potential increases with *higher* plant and disease relatedness, and the culture relatedness value is in the denominator so that the plant's potential increases with *lower* culture relatedness. It must be emphasized that this is merely an ad hoc formula proposed to meet the assumptions of the hypothesis, but the actual equation will have to be modified as in Equation 8 with weighting factors, power factors, and/or constants added to it to model the actual data as closely as possible.

The potential could be summed across all cultures to find the universal potential $P_{s,d}$ of a species *s* to treat disease *d*:

$$P_{s,d} = \frac{1}{N_c} \sum_c P_{s,d,c} \quad \text{Eq. 2}$$

where N_c is the number of cultures involved. These potentials could be summed over all diseases to determine the universal potential of species *s*:

$$P_s = \frac{1}{N_d} \sum_d P_{s,d} \quad \text{Eq. 3}$$

where N_d is the number of diseases. Further reductions of this potential are possible:

$$P_{c,d} = \frac{1}{N_s} \sum_s P_{s,d,c}, \quad \text{Eq. 4}$$

$$P_d = \frac{1}{N_s} \sum_s \frac{1}{N_c} \sum_c P_{s,d,c}, \quad \text{Eq. 5}$$

and

$$P = \frac{1}{N_s}\sum_s \frac{1}{N_d}\sum_d \frac{1}{N_c}\sum_c P_{s,d,c} \qquad \text{Eq. 6}$$

where N_s is the number of species, $P_{c,d}$ is the potential of culture c to treat disease d, P_d is the potential of disease d to be cured by any herbal remedy in the dataset, and P is the overall potential of an entire study. This study potential P is a possible way to compare different studies overall success.

Potentials could be summed over all the species in a family or other taxa to determine the values of a given family, P_f, of course normalized to the number of species in the family, or using other techniques such as residuals (Moerman 1991):

$$P_{f,d} = \frac{1}{N_{s-family}}\sum_s^{family} P_{s,d} \qquad \text{Eq. 7}$$

where $N_{s,f}$ is the number of species in the family. This should correspond well to previous studies' pinpointing of families with high probability of usefulness or effectiveness for medicinal plants.

Informant consensus techniques could be used within each culture studied to determine reliability weights w_s for each culture that can then be used when summing potentials across cultures or for each plant use:

$$P_{s,d,c} = \frac{1}{N_s N_d N_c}\sum_{s',d',c'} w_{s'}\frac{R_{s,s'}R_{d,d'}}{R_{c,c'}} \qquad \text{Eq. 8}$$

Alternatively, if informant consensus values are not available for a particular species, disease, or culture because only one or a few healers were interviewed, the normalized length of time the plant remedy has been used by the healers can act as a stand-in to represent how well tested the remedy might be:

$$P_{s,d,c} = \frac{1}{N_s N_d N_c} \sum_{s',d',c'} \frac{t_{s',d',c'}}{t_{\max}} \frac{R_{s,s'} R_{d,d'}}{R_{c,c'}} \quad \text{Eq. 9}$$

Where $t_{s',d',c'}$ is the average reported (in interviews) length of time that species *s'* has been used to treat disease *d'* in culture *c'* in a particular time unit (most likely years), while t_{max} is the maximum amount of time in the same units that any plant has been used in the entire dataset. This would ensure internal consistency within cultures by giving a higher weight to plants that have been used longer and improve the accuracy of the data by raising the probability of experimentation and validation within a culture for a particular plant use. If available, informant consensus values would be more accurate as an unbiased percentage of informants who spontaneously mention a plant use vs. the length of time used, which is self-reported and therefore more prone to errors as a weighting measure. In my own interviews, I asked each healer how long they in particular had used each remedy and how long they remembered it being used by people in their village; this was a backup in case the total number of healers interviewed was too low to use informant consensus on any one remedy. In cases where only a few healers recognized a disease, the informant consensus would most likely not be valid, and the length-of-time-used measure would be used instead for weighting.

An example is in order here to demonstrate how these formulae work. Take diseases *X* and *Y*, and the plant species *A*, *B*, and *C* used to treat them in cultures *M* and *N,* as illustrated in Table 2.1.

Table 2.1. An example of uses of plant species A, B, and C to treat diseases X and Y by cultures M and N, with a '+' if there is a record of a plant being used to treat that disease in that culture and a '-' if there is no such record.

Culture M			
Plants	A	B	C
Diseases			
X	+	+	-
Y	-	+	+

Culture N			
Plants	A	B	C
Diseases			
X	+	-	-
Y	+	+	-

If the relatedness between plants is defined as $R_{AB} = 0.5$, $R_{AC} = 0.7$, and $R_{BC} = 0.5$; the relatedness between cultures is $R_{MN} = 0.75$; and the relatedness between diseases $R_{XY} = 0.3$, then

$$P_{A,X,M} = \frac{1}{N_s N_d N_c} \sum_{c'=M}^{N} \left(\sum_{d'=X}^{Y} \left(\sum_{s'=A}^{C} \frac{R_{A,s'} R_{X,d'}}{R_{M,c'}} \right) \right) =$$

$$\frac{1}{N_s N_d N_c} \left(\frac{1}{R_{M,M}} \left(R_{X,X}(R_{A,A} + R_{A,B}) + R_{X,Y}(R_{A,B} + R_{A,C}) \right) \right.$$

$$\left. + \frac{1}{R_{M,N}} \left(R_{X,X}(R_{A,A}) + R_{X,Y}(R_{A,A} + R_{A,B}) \right) \right) =$$

$$\frac{1}{3 \cdot 2 \cdot 2} \left(\frac{1}{1} \left(1(1 + 0.5) + 0.3(0.5 + 0.7) \right) \right.$$

$$\left. + \frac{1}{0.75} \left(1(1) + 0.3(1 + 0.5) \right) \right) = 5.06$$

and so on through the table, yielding Table 2.2 for the calculation of $P_{s,d,c}$.

Table 2.2. The calculation of the disease-treating potential $P_{s,d,c}$ for the hypothetical example of plant species A, B, and C being used to treat diseases X and Y in cultures M and N.

Culture M			
Plants	A	B	C
Diseases			
X	0.316	0.268	0.230
Y	0.313	0.346	0.312
Plant total	0.629	0.614	0.542
Normalized ($1/N_D = 0.5$)	0.314	0.307	0.271

Culture N			
Plants	A	B	C
Diseases			
X	0.328	0.271	0.272
Y	0.333	0.354	0.324
Plant total	0.661	0.625	0.596
Normalized ($1/N_D = 0.5$)	0.330	0.313	0.298

Table 2.3. The calculation of $P_{s,d}$, found by summing and normalizing the calculations of $P_{s,d,c}$ of culture M and culture N using plant species A, B, and C to treat diseases X and Y.

Both Cultures			
Plants	A	B	C
Diseases			
X	0.322	0.269	0.251
Y	0.323	0.350	0.318
Plant total	0.645	0.619	0.569
Normalized ($1/N_D = 0.5$)	0.322	0.310	0.284

When these two tables are summed and normalized for the number of cultures (2), this yields Table 2.3 for $P_{s,d}$. From Table 28 we can see that species *A* has the highest

potential (0.322) to treat disease *X*, and species *B* has the highest potential (0.350) to treat disease *Y*, while species *A* has the highest overall potential (0.322) and would probably be the first of the three species we would want to analyze in the laboratory.

If disease *Y* had not been studied in this example, the potentials would have come out as $P_A = 0.291$, $P_B = 0.240$, and $P_C = 0.240$, which we can see is not much different from the normalized row (divided by the number of diseases) in the above table. The potentials have the same rankings of the species and the same magnitude. This shows how the potential is not affected by the size of the dataset (the total number of diseases studied, in this case). Species *A* still scores as having the highest overall potential of the three.

2.3.1. Synergy

Mixtures of plants are found in many herbal medicines (Balick, Kronenberg et al. 2000) and the synergy involved in plant mixtures is apparent, among many examples, in the Amazonian hallucinogenic drink *ayahuasca*. Usually this is a mixture of

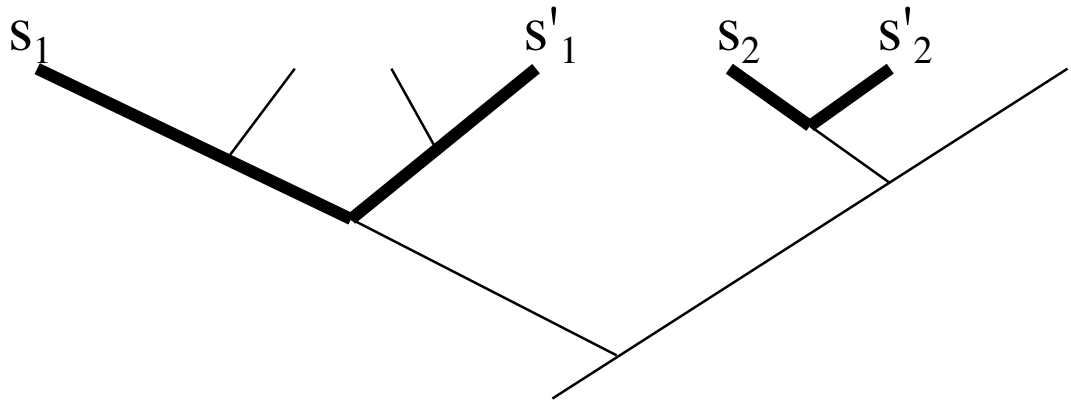

Figure 2.1. Species used in medicinal plant mixtures may come from similar phylogenetic clades, reinforcing the idea that they are adding similar synergistic phytochemicals to the mixture. Species s_1 and s_2 are from mixture A, and s_1' and s_2' are from mixture B.

Banisteriopsis caapi (Spruce ex Griseb.) C.V. Morton [Malpighiaceae] containing the monoamine oxidase inhibitors (MAOI) harmine and harmaline, and *Psychotria viridis* Ruiz & Pav. [Rubiaceae], containing the endogenous neurotransmitter dimethyl tryptamine (DMT). Neither plant would have much effect ingested on its own, as the DMT gets broken down in the digestive tract by monoamine oxidase (MAO). However, the MAOIs in the *B. caapi* blocks the breakdown effect of the MAO, allowing the DMT to enter the brain, creating one of the most powerful natural hallucinogens known. In another example of synergy among multiple active compounds in one plant, Lewis et al. (1999) found that the combination of two anti-malarial compounds from a Peruvian plant used by the Aguaruna had a 25-33% higher malarial-inhibition effect than the sum of the inhibitions of the individual compounds, i.e., over a quarter of the activity of this compound mixture was synergistic. Within one plant, 5'-methoxyhydnocarpin, found in several species of *Berberis* [Berberidaceae], stopped multiple drug-resistant pumps found in *Staphylococcus aureus* from pumping the antimicrobial berberine alkaloids, also found in these same *Berberis* species, out of the cell, the two compounds in combination being much more effective against the microbe than either compound on its own (Stermitz, Lorenz et al. 2000). Raskin and Ripoll (2004) give a good review of the many synergistic antifungal, antimicrobial, and other activities currently known for plants and say there is a great need for such synergistic plant medicines, for instance for multiple drug-resistant pathogens and AIDS. Even the United States Food and Drug Administration is accepting clinical trials of botanical drugs with multiple plant components in their Guidance for Industry Botanical Drug Products (U.S. Department of Health and Human Services and Food and Drug Administration 2000), a change from their former oppositional stance

towards botanicals and the difficulty of getting drugs approved with multiple components creating potentially complicating synergistic effects. With all these clear cases of powerful synergistic medicinal effects in plants, how can we deal with the confusing non-linearity of multi-compound and even multiple-plant mixtures with hundreds of potentially active compounds?

The plant potential equations above can be adapted to highlight cases in which plants are used synergistically, where plants from one phylogenetic clade are often present in a mixture along with plants from another clade, showing the former plants to be important admixtures even if they never appear alone as a medicine. This would imply that the compounds common in one clade are working together with compounds common in the second clade, one either reinforcing the other or subduing toxic side effects (see Figure 2.). As we previously compared sets of two species, one pair at a time, and then summed the calculated potentials over all possible pairs, so too we must start the plant mixture potential calculation by comparing two mixtures. As the simplest case, to compare a mixtures of two plants, s_1 and s_2, with another mixture of two plants s'_1 and s'_2, the equation would be

$$P_{(s_1 s_2),d,c} = \frac{1}{N_{s'_1 s'_2} N_d N_c} \sum_{(s'_1 s'_2),d',c'} \frac{R_{s_1 s'_1} R_{s_1 s'_2} R_{s_1 s'_2} R_{s_2 s'_2} R_{d,d'}}{R_{c,c'}} \qquad \textbf{Eq. 10}$$

with $R_{d,d'}$ and $R_{c,c'}$ being as above, R_{s_x,s'_y} being the relatedness of species s_x and s'_y, and $P_{(s1s2),d,c}$ being the potential of plant mixture s_1 and s_2 for disease d in culture c. This process can be extended to mixtures of n plants with the equation

$$P_{(s_1 s_2 \cdots s_n),d,c} = \frac{1}{N_{s'_1 s'_2 \cdots s'_n} N_{d'} N_{c'}} \sum_{(s'_1 s'_2 \cdots s'_n),d',c'} \frac{\prod_{x=1,y=1}^{N_s, N_{s'}} R_{s_x s'_y} R_{d,d'}}{R_{c,c'}} \quad \textbf{Eq. 11}$$

with the combinatorics of plant-plant relatedness products increasing rapidly with the number of the plants in the mixture. Yet this should still be a tractable way to pinpoint those plants that are aiding the action of another plant in a medicinal mixture, i.e., those plants that appear in high-potential mixtures but are not recorded as being used alone for the same disease.

2.3.2. Taxonomic Uncertainty

Often in ethnobotany, not all the medicinal plants collected cannot be identified to species with confidence; for example, collaborators may just give the researcher the ground-up leaves or roots of a plant to identify (Balick, Kronenberg et al. 2000). These data can still be used, however, by employing approximate randomization statistics methods. Most ethnobotanists eliminate data on plants they cannot fully identify, but if a plant is identified as a particular genus, or from the plant's common name it can be inferred that it is one of several possible unrelated species, this information can be used to derive medicinal efficacy potentials for the plant. Common-name uncertainty is much more difficult to use than uncertainty of several species within one genus, as the actual species corresponding to a common name could be in any of several disparate genera or families, or just completely misidentified by the collaborator.

The potential value calculated will of course not be as exact as if there is a species-level identification for the plant. Instead, it will have a range of values or confidence intervals derived using approximate randomization statistics techniques, where the

potential efficacy of each collected plant is calculated thousands of times by resampling from collected data to give potential values for the different combinations of possible plant species identifications. These thousands of calculated potentials are then used to find an average potential and an error range for those unknown plants. In the case of common-name uncertainty, if the dataset is small and the uncertain species are key to the potential calculations (i.e., when one of the possible species is used to treat a disease closely related to other diseases and is closely related to many other species in the dataset), the calculated potentials may be in several discrete ranges rather than one as the input species' relatedness values would be quite disparate. The more incompletely identified plants there are in a dataset, the more uncertainty there is (e.g., from many possible species corresponding to a common name), and the greater the error ranges of the plant potential will be, but this will still often be enough to rank it in a list of plants with the highest potentials. This usage of incomplete data in ethnobotany would be quite useful in many studies.

2.3.3. Non-native species

Medicinal plant uses that have been introduced into a culture from another culture must be eliminated from the data, as they are less likely to be *independent* discoveries of the same plant use. We do not want cases such as jambol (*Syzygium cumini* (L.) Skeels [Myrtaceae]) being used in European herbal medicine to treat diabetes, as this is a native of Asia and therefore a case of transmitted information and not independent discovery. This requires a good understanding of the introduced vs. traditional plant species and uses, and how this medicinal knowledge is disseminated. Introduced plant uses can be determined by comparing current plant use data gathered to any previous ethnobotanical

surveys done in the cultures being studied to see if their uses are changing over time. If the collaborators in the studied cultures describe these new uses as being from outside their culture, as Campos et al. (2003) has documented with the Yawanawá and Kaxinawá in Brazil, these should be considered introduced uses, rather than self-discovered uses, and eliminated from the data. However, Alexiades (Alexiades 1999) found that the Ese Eja identified some native species as being alien and vice versa, so care must be taken with this data, and multiple sources should be referenced. If no previous ethnobotanical surveys exist for these cultures, floras of the area which describe whether plants are native or introduced can be used to eliminate any uses of introduced plants from the data.

Newly-discovered medicinal uses of *introduced species*, as opposed to *introduced uses* of plants, might be worth keeping in the database if the two subtle cases can be differentiated, as they may be tested uses and just as valid as uses of native species. An introduced species being used in its introduced area A for a disease X related to the disease Y for which it is used in the species' native area B is even more ambiguous, as one must definitively determine that the culture of area A does not consider diseases X and Y to be related and therefore that this is an introduced use. Bennett and Prance (2000) discuss introduced medicinal species at length, saying they are well represented in the pharmacopoeia of northern South America. This is not to claim that introduced species are ineffective, but rather that it is difficult to say how long they have existed in a certain area and therefore whether they have been truly tested there and if this use is an independent discovery from the uses in the plants' native area. Clues such as local plant names and introduced species' ranges cannot clearly date the species' introduction and therefore the amount of experimentation with the plant.

The local plant names can be some indication of an introduced use, i.e., local plant names not in the local language provide some evidence, albeit not definitive, that the use has traveled along with the name from the plant's native area to the introduced area (Balée 2003; Johnson 2006). Introduced uses moving from one native area of a plant species to another native area of the species where there are local names in both areas complicate the situation further, as the use may transfer without the name. Therefore, it is important to look at the natural range and local names of the plants considered.

2.4. Plant relationships

The idea that certain taxonomically related plant groups (taxa) have a higher occurrence of medicinally active compounds in them we will call "taxon predominance" here. The measurement of taxon predominance is problematic because taxonomic rank is a somewhat arbitrary objective construct that leads to anomalies: when simply counting medicinal plants in certain families, it emphasizes larger families, and when finding percentages of medicinal plants in families, it emphasizes small families (Moerman 1991). Although these anomalies can be resolved somewhat by looking at residuals in predicted percentages, as Moerman has, there are still a few problems.

First, considering if two plants share a taxon is a binary question– are plants *A* and *B* in the same family or not? – when what we would really like is a continuous measure that would give us more information from which to derive descriptive statistics, i.e., how closely related are plants *A* and *B*? We would like to look at it as "clade predominance" rather than "taxon predominance," to retain that continuum of information. Second, these residuals are not normally distributed, making it difficult but not impossible to analyze

the significance of the differences in residuals as they violate the assumptions of standard statistical analyses (Lewis and Elvin-Lewis 2006; Manly 2006).

A randomization technique such as Monte Carlo simulation or approximate randomization statistics may get around the latter issue, and this has been done on a large database of Native American medicinal plants, yielding the conclusion that plant families with statistically significantly low or high numbers of medicinal species do not correlate with the families' species number and that most likely certain families evolved chemical adaptations suited to their ecological setting that lead them to be selected more often by the Native Americans as medicinal plants (Moerman and Estabrook 2003).

However, despite the statistical rigor of this last approach, the third problem is that looking at taxon predominance deals with taxa, which do not circumscribe consistent degrees of relationships at the same ranks (taxa levels), i.e., two plants that are in family *A* are not necessarily as closely related as two plants in family *B*. If a taxonomist prefers to split large plant families, two species in different families may in fact be more closely related than two species in the same genus in another large family, as Avise and Johns (1999) have discussed with the relatively recently evolved, small group of primates (7 species) being split across several families, compared with the speciose, earlier-branching fruit flies with 12 species being grouped into one genus. Avise and Johns have attempted to address this by proposing a system in which different taxonomic ranks would strictly represent a measure of evolutionary relations by indicating the time since divergence from the taxa's common ancestor. For instance, genera would be species groups that branched off from each other 5 million years ago, families would have a branch point of 20 million years ago, and orders 45 million years ago. This proposed system, however,

does not take into account different rates of evolution in different branches under different evolutionary pressures.

To implement this we would have to know the time of the evolutionary branch points for all described plants to be able to put them in the correct taxa in a system of time-determined rank, but this information is available for only a small percentage of known plants. One technique that has had some success in putting dates on evolutionary events uses molecular clocks to date divergence points, where the rate of DNA or RNA mutation since a divergence point is measured and calibrated against a known standard mutation rate (Renner and Won 2001; Richardson, Pennington et al. 2001). This seems to work best only in small plant groups, however, as the supposed constant rate of DNA mutation seems to break down across large groups, considering the fact that plants evolve at different rates at different times in history under different environmental conditions (Clegg, Gaut et al. 1994; Sanderson 1997). Inventive fixes for this variation can be performed, such as using plant fossil data and the proposed phylogenies of the flowering plants (Soltis, Soltis et al. 2000) to estimate (and often push back) the divergence date of sister plant taxa that are known to share a common branch point (Magallon, Crane et al. 1999). The "Deep Time" project (Soltis 2002; Sun, Ji et al. 2002), an attempt to bring together data from molecular and morphological systematics, paleobotany, and geology to date many of the angiosperm evolutionary divergence events, will collect much of the data needed in one place for finding plant relationships by their date of divergence from their most recent common ancestor. In the short term, the inaccuracies of this system would allow it to be used only for higher ranks such as families or orders, so for lower ranks another system is needed.

A simple technique for measuring the genes common to two plants, like *re-annealing* (Purves and al. 1998) where the time for separated DNA strands from the two plants to reconnect or "re-anneal" with each other is determined, could be performed for plants in this study. This is a fast procedure, and the re-annealing times derived from this could be used as a measure of plant relatedness, but the number of experiments necessary would quickly skyrocket as the combinatorics of comparing each plant to all the others in the study increases with large sample sizes. Comparing *n* plants would require *n!/(n-2)!2* physical experiments in the test tube, re-annealing each species' DNA with every other species' DNA, which for even 50 plants would mean 1225 physical comparison experiments.

Chemical fingerprints or metabolite profiles (Fiehn 2002) of all the plants in the study can be created quickly using high-performance liquid chromatography (HPLC) (Robinson 1991; Merken and Beecher 2000), which monitors the diffusion time and spectra of compounds in a plant extract as they diffuse in a solvent through a column filled with different substrates, or diffusion-ordered (DOSY) nuclear magnetic resonance (NMR) imaging (Delsuc and Malliavin 1998; Gostan, Moreau et al. 2004) which uses NMR to create a fingerprint of multi-compound plant extracts by mapping how these compounds diffuse over time under magnetic excitation. Either of these techniques would be a relatively easy way to avoid the proliferating combinations of plant comparisons in re-annealing, as the fingerprints for each plant are recorded on a computer in the form of diffusion spectra over time, where calculating the relationships among all the combinations of plant fingerprints could be done in minimal time. For 50 plants, only 50 physical readings need to be done with HPLC or DOSY NMR, and the 1225 comparisons

could be calculated in the computer using the output of these 50 tests, i.e., the data can be re-used. With re-annealing, every comparison of two plants must be done physically in the test tube.

The chemical-fingerprint approach to determining the relationships of plants has two advantages over a phylogenetic molecular-clock approach. First, a chemical comparison gets more directly at what we are looking for in the plants – are two plants sharing some secondary metabolites that would act in a similar way in the human body to treat a disease? – rather than using the proxy for metabolite similarity of genetic similarity that phylogenies represent. There are many steps (promotion, transcription, deletion, folding, and synthesis pathways, to name a few) that separate similar DNA from similar metabolites. Second, for plants for which there is no existing description of relationships – chemical, phylogenetic, or otherwise – it is much easier to derive metabolite similarity of a random sampling of plants from across the plant kingdom through fingerprints than to derive a dated phylogeny for these isolated plants without the context of their genera, families, and orders. The fingerprint approach has the disadvantage that one collection of a plant may only represent the metabolite fingerprint for that time of day, season, location, stress level or plant part, as metabolites can vary widely in the same species with all these dependent variables (Robinson 1991). This problem can be somewhat alleviated, however, by sampling the same plant part, time, and location as the collaborators do, since this represents the metabolites they are using in their herbal medicines, or by taking a cross-section of all the parts, times of day, seasons, and locations available to the healers and grouping all these samples of one species as one plant when doing the fingerprint, as a way to try to get all the possible metabolites that

might be present in this species over all conditions. The latter scheme may be impractical due to the immense amount of collection time necessary and arrive at the same combinatorial problem as we found with re-annealing, however,

Metabolite fingerprinting will work best with closely related plant species, as plants in different families or orders can often have such different secondary compounds that the extraction methods must be quite different and fingerprint data will have little or no similarities. However, the fingerprint method of determining plant relatedness will complement the dated phylogeny method, as dated phylogenies have been determined mainly for the broader scale of orders and families, but not between genera and species as of yet. Therefore, the dated phylogeny method of relatedness should be used to determine broader-scale relationships for those families for which it exists, and, if needed, the fingerprint method can be used to fill in at the smaller scale. A calculation of both phylogenetic and metabolite relatedness for the same set of species could be used to calibrate these different systems to each other, if there is some area of overlap.

Another assumption of metabolite fingerprinting is that phytochemicals are conserved across genera, families, or orders; there is a basis for this, as phytochemicals have been used in the past as a trait to create phylogenies in the field of plant chemosystematics (Harborne and Turner 1984). Many compounds are found throughout entire families or orders, such as cyclopentenoid cyanogenic glycosides found in the Achariaceae, Passifloraceae, Turneraceae, and Malesherbiaceae within the order Malpighiales; betalains in the Caryophyllales; and the sesquiterpene lactones common in the Asteraceae (Judd, Campbell et al. 1999). Some compounds are found only in certain genera, as with hypericin in *Hypericum sp*. [Clusiaceae] (Evans 1996) and betulin in the *Betula* genus

[Betulaceae] (Judd, Campbell et al. 1999). This most likely explains traditional peoples' tendency to concentrate their medicinal plants in certain families (But, Hu et al. 1980): they are realizing that some effects (Johns 1990) or tastes (Shepard 1999) of a group of plants are similar and therefore they are using other members of that plant group to treat their diseases as they most likely contain similar disease-treating components. Balunas (2006) has done an extensive analysis of how the percentage of active plants and average 50% effective concentration (EC_{50}) values of anticancer activity in plant species from around the world vary with the plant part, collection location, and plant family, showing the interesting trends that percent of active plants is not higher in areas with higher biodiversity, but is higher in the Clusiaceae, Elaeocarpaceae, Meliaceae, and Rubiaceae than other families and higher in roots and below-ground collections than above-ground collections.

2.5. Disease relationships

Little to no research has been done that considers the issue of treating related diseases with related plants. Some diseases in past studies may be connected, such as different types of infectious diseases like wound infections and thrush, which can actually be caused by different taxa of bacteria. If we look more deeply into the Western classification and causes of diseases (Isselbacher 1980), we realize that seemingly unrelated diseases may have the same underlying cause and be treated in similar ways. For instance, it would appear that eczema, diabetes, and asthma are very different diseases, but they are all in fact autoimmune syndromes– the body turning against and attacking itself, in one case in the skin, another in the pancreas, and the third in the respiratory system (Cookson 1999). Once again, for the proposed approach we need to be

able to measure the relatedness of the diseases, regardless of whether they are due to genetic, infectious, or environmental causes.

The relatedness of two diseases is perhaps the hardest of the three relatedness measures to delineate, as diseases did not all evolve from a common ancestor and therefore are not linked by a phylogeny as cultures and plants are. We can say that two different bacterial infections are closely related, but how can we say how closely a bacterial infection and sickle cell anemia are related? One is caused by an invading organism and the other by genetics. These disease categories can be analyzed separately, but as we will see, more accurate predictions can be made by finding a way to link the categories.

Exacerbating this problem is the fact that Western doctors classify diseases mainly by the body system affected, such as cardiovascular, brain, or bone diseases, because doctors use the symptoms within these body systems to diagnoses diseases rather than their underlying causes (Isselbacher 1980). Some diseases are grouped together by their causes, such as autoimmune diseases, but as this is usually not the case. One approach to linking diseases with their different base causes is to look for patterns in the existing medicinal plants that are laboratory-proven to effectively treat different diseases in order to reveal the related mechanisms of cause and treatment of diseases. Specht (1996; 1997) has done this type of analysis using cladistic computer programs using the parsimony algorithm to determine how plant families are related by the diseases they are used to treat (a method that could be termed pathotaxonomy, analogous to chemotaxonomy), and how diseases are related by the plant families used to treat them (which we will call "plant-based disease taxonomy" or PBDT).

There is some evidence of similar diseases being treated with closely related plants, as Lukhoda, Simmonds et al. (2006) have shown by looking at the problem pathotaxonomically that similar disease-treating characters (e.g., immunosuppressant, immunostimulant) group together on a phylogeny of *Plectranthus* species [Lamiaceae]. Senchina et al. (2006) performed a similar phenetic analysis of several *Echinacea* species [Asteraceae], showing that some of the immunomodulatory characteristics of the species align with one interpretation of *Echinacea*'s phylogenetic clades if both immune-boosting and immune-suppressing characteristics are taken into account, showing that we must look at not just one but rather many medicinal actions to see a correlation with the plant phylogenies, and to be able to use the plant phylogenies as an indication of a shared disease treatment mechanism or vice versa. Daly and Stevenson (1998) have extended the PBDT method to grouping diseases by the plant species used to treat them. In this case, if a subset of the same plants is used to treat the same diseases, the diseases are more likely to be caused by a similar metabolic system in the human body that is being affected therapeutically in similar ways by these similar plants. This technique uses algorithms borrowed from plant taxonomy to find patterns of related diseases where diseases are treated by the same plant. For instance, in Guatemala, the Neotropical herb *tres puntas* (*Neurolaena lobata* (L.) Cass. [Asteraceae]) is used there (personal observation, 2000) and shown in the laboratory to treat malaria, diabetes, and dengue fever (Franssen, Smeijsters et al. 1997; Lentz, Clark et al. 1998; Berger, Passreiter et al. 2001; Fujimaki, Kamachi et al. 2005). Therefore, under the shared-plant-treatments technique, these three diseases would be considered to have some relation, depending on other plants also used to treat these diseases.

In order to avoid circular reasoning while using the PBDT method to find disease relatedness values, the plants in the main study dataset should not be used to find disease relations, but rather plant species that have already been studied in the laboratory and have been shown to be effective against the diseases being studied. The NAPRALERT (Pharmacological Sciences (PCRPS), College of Pharmacy, University of Illinois) and MEDLINE (ProQuest LLC 2007) databases are good sources on laboratory and clinically tested medicinal plants for this technique. Using laboratory-proven medicinal plants to find these patterns could permit the creation of a broader disease taxonomy and estimate disease relatedness. If there is not a sufficient number of laboratory-tested plants to link the diseases being studied, the diseases analyzed would have to be limited to comparisons of groups of phylogenetically related diseases, such as among the infectious Protista kingdom parasite diseases (malaria, Chagas' disease, African sleeping sickness, and leishmaniasis). In fact, one good test of the PBDT technique is to compare the relationships it determines for the set of infectious Protista diseases to the phylogenetic relationships determined by systematists for the Protista species. If this comparison of the PBDT and phylogenetic methods of determining disease relatedness validates the PBDT method, it can be used to tie together all the diseases, otherwise the analysis should be limited to within the easily related disease classes.

A third option for determining disease relations is the relatively new genetic drug-disease connectivity map (Lamb, Crawford et al. 2006) derived from the human genome project data that show how diseases and pharmaceuticals that are used to treat them affect similar genes. This would only work for the diseases with a genetic basis or predisposition (uterine fibroids, eczema, asthma, and diabetes in this study) and a

measure of relatedness would have to be derived from the connectivity network. The relatedness values from this system can again be compared to the relatedness values derived from the PBDT as a way to validate and calibrate this method.

2.6. Cultural relationships

To determine cultural relatedness, it has been suggested to me (Rick Stepp, personal communication, 2000) to simply look at geographic distance between the two cultures, but this is problematic as geographic barriers such as mountains and oceans that slow the transmission of cultural knowledge are hard to factor in. Are the indigenous groups of southern Argentina and Chile really as similar to those of South Africa, at 7,100 km distance, as they are to the people of Costa Rica, also 7,100 km away? These cultural barriers are not very easy to quantify.

Alternatively, evolutionary language trees could be considered, as they are a fairly complete record of the intermingling of different cultures and passing of information such as herbal remedies. Glottochronology is a technique that can be used to date language phylogenies using word roots shared between languages, called *cognates* (Cavalli-Sforza, Menozzi et al. 1994), but glottochronology is not considered valid past 5,000 years ago for native North American languages (Foster 1996) and not past 6,000 years ago for Indo-European languages (Nichols 1997), including English and Hindi. This means that glottochronology would not work for the distant cultures of Peru and Mali being considered.

Cultural phylogenies have been developed based on multiple genetic comparisons that are probably valid past earlier dates. These genetic phylogenies match quite closely with language phylogenies and actually may be a better indicator of cultural knowledge

transmission than language phylogenies as languages can hybridize quite rapidly, e.g., creoles and pidgins (Cavalli-Sforza, Menozzi et al. 1994). Given that these genetic cultural phylogenies are dated, they can be used to calculate cultural relatedness by using a metric such as 1/time to the most recent common ancestor of the two cultures. This genetic cultural-relatedness method appears to be much more viable for the distantly related cultures under study than glottochronology, and is currently being updated with National Geographic's Genographic project (Underhill, Shen et al. 2000; Wells 2006) which should cover the Asháninka and Malinké groups that I have researched, groups that Cavalli-Sforza has not included.

2.7. Discussion

In the end, the best data on relationships will come from a combination of metabolite fingerprinting and dated phylogenies like "Deep Time" for plants; disease descriptions, phylogenetic relationships (where possible), and shared-plant treatments for diseases; and genetic phylogenies for cultures. Advances in these techniques will likely come up that can be integrated as well as the following extensions of relations of plant parts used, using existing databases, model validation and prediction. It should be pointed out that some measures of relatedness will be more accurate than others, and only some of these potential measures are described here out of a realm of many possibilities; on the other hand, the equations that synthesize these relations into measures of potential medicinal efficacy of each plant should function regardless of how the relations are measured.

It may be possible to add additional factors for the relatedness of the plant part used (root, bark, wood, leaves, flowers, fruit, seeds, or combinations thereof), extraction method (decoction; alcohol, water, or oil tincture; infusion; entire plant), season

harvested, companion plants, and growth habit, to the above equations in order to refine their accuracy if a suitable measure of these relations can be determined. There is no immediately obvious metric, for instance, of how the different compounds found in the roots vs. the leaves of different species might be related, analogous to the relatively simple metrics of the phylogenetic distances for species, culture, and diseases. Unless many different plant parts from unrelated species can be tested for efficacy to derive some measure of the average relatedness between the compounds in leaves and roots, for instance, it may be difficult to include factors such as this in the calculations.

Any published ethnobotany study or database can be integrated into the data to broaden the coverage and increase the accuracy of the data. For instance, the United States Department of Agriculture's phytochemistry and ethnobotany database (Duke 1994), the Native American Ethnobotany Database (Moerman 1986), and the culturally more similar International Ethnobotany Database (Skoczen and Bussmann 2006) would allow different cultures that have not been studied firsthand to be included in the medicinal potential analysis. However, different interview and research methodologies may cause problems in a unified analysis of these databases, since the best analysis would be on plant-use data that has been collected in a completely consistent manner in the different cultures; if we interview one group about plants used to treat symptoms of a disease while solely naming the disease in interviews with another culture, it is likely they will be describing plants used to treat different diseases, and there will be no consistency in the data.

Once data has been collected on a sufficient number of medicinal plant species used by diverse cultures to treat related diseases, how the three factors of plant, disease, and

cultural relatedness interact in the mathematical model can be assessed. The formulas presented above are ad hoc and therefore need to be validated or modified. This can be done by performing a consistent evaluation of each plant species' medical efficacy, via either bioassays or literature searches, and seeing how the efficacy correlates with the relatedness of the plants, diseases, and cultures. Existing studies have tried to make a standard measure of efficacy by grading previous lab or clinical studies on plants from the literature as "not effective," "effective," or "highly effective," but of course this always introduces the grader's bias (Trotter and Logan 1986). With possible access to one of the large ethnobotanical databases such as those of Duke or Moerman, a quick verification of the system could be performed using literature studies as a sort of verification, but the vast differences in the way plants' medicinal efficacies are tested in the existing literature makes this approach problematic. Instead, a consistent set of efficacy studies on the plants would give more reliable verification of the system. It would also be interesting as another form of validation to see how the index proposed here correlates with other ethnobotanical indices such as informant consensus values and relative importance as has been done with several existing indices (Albuquerque, Lucena et al. 2006; Reyes-García, Vadez et al. 2006).

Measuring efficacy across diseases can be difficult as, for instance, one cannot reliably compare EC_{50} values from an antimalarial assay to the EC_{50} values for a diabetes assay. General disease-treating efficacy could be measured using bioassays such as the brine shrimp assay for bioactivity, which can be used across different diseases (Trotter and Logan 1986), but it is inaccurate as it only tests for certain types of biological activity that might occur in the human body. Therefore, for validation purposes, bioassay tests

should be used for comparison of plant efficacy activity only within one disease, and though difficult, literature review such as Trotter and Logan's (1986) should be used to compare between different diseases. One way to adjust for differences between diseases is to factor in the efficacy of the dose a healer usually administers for a particular disease, or calculate how this efficacy compares with the effective dose of a proven standard pharmaceutical, i.e. how close does the dose of a plant given traditionally come to an effective dose.

Prediction of unexplored but effective medicinal plants will be possible, perhaps for the first time in this field, as the potential of any plant in a dated phylogeny can be calculated, not only those that are currently reported as being used in treatments. Plant species with no reported medicinal use can easily be plugged into the quantitative system just as any other known medicinal plant species is, based on their relations to other plants with known uses, producing a measure of the medicinal potential for the unreported plant that may be within the range of potentials for reported plants. If these plants are in the top of the range of computed potentials, they should be considered for laboratory analysis for true efficacy and they may turn out to be just as effective if not more so than reported plants.

2.8. Conclusion

A new theoretical mathematical methodology of "relational efficacy" is introduced that ethnobotanical researchers can use to estimate the potential of the plants they have studied before the plants have been fully analyzed in a laboratory. Once this system is validated, it should also allow effective comparison between studies by looking at the difference in the overall potential of all the medicinal plants in each study or the potential

of particular species occurring in more than one study. Thus, this system will be able to synthesize many cultures' medicinal plant knowledge to indicate plants with a high potential for being medically effective, save limited laboratory time and resources, and predict species that may have great disease-treating potential that have never before been considered in any culture.

Chapter 3. Ethnology of the Asháninka and Malinké

Though probably having no direct communication and sharing little in terms of local climate and plant species, the Asháninka in Peru and Malinké in Mali share significant aspects of their view of relations within and outside their cultures, and how this affects their use of traditional medicine. That, plus a surprising flora overlap given their geographic distance, make them excellent candidates for this thesis on relations of plants, diseases, and cultures. This chapter is based largely on literature with some supporting points from my own experience, given my limited time in the field with each group to do my own ethnographic work. The two groups have very different histories, the Malinké much longer and more geographically stable and the Asháninka with a much shorter written history if not absolute history and a more nomadic lifestyle, yet they have come to remarkably similar views of uses of their medicinal plants. This may be related to both groups having had to deal with colonialists, enslavement, and therefore being forced to learn to become self-sufficient and a need to strengthen bonds within their group. The Asháninka have had a more tumultuous recent history, having conflicts with the communist terrorist groups, *Sendero Luminoso* and *Movimiento Revolucionario Túpac Amaru* (MRTA), which forced them farther in the jungle and away from society. This has reduced their apparent use of alien plants compared to the Malinké, who generally live in larger towns, with many introduced plants growing all around them and comprising their diet. Instead of introduced plants, the Asháninka have had to deal with many diseases to which they had no resistance introduced by the conquistadors such as malaria, smallpox, and measles to which they have had to adopt their local herbal pharmacopoeia. Each

group has learned how to integrate the familiar and the foreign, self-sufficiency and dependence on those within their culture, and relations to their natural world that have helped them thrive.

3.1. Malinké

The word Malinké comes from the Fulani for "people of Mali", the greater than 5 million Malinké are spread in countries throughout West Africa, as these areas are covered by the former Malian Empire of the 13-15th Centuries. There are large groups of Malinké in Guinea, Ivory Coast, Mali, Senegal, the Gambia, and Guinea Bissau, with smaller populations in Liberia, Sierra Leone, Burkina Faso, and northern Ghana, with the largest population in Guinea. They are no longer the majority in Mali as they were in the past. The Malinké are alternatively called Mandinka, Maninka, and Mandingo, the latter referring to Malinké in the Manding Highlands near upper Niger River in Mali, and they are part of the larger Mande group, of which Malinké is the most widely spoken language. This tonal language (like Mandarin Chinese, where a rising and falling tone of the same phoneme has different meanings) is considered clear and easy to learn and thus has been widely adopted (Nwanunobi 1996). Languages in the Mande family, which are 5000 to 7000 years old, such as Bamanakan, Bozo, Bobo, Jahanka, Jowulu, Jula, Soninke, and Maninkakan, the Malinké language, are spoken from Senegal to Nigeria and from the border of the Sahara to Guinea coast today with the center of this area in Mali (McCall 1971, p.28). The proto-Mande language originated in the Arawan area of Northern Mali (McCall 1971, p.72).

There are a few ethnographies on the Malinké, though they are more prevalent in the French literature (Adjanohoun, Aké Assi et al. 1980; Rozat 1981). Hopkins' (1971) edited

volume is the most comprehensive, with chapters covering the language, political, and social structure. Schaffer (1980) is the most complete in terms of ethnobotany, including many plant descriptions for medicine, ritual, and food, though it mainly covers boys' circumcision rituals. Nwanunobi (1996) has the most updated coverage of the Malinké aside from the more recent journal articles (Duvall 2003; Imperato and Imperato 2006), while Adjanohoun (1980) and Rozat (1981) cover the medicinal plants of the area, though not solely those of the Malinké.

3.1.1. Geography and Vegetation

Mali (see Figure 3.1) is sometimes referred to as the "The Bright Country" due to the savannas that dominate the landscape. These are sparsely dotted with very tall trees that are landmarks and gathering places in villages for festivities. The original area of Mali is around the upper Niger (called Joliba by the Malinké) and its tributaries the Sankarani and Baoulé. The Niger is the second longest river in Africa and provides a major source of water, floodplains to irrigate crops, food in the form of fish, communication and transportation. Niani near the Sankarani River was the old capital, as it had resources of gold and many soldiers to draw from, and was easily defended.

Mali is between the Guinea-Sudan and Sahara climatic regions and consists mostly of Sahel Dry Zone, divided into 4 regions (Adjanohoun, Aké Assi et al. 1980, p.15):

Figure 3.1. Kita, Mali. The Malian field site Kita is located in the west of Mali, in West Africa, in savanna vegetation.

Sudano-Guinean; clear forests and savannas with Stereospermum kunthianum [Bignoniaceae] , Loeseneriella africana [Hippocrateaceae] , Cassia nigricans [Fabaceae] , Morelia senegalensis [Rubiaceae] , Brachiaria xantholeuca [Poaceae] , Cienfugosia digitata [Bombaceae] in.

Sahelo-Sudanian; savanna woodlands with Guiera senegalensis [Combretaceae] , Dalbergia melanoxylon [Fabaceae] , Pterocarpus lucens [Fabaceae] , Acacia seyal [Fabaceae] , Combretum glutinosun [Combretaceae] , Leptadenia pyrotechnica [Asclepiadaceae] , Bauhinia rufescens [Fabaceae] , Brachiaria ramosa [Poaceae] ,

Loudetia togoensis [Poaceae] , Elyonorus elegans [Poaceae] , Panicum subalbidum [Poaceae] , Alysicarpus ovalifolius [Fabaceae].

Sahelo-Saharan; steppes and scrubland covering more than 200,000 km^2 and dominated by *Acacia ehrenbergiana* [Fabaceae] , *Euphorbia balsamifera* [Euphorbiaceae] , *Combretum aculeatum* [Combretaceae] , *Boscia angustifolia* [Capparaceae] , *Hyphaene thebaïca* [Arecaceae] , *Sclerocarya birrea* [Anacardiaceae] , *Balanites aegyptiaca* [Zygophyllaceae] , *Boscia senegalensis* [Capparaceae] , *Acacia nilotica* [Fabaceae] , *Acacia albida* [Fabaceae] , *Geigeria alata* [Asteraceae] , *Cleome tenella* [Capparaceae] , *Tephrosia obcordata* [Fabaceae] , *Euphorbia scordifolia* [Euphorbiaceae] , *Abutilon fruticosum* [Malvaceae] , *Aristida hordeacea* [Poaceae] , *Aristida mutabilis* [Poaceae] , *Aristida stipoides* [Poaceae] , *Schoenefeldia gracilis* [Poaceae] , *Schizachyrium gracilis* [Poaceae] , *Cenchrus biflorus* [Poaceae] , *Tetrapogon cechriformus* [Poaceae] , *Sporobolus helvolus* [Poaceae].

Saharan; flora localized in valleys from groundwater with *Farsetia ramosissima* [Brassicaceae] , *Pergularia tomentosa* [Asclepiadaceae] , *Schouwia schimperi* [Brassicaceae] , *Hyphaene thebaica* [Arecaceae] , *Cympopogon proximus* [Poaceae].

Duvall (2003) argues that although some previous researchers called the area where *Gilletiodendron glandulosum* (Port.) J. Leonard [Fabaceae] and *Guibourtia copallifera* Benn. [Fabaceae] grow in Mali "Sudanian dry forest," this is not an appropriate term for these areas, as the two species never grow in exactly the same place. Instead, he claims that *Gilletiodendron glandulosum* forest corresponds to Lawesson's (1995) "Sudano-Guinean gallery forest", and *Guibourtia copallifera* forest is a subtype of this form that has been isolated by the microclimates of the Manding plateau and not, as others have

claimed, a vegetation subtype created by human influence. This discussion contradicts the controversial argument of Balée (1989) that there are really fewer primary forests in the Amazon than previously thought, as most areas have been shaped by human management or influence over time due to large Amazonian populations.

A culture of migration has developed among the Malinké when there are no crops to farm, but people return home during harvest time, so they are not considered nomadic. In the past, some traders went as far as Nigeria and settled there as the Dyula. The cycle of rainy and dry seasons, with more rain in the south, means more yams are grown in the south and more millet and sorghum in north. The first month of rain requires a lot of labor for sowing. The dry season is November to April, with the early dry season, through December, being harvest season (Nwanunobi 1996).

3.1.2. History

As with many West African groups, this several-century-long history among the Malinké is passed on through *griots* (or *jeli* in their language) who play *kora* (a stringed instrument plucked with both hands) or *tam-tam* (a large handheld drum) and sing stories of past and current news. Strangely, despite their role, griots still have a low social status and cannot marry outside their group.

The Sundiata is one of the most widely told stories by the griots about the renowned Malinké leader of the same name who founded the Malian Empire in the 1200's.

Before 1200, the Do, Malal, Sibi, Toron, Traoré, and Dalikimbon communities, united by language, came together as a means of defense against the Soninke and Soso. My field site of Kita won independence from the Traoré of Bafing (Hopkins 1971, p.101).

Islam, which reached the area around 1000, had a big influence on Bilali Bounama, ancestor of the leader Sundiata (Nwanunobi 1996), which spurred him to spread the religion. The Sudan from the Atlantic coast to lake Chad became part of Islam in the 11th Century (McCall 1971, p.28).

The current Republic of Mali is not the same as the ancient empire of Mali of the years 1240 to the early 1400s, which extended to the West Coast of modern Senegal and was not landlocked (Nwanunobi 1996). With the dissipation of the empire in the 1300's, the Malinké dispersed and intermarriage with other groups increased to aid in trade, mobility, and promoting ties among cultures, especially in Southwest Africa (Nwanunobi 1996). By late 1600s, only the small state of Kangaba was left in the Malian Empire. Subsequent kingdoms were led by a series of warlords or *faama*.

The colonial period saw further dissolution of Malinké leaders control over their land, trading, and taxation with increased slave trade and controls by French colonialists. All Malinké countries gained independence by the early 1960s, specifically in 1959 for Mali (Nwanunobi 1996).

In pre-Islam times, deities and spirits were connected to the environment. The leader of a community, called a *mansa*, had spiritual and secular powers through the *mansa*'s ancestors. The *jinn* are magical beings that take on human forms and *gri gri* are charms and talismans used to bring good luck to journeys or projects. The conversion to Islam led to many changes in Malinké culture, including the banning of pork, introduction of halal meat, pilgrimages to Mecca, and the reading of the Koran among others. A slow power shift from the *mansas* to the imam (Islamic community spiritual leader) and Islam

occurred, but the Malinké religion is still present within Islam in Mali (Nwanunobi 1996).

3.1.3. Customs

The Malinké are patrilineal as status is determined through the father's family (Nwanunobi 1996), with a possible distant matrilineal past as evidenced by Voltaic neighboring groups (Adjanohoun, Aké Assi et al. 1980, p.51). Extended family units make up villages, with polygyny (men having multiple wives) being common (Nwanunobi 1996).

Hopkins (1971, p.99), who did his fieldwork in Kita, as did I, studied clan lineages and circumcision rituals among the Malinké. He found that though what he calls polygynous marriage exists there, it is not necessarily simultaneous, but rather a man may have several successive wives over his entire life, and therefore it should be called serial monogamy instead. Children of same mother are called *baden* (from *badenya* - "kinship") and of same father *faden*. *Faden* half brothers are often hostile to each other (Hopkins 1971, p.99). As Hopkins states, "patrilinearity and territoriality are the warp and woof of Maninka social structure." (1971, p.103).

Hopkins observed four levels of lineage in Kita:

- clan (*diamu*) , all with same clan name;
- lineage (*kabila*), an exogamous unit whose members may inherit from one another, named for common ancestor;
- sublineage (*babunda*, or "door of the mother's house"), localized in a village rather than a canton and not related to marriage or inheritance;

- household (*lu*), the basic unit of village life of 18-100 people, in a common taxpaying and crop-growing residence.

The dominant clans in 1971 in Kita were the Keita, Tounkara, and Kamara, which make up about two-thirds of the Kita population, less than in pre-colonial times (Hopkins 1971, p.101).

Outside the lineage system there are the *nyamakala* or vocation-based groups: the *tontigi* who produce food, *numu* who make iron, wood and clay tools and perform circumcision, and the *dieli* or *djeli* who recite legends like the Sundiata, play music, and lubricate society. Sometimes these all happen in conjunction with the *djeli* drumming and singing to spur on the *tontigi* to thresh while the *numu* repair tools. These groups are outside the central kinship lineages and are apolitical so they can propose changes to the village structure without being seen as being politically competitive (Hopkins 1971, p.107).

Marabouts or *mori* are Muslim spiritual leaders that developed in an otherwise pagan area. They get their prophet status and power to preside at rituals and pray for others either through religious study and charisma or inheritance from their parents, but sometimes both (Hopkins 1971, p.108).

Strangers (*dunan*) are accepted into Malinké communities readily, as they add to the community's size and prosperity, as long as they are accompanied by a community member (*diatigi*). Eventually they receive community member status (*duguren*), can have a voice in village councils, and can marry into sublineages of their hosts (Hopkins 1971, p.109). This is one possible explanation, though not the only one, as to why alien plants like neem (*Azadirachta indica* A. Juss. [Meliaceae], discussed below),

Madagascar rosy periwinkle (*Catharanthus roseus* (L.) G. Don [Apocynaceae]), and teak (*Tectonia grandis* L. F. [Verbenaceae]) are accepted readily into the area, and eventually into heavy use if they prove to be useful. This, or the longer history of trade routes with Europe and the East, might also explain why most food plants in Kita were introduced crops to the area. From my personal observation (2003 and 2004), the majority of food plants sold in markets in Mali were introduced species (*Mangifera indica* L. , *Anacardium occidentale* L. , *Lycopersicon esculentum* Mill. , *Solanum melongena* L. , *Solanum tuberosum* L. , *Phaseolus vulgaris* L. , *Amaranthus sp., Musa x paradisiaca* L. , *Oryza sativa* L. , *Triticum aestivum* L. , *Arachis hypogaea* L.) with few of the native foods in evidence (millet, *Pennisetum glaucum* L. and sorghum, *Sorghum bicolor* (L.) Moench), whereas the majority of food plants sold in markets in Peru were native. This may also be due to the fact that Peru is a center of many food crop origins and domestication while Mali is not, as an alternate explanation for the above observations (Vavilov 1951).

The chief (*diamanatigi*), usually the senior man of the core lineage, has little coercive power, and acts more or less merely as a figurehead or "human symbol for the community," as he could be the poorest and least influential person in the village (Hopkins 1971, p.110). Instead, the big man (*togo tigi*) leads by virtue of wealth, having many followers, firepower, oratory, or reputation, but even they could not easily go against popular village sentiment (Hopkins 1971, p.111). Clearly, power is more important than authority in leadership of the village. The interplay of all these different lineages, sublineages, and epilineages inherent in Malinké society (Hopkins 1971, p.113)

has an analogy in the interweaving of plant, disease, and cultural phylogenies or lineages used in the relational efficacy technique explored in this thesis.

Male and female circumcision (*kwiyung*), Schaffer's main topic of study (Schaffer and Cooper 1980) is performed on both girls and boys, not at a set age. It is a focus of children's lives so older uncircumcised children are often teased. For boys 6-13 years old, the ritual is every 5 yrs (Schaffer and Cooper 1980, p.95). Every boy is circumcised at the same time and all boys age 10-14 are considered *twins* and have a strong bond or class called a *fula ton* or *kari*. After circumcision, the blood from the wound is clotted with outside of millet stalk, and later wrapped with *pelinkumfo* (*Aframomum sceptrum* [Zingiberaceae]) leaf or *tabo* (*Nauclea latifolia* [Rubiaceae]) bark, or a western ointment to aid healing. A leaf dance is performed in celebration by the boys' guardians with *fara* (*Piliostigma thongii* [Fabaceae]), which is used to make the *kangkurao* and *jambakatao* (*Combretum glutinosum* [Combretaceae]) meaning "bitter leaf" which is a popular purgative. Both represent unity of man and nature because of these uses. The guardians sing "The *fara* tree and the *jambakatao*; all for one and one for all." A lodge is then built by the guardians and older men under the *tabo* (*Cola cordifolia* [Malvaceae]) tree (Schaffer and Cooper 1980, p.95).

Female circumcision takes place every 2-3 years under the *tabo* (*Cola cordifolia* [Malvaceae]), *jungo* (*Mitragyna inermis* [Rubiaceae]), and *jalo* (*Khaya senegalensis* [Meliaceae]) trees, the latter two of which are thought of as women's trees since the *Jalo* is used to make mortars, pestles, and cooking stools while the *jungo* is used to make the handle of the woman's rice hoe (Schaffer and Cooper 1980, p.99).

In addition to and sometimes in conjunction with ritualistic plants, true twins (not merely circumcision "twins" who are unrelated) are highly revered in Malinké culture. The family of twins keeps an altar called a *sinzin* in the home of twins, on which offerings are made of chicken blood, cola nuts, millet paste and millet beer to protect the twins. Bowls called *flani daw* ("twin bowls"), which are carved from the wood of the *sunsun* tree (*Diospyros mespiliformis* Hochst. ex A. DC. [Ebenaceae]), the *balanza* tree (*Faidherbia albida* (Delile) A. Chev. [Fabaceae], formerly *Acacia albida*), and a few other trees are made for twins as well (Imperato and Imperato 2006).

3.1.4. Village Life

The village (*dugu*) and canton are the historical levels of Malinké organization, but the canton became less important in post-colonial times. The village is the unit for land ownership and for ceremonies with the associated shrine or mosque. The village lineage or sublineage is responsible for founding the village and can therefore claim the chiefship of the village (Hopkins 1971, p.103).

Sacred trees are often associated with the village founder. e.g., *lenko* (*Afzelia africana* [Fabaceae]), *kitarao* (*Schrebera arborea* A. Chev. [Oleaceae]), *sito* (*Adansonia digitata* [Malvaceae]), and *tabo* (*Cola cordifolia* [Malvaceae]) (Schaffer and Cooper 1980, p.69). Houses are made of mud brick "banco" with thatch (Nwanunobi 1996).

Relations between adjacent villages and lineages are based on their shared ancestors and viewed as a form of "foreign relation". Some are linked, while others are hostile if they have no common ancestors (Hopkins 1971, p.9104). I observed this in Kita in that Malinké Touré, Kamissoko, and Traoré families would associate more with the families

of the same names in neighboring villages more than they would associate with the Christian, Peulu, Fulani, and other clans within Kita.

Family-owned farmland produces most of what the Malinké village family eats, with some land being individually owned and other land being collectively owned by the family and managed by elders. Each family member works family land together five days per week and individual plots two days per week. Men clear undergrowth, prepare land, manage male-associated crops (e.g. millet and sorghum), hunt, herd, do leatherwork, blacksmith, weave, and construct houses. Boys care for men's crops and cattle and scare off birds from cattle until the boys have reached circumcision.

The women's role is more in the house dealing with child care, trade, arts, spinning thread (but not weaving), rice, and daily food, which commonly consists of boiled millet flour and rice with cinnamon, while rice, meat and peanuts are more special. Girls pound grains, spin thread, and fetch firewood and water.

Men and women generally eat separately using their hands to scoop up bite-size pieces of the hot sticky rice dishes from a common bowl placed on the ground in the middle of a circle of people sitting on low stools.

A number of interesting proverbs regarding plants, food, and relations give some insight into Malinké village life:

- "maxafeno le se kinoo diyaa" means, "The sauce makes the dish delicious", given the strong adoption of palm oil and the non-native peanut sauces in Malinké dishes.
- "kanijuo man kunan bari a dino ye kunan" means "the spice plant is not pungent, but its shoots are", i.e. sons are not always like their father.

- "moxo mee i buloo bula saanaa to" means "A person should not put his hands in snake poison," i.e., do not take pointless risks without reward.
- "kuma se mee daa fula le to" means "In two mouths conversation lives long." i.e. it is easy to maintain a friendship when both people give and trust equally (Nwanunobi 1996). This echoes my experience in setting up fieldwork with the Malinké in Kita in that I had relations with the Département de Médicine Tropicale in Bamako that made me more trusted with the Kita healers association. They felt they had been betrayed by a researcher who had been there about 10 years before who gave them little in exchange for their interviews, so they asked for $30 for each 3–4 hour interview to ensure there was some give and take on my part. Thus it was clear to me that strong trusted relations are important to the Malinké as they are to the Asháninka.

3.1.5. Malinké ethnobotany

The Malinké currently suffer a 30-50% infant mortality rate and a rise of parasitic diseases due to development. Malians depend primarily on traditional medicine; like 70% of the world (Adjanohoun, Aké Assi et al. 1980 p. 9). The World Health Organization now estimates that that as much as 80% of Africa's population uses traditional medicine and 60% of children are first treated with herbal medicines for malaria fevers in Ghana, Mali, Nigeria and Zambia, while in the rest of the world, traditional medicine use ranges from 40-80% (United Nations World Heath Organization 2003). Adjanohoun has data on many medicinal plants used in Mali, although this research was done in only three weeks around Bamako, Sikasso, and Narena (1980, p. 25).

Cola nuts (*Cola nitida* (Vent.) Schott & Endl. [Malvaceae]) comprise a key stimulant and ritualistic plant in West Africa, are brought to the parents of a woman a man wants to marry and the parents accept the nuts if the proposal is accepted. Cola nuts are also used to block bad luck in sand reading (divining) (Nwanunobi 1996) and are brought as a gift when visiting someone's house, but only in odd-numbered quantities, usually five or seven (personal observation, 2004). It is interesting that cacao (*Theobroma cacao* [Malvaceae] and subfamily Sterculioideae), in same family and subfamily as cola, is endemic to the Neotropics but similarly to cola also produces stimulant methyl xanthine alkaloids (i.e. caffeine and theobromine, vs. cola's caffeine) (Bletter and Daly 2006), is used as a form of currency, and is valued in odd numbers (the 5 rows of seeds covered in pulp that are visible in the cross-sectioned fruit give the cacao its ritual significance as this is a sacred number) (McNeil 2006). It can only really be taken as a case of convergent culture evolution surrounding a stimulant plant rather than a custom that was transferred between the cultures, as there is no clear route of cultural transfer between Mesoamerica and West Africa. Although there are significant rituals surrounding other stimulant plants around the world, such as coca (*Erythroxylum coca* Lam.), mate (*Ilex paraguariensis* A. St.-Hil.), guayusa (*Ilex guayusa* Loes.), guaraná (*Paullinia cupana* Kunth), khat (*Catha edulis* Forssk.), coffee (*Coffea arabica* L.), tea (*Camellia sinensis* (L.) Kuntze), and betel nut (*Areca catechu* L.), none of these rituals seem to have as much in common as cacao and cola, so this seems to be special case of cultural convergent evolution. There is use of tea as money (Bertsch 2006)and being used in odd numbers in Yunnan, such as seven puer tea cakes being packed together (Selena Ahmed, personal communication, 2008), and of khat being used as currency in modern

times to pay drug dealers in Somalia (Stevenson 1992), so this may just represent the immense value that stimulant plants have to cultures in general.

The *fara* tree's red bark (*Piliostigma thonningii* (Schumach. & Thonn.) Milne-Redh. [Fabaceae]) is used to cover the body of people playing the part of the Kangkurao', the demon-figure that is the center of a cult. One mask is called the *mumbo jumbo* and is the origin of the English word for black magic and obfuscation (Schaffer and Cooper 1980, p.9).

Matt Schaffer and Christine Cooper did extensive fieldwork among the Malinké of Pakao Dar Silamé, Senegal from 1971 to 1976 and their ethnobotanical collections are kept in the Oxford herbarium (OXF) (Schaffer and Cooper 1980, p.16). Of the Malinké, whom they refer to as the Mandinko, they say, "Mandinko rely heavily on plant cures and have a sophisticated medical vocabulary." (Schaffer and Cooper 1980, p.16).

The Malinké staple crops are rice, millet, peanuts, corn, and *findo* (*Digitaria exilis*), a native grass whose seeds are eaten as a porridge. Rice, which is a favorite food of the Malinké, often flavored with a peanut gravy, is cultivated only by women, while men cultivate millet and peanuts (Schaffer and Cooper 1980, p.28). The non-native crops corn and tomato are intercropped as corn ripens early enough in September for planting of a tomato crop. The corn is eaten roasted, as meal or as couscous, and tomatoes are used for making sauces. Fields are left fallow for 1-5 year periods, with peanut crops used as natural fertilizer supplemented with chemical fertilizers. Long-term storage of grains is done in large baskets sealed with ashes and cow dung. Peanuts are also an important source of cash, sold through cooperatives at 40 CFA/kilo (*Communauté Française d'Afrique*, Malian currency) in 1974 ($0.16/kilo in 1974 dollars), bringing $17,736 for one

Senegalese Malinké village in 1972. This money is used to buy refined sugar for millet broth flavoring, cola nuts, salt, oil, tobacco, cigarettes, biscuits, and kerosene (Schaffer and Cooper 1980, p.30). Other crops include okra, red peppers, tomatoes, oranges, and mangos (Schaffer and Cooper 1980, p.32).

It is clear from these crops, from other ritualistic plants mentioned in this chapter, and from my own research that the Malinké have extensive experience and uses for their native plants while incorporating non-native plants readily into their food and medicinal plant vocabulary, if not into ritualistic uses.

3.2. Asháninka

The Asháninka may have fewer records of their long-term history than the Malinké, but their links with the Rubber Boom and their entanglement in 20th Century Peruvian history has created many records of their recent history aside from their ethnobotany. Spreading through several departments of Peru and into neighboring Brazilian states (see Figure 3.2), the Asháninka number about 60,000 and are currently the third largest indigenous group in Peru after the Quechua and Aymara, and the largest group in the Amazon (Keplinger, Laus et al. 1999) with the four subgroups of the Pajonal, Perené, Ucayali, and Pichis (Gordon 2005). They live mostly as migrant farmers along Ene, Tambo, Tambopata, Perene and Pichis rivers (Keplinger 1993) in the Southwest Amazon floristic zone (Daly, Silveira et al. 2007) and into the cloud forest areas at higher elevations (Gentry 1996) as seen in Puerto Bermudez, Ucayali (see Figure 3.2). According to Lenaerts 76.7% to 88.6% of the forest flora is used medicinally (Lenaerts 2006). In Paititi where I found 78 species being used out of a probable 4000-5000 species in the Yuruá area (Daly, Silveira et al. 2007) this would lead to a figure of about 1-2%

use, but this is hard to compare since I did not survey all medicinal plants, nor did I do an exhaustive survey, and the species accumulation curves were still increasing steeply after 8 healer interviews.

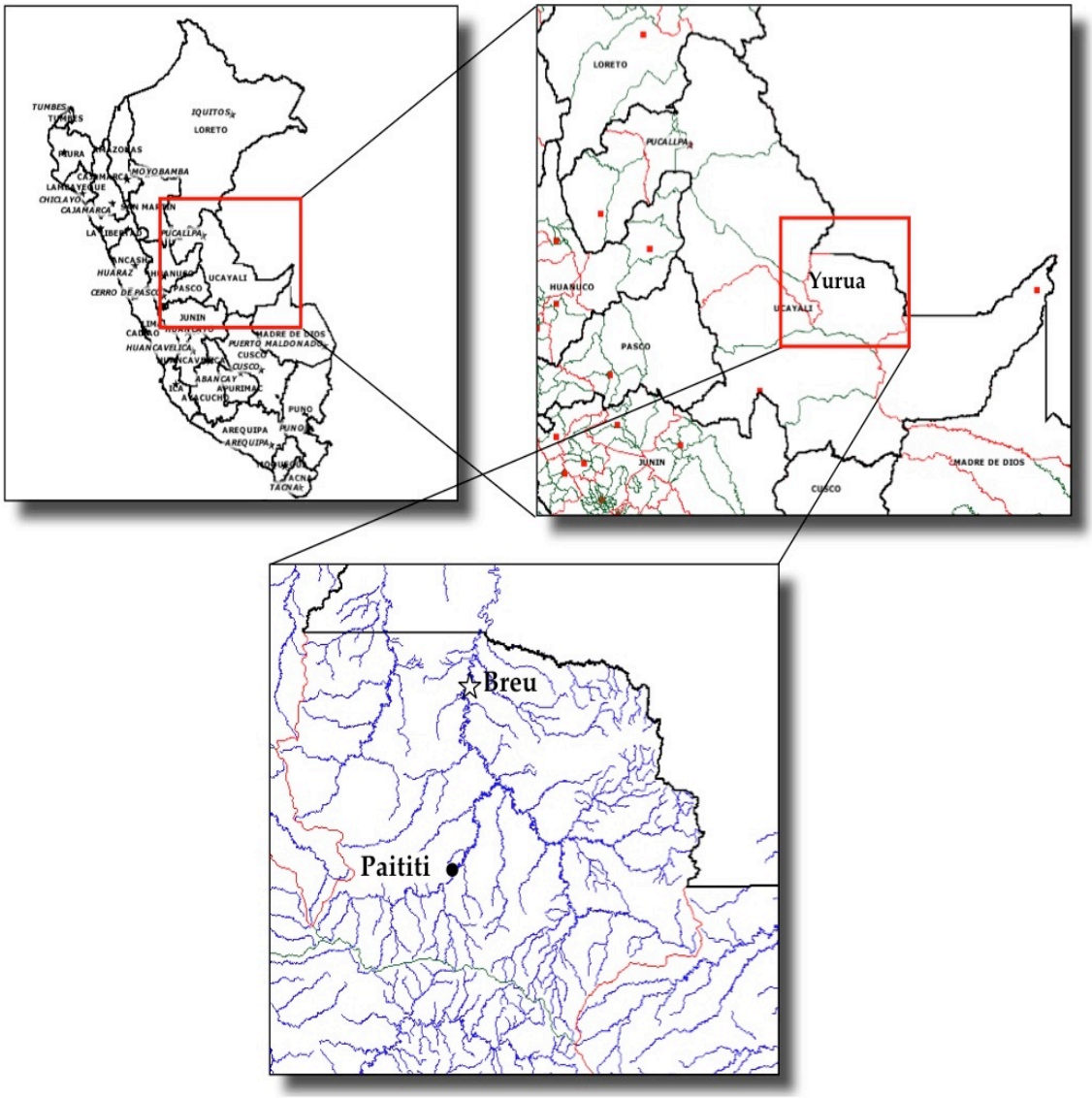

Figure 3.2. Paititi, Peru. The Peruvian study site Paititi is in the Amazonian district of Yuruá in the department (state) of Ucayali, along the Huacapishtea River.

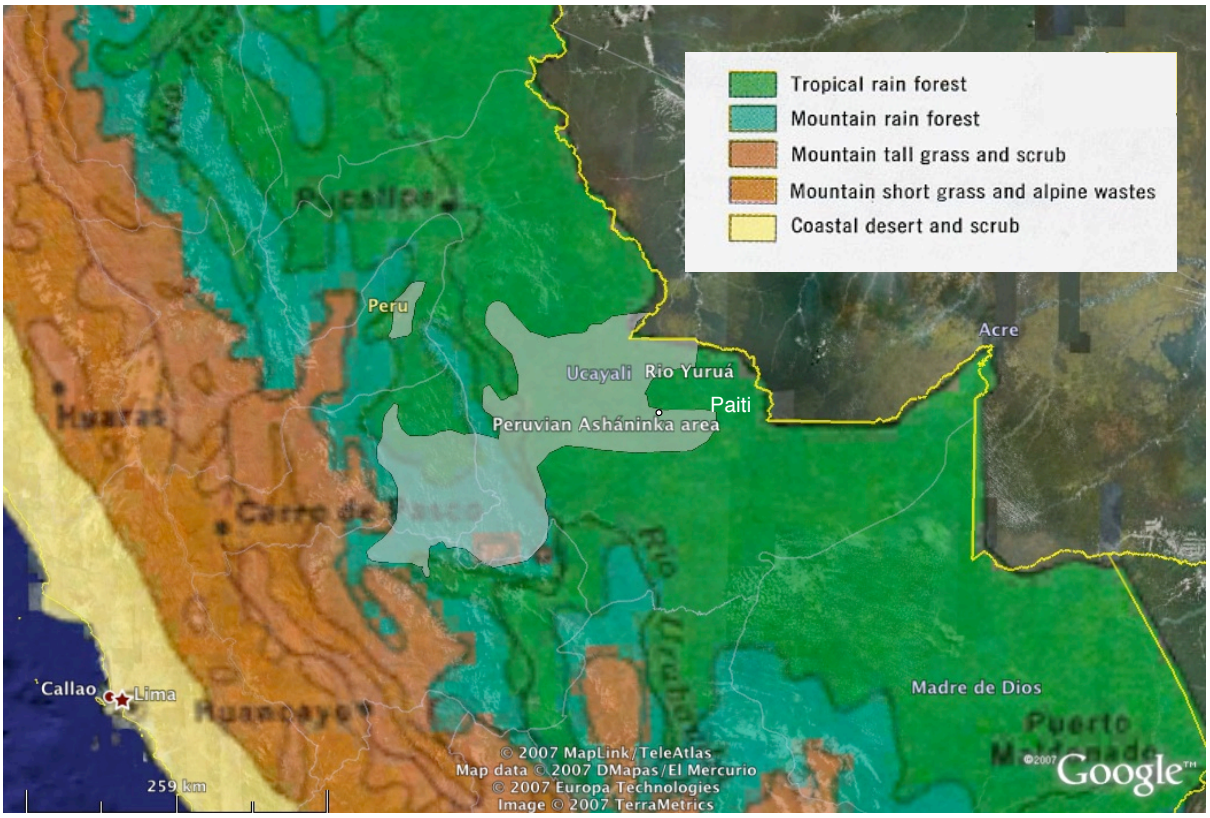

Figure 3.2. The Asháninka area of Peru (white) overlain by the vegetation zones and the location of the field site, Paititi along the Huacapishtea tributary of the Yuruá River.

3.2.1. Language

Asháninka is part of the Arawak language family, along with Machiguenga, Mashco Piro, Nanti, Nomatsiguenga, Yanesha, and a few other minor languages (Gordon 2005); it is divided into four dialects that correspond to the subgroups mentioned above. They have no writing or texts. Old people and women still know the names of some symbolic designs in their crafts but do not see it as a language that can combine into signs (Keplinger, Laus et al. 1999). The shaman of the Asháninka and the closely related Matsigenga (alternatively Machiguenga or Machigenka) have a special writing system

that is painted on cloth. The shaman Daniel, of the closely related Machiguenga tribe, reported that they can write or paint the symbols only after taking an (unspecified) drug. *Sancoshi* or *shanspchoshi* is the word for holy men who can produce this writing. Keplinger (1999) thinks this is similar to *sanquenaantsi* which is the word for writing, though it sounds more like the *sancashi* (*Vernonia* sp.) plant. This would not make much sense, however, only if it were used in the aforementioned drug. There are known psychoactive plants in this genus (Sobiecki 2002), and in the related *Lactuca* genus in the same subfamily Cichorioideae (Schultes and Hofmann 1980).

3.2.2. History

Prior to Western contact, the Asháninka traded stone tools (axes) and salt with the Andean Quechua for clothing, and have tales of working the forest and fields with dull wooden and stone tools. This led to some of the influence of Inca culture on the Asháninka: there are a few Quechua borrowed words, mostly introduced after the Spanish conquest, an interesting example of which is "money" (*coriqui*). The fact that they borrowed the word for money implies that they either had no word for money or they did not use money at all before this concept was introduced by the Quechua, according to theories of introduced words and their uses (Balée 2003), though the traded stone tools and salt items could just have easily served as a form of money, having ranked value and being portable and divisible. So it seems more likely that they adopted this Quechua word for money either because they did not have an appropriate word for the concept.

There was already some contact with the Asháninka before Franciscans arrived, and some Christian converts by 1570. Metal tools were traded with missionaries for goods

and services which changed the Asháninka social structure by changing ideas of ownership and value as well as teaching them a “work discipline” which could them be later taken advantage of by rubber tappers using the Asháninka as slave labor (Narby 1989, p.54). Metal tools, domesticated animals, non-native trees, and traditional Spanish stories are some of the few legacies of colonial period.

Eventually, the Asháninka started to reject outside rule and law. Chiefs led rebellions in 1674 and 1723 against the Catholic Church's limits on freedom (e.g., polygamy), not wanting to change old customs, fear of quickly spreading diseases (two thirds of the Asháninka were killed in epidemics close to missions), and threats to salt trading business. There was a successful rebellion in 1742 led by Inca/Quechua/Spanish leader Juan Santos Atahuallpa Apo Inca, who hoped to drive Spanish out of Peru. Spanish contact with Asháninka ceased after this until 1850. These rebellions gave Asháninka the reputation and name of *Campas Bravas* ("fierce Campas", where *campas* was the Spanish term for the indigenous groups closely associated with the Asháninka). The closely related Machiguenga were referred to as peaceful Campas. Western contact with the Asháninka resumed in 1847 with the construction of a fort at San Ramón, Junín, but this was met with some violence. Other areas remained isolated until the 1870's, with the Gran Pajonal area completely uncontacted until the 1950's.

The Amazonian Rubber Boom caused great harm to the Asháninka with enslavement, invasion of their territories, the spread of disease, and dissolution of their customs. Asháninka expanded from Atalaya to the lower Yuruá from 1904-1910 following the rubber boom, and then around 1945 down the Yuruá and Huacapishtea (Lenaerts 2005) where my field site Paititi is located.

Asháninka land has been taken over by missionaries, colonists and government officials since the end of the Rubber Boom (Narby 1989, p. 9). The missionaries, especially the Adventists, changed the Asháninka view of the land with communal ownership and spirits walking on the sky and lake dwellers living under their feet, towards a more secular vision, and moving them away from their subsistence yet comfortable agriculture. The Adventists, however, were not able to change the spiritual landscape vision of the Asháninka, which remained very much alive, at least in Quirishari (Narby 1989, p.263). There has been a recent increase in schooling among the Asháninka, leading to a decrease in time for traditional activities, such as spinning thread and weaving for girls and hunting for boys (Keplinger, Laus et al. 1999). Moreover, many parents of the Asháninka in Quirishari had been killed by epidemic disease in the 1940s (Narby 1989, p.274).

The history of Paititi as an Asháninka town is fairly short, according to the accounts of Paititi's chief Raul Casanto Shingari as well as the oldest Asháninka in the town and the true town founder, Frederico Pinedo Sanchez. Supposedly this was a Yurunahua occupied area until about 1998 when they heard rumors that the *Sendero Luminoso* (Shining Path communist guerilla group) were nearing the village highly armed to take it over. Having heard how the *Sendero Luminoso* had decimated other uncooperative villages, the Yurunahua were scared off and abandoned the village in the middle of the night. The rumors may have been faked by neighboring competing groups such as the Murunahua since the *Sendero Luminoso* never seemed to materialize, but the Yurunahua had already abandoned the town and were ready to move on. They had known about Raul from when he was head of the indigenous peoples group *Asociación Interétnica de*

Desarrollo de la Selva Peruana- AIDESEP ("Interethnic Development Association of the Peruvian Jungle") and knew he was living in Pucallpa, so they offered the town to him.

Raul asked Frederico, an elder Asháninka from Dulce Gloria, a largely Asháninka town of 300 people at the confluence of the Yuruá and the Huacapishtea a few days travel from Paititi, to scout out the town and establish residence there. In my two visits to Paititi in July of 2003 and November of 2004, Frederico was no longer living there but made frequent visits from Dulce Gloria. Raul was not living in Paititi at all in 2003 and did not seem to have much desire to, saying he had to stay in Pucallpa for medical attention, so his wife Nellie managed the duties of leading the town. In 2004, he had just started living in Paititi full time about a month before I arrived in November, taking over leading duties from his wife. In late 2006 I received news from him via email that he had returned to Pucallpa and was having heart problems for which he was seeking medical attention. In early 2007 when I attempted to call his daughter in Pucallpa, she informed me that Raul had left Pucallpa but could not tell me where he had gone. With sporadic contact with two former teachers from Paititi, Juan Quinchuvia, an Asháninka, and Sedequías Anco'n Chavez, a Shipibo, I have heard that a small group of people remains in Paititi as of this writing. This history demonstrates the fluidity of Asháninka and other Amazon groups' settlements, often lasting for only a few years (Narby 1989).

3.2.3. Ethnobotany

Lenaerts (2006) states that the Asháninka use of medicinal plants is based more on relations of the plants and people using them, than the actual medicinal efficacy of the plants, as Western medicine does. Therefore a good a healer does not need to be a good botanist but rather focuses on the network of relations holding the living world together,

as it is from this network that health and illness derive. Because or despite of this, knowledge of medicinal plants is spread throughout Asháninka communities yet it is not highly valued by these communities. The Asháninka had higher percentages of plants with indigenous names and uses than the Shipibo (Lenaerts 2006), and, although Lenaerts states he has no way to prove it, not having done any laboratory tests on the plants, he feels that the Asháninka plants are just as medicinally effective as the Shipibo's.

Much borrowing or transfer of plant knowledge goes on between the neighboring groups of the Amazon, and though this borrowing does not seem to rely on the efficacy of the borrowed remedies, it does follow a chain of transference from those groups closer to urban Peruvian centers. The Chitonahua and Yora (or Nahua) borrow from the Yaminahua, who borrow from the Asháninka, who borrow from the Shipibo who are nearest urban centers such as Pucallpa. This simplified network has replaced a more complex reticulate network that existed in the past (Lenaerts 2006). Though the network of people is key to recommending a plant's continued use, it is also getting into the point of view of the natural elements (animals, plants, spirits) that may be causing a particular disease that truly informs the use of the plants, so all these relations, of the people, animals, plants, and environment are important.

Rather than how Western medicine may try to explain "magical" medicinal uses with "real" physical effects such as that medicines treating black water spirits are actually treating parasites found in stagnant water, the Asháninka believe the opposite, that the magical effects explain the apparently physical effects (Lenaerts 2006). For example, if someone is bitten by a snake, the snake, which still embodies the will for the victim's death, must first be found and killed to restore the necessary relationships before the

medicine to treat the snakebite can work at all. In order to rectify these relationships, the shaman must disembody themselves and embody as the animal, spirit, or human that is causing the disease to momentarily gain that disease agent's perspective. It is this skill at rectifying relationships rather than having wide knowledge of many plants that an herbalists may have. Therefore the Asháninka shaman's training is in this step-by-step disembodiment and re-embodiment using the widespread Amazonian psychoactive plant mixture ayahuasca, made from *kamarámpi* (*Banisteriopsis caapi* (Spruce) Norton, [Malpighiaceae]) and usually *huruúwa* (*Psychotria viridis* Ruiz & Pav., [Rubiaceae]). Next in the sequence of the shaman's training comes tobacco (*Nicotiana tabacum* L., [Solanaceae]), *datura* or *sáárо'*, (*Brugmansia candida* Pers., [Solanaceae]), and *thonénto* (*Cavanillesia hylogeiton* Ulbr., [Bombacaceae]) and *kasáwi* trees (*Duroia hirsuta* (Poepp. & Endl.) Benth., [Rubiaceae]), with each embodiment with a different creature calling for a different plant ally. The herbalist viewpoint is quite different, however, depending on limited ailments or externally evident symptoms, from which the herbalist can guess what the disease is and collect a plant from the forest that will be strong enough to fight it. Thus the healing process of the herbal healer is that of countering diseases and the shaman's may be seen more as rebalancing of relationships or as Lenaerts says, "While the Western approach focuses essentially on chemical effectiveness of the plants themselves, Asheninka [sic] people pay much more attention to relational aspects. " (Lenaerts 2006).

Jeremy Narby (Narby 1989) did his Ph.D. fieldwork with the Asháninka in Quirishari, Pichis Valley, in the Central Peruvian Jungle, Pasco state from 1984-86. Quirishari means "place of palms," as *quiri* is the Asháninka word for *Mauritia flexuosa*

L. f. [Arecaceae] or *aguaje* in Spanish (Narby 1989, p.269). The Pichis Asháninka speak a different dialect of the language from the Yuruá Asháninka I worked with in Paititi. Carlos Perez Shuma, an Asháninka healer and former ACONAP president, mentioned using "ayahuasca and other plants" to nurse himself back to health after a snakebite in 1980 (Narby 1989, p.14), and agreed to teach Narby more about Asháninka culture and plants.

One of the Quirishari men, Abelardo, used the hallucinogenic vine *sanango* (most likely *Tabernaemontana* spp. [Apocynaceae] or *Brunfelsia* spp. [Solanaceae]) as a backache remedy. When collecting this plant, Abelardo scared off a poisonous *jergon* snake. Nearby they found the treatment for *jergon* bite, a "cousin" plant to the *sanango* with white fang-like lobes, a clear case of the Doctrine of Signatures (DOS) where a plant looks similar to the organ or disease it treats Narby, 1989, p.270. DOS can be a confounding effect on relational efficacy depending on how it is viewed, as is described in Chapter 2.

There are several informative Asháninka sayings or notions about plants (Narby 1989):

- "Corn calls its friend the moon to tell the clouds to rain, so it rains." meaning that it usually rains the same night one plants corn.
- "What you have just sowed with your hands is as if it was your own body," so that one should not plant seeds soon after drinking alcohol or using a fire since these both burn the body and therefore the body touching the seeds would burn the seeds.

3.2.4. Customs

Instead of seeing a horizontal or political division of the races of the world, the Asháninka see vertical divisions, with the spirits right above the Asháninka, walking on the sky. The white people or *viracocha* in the underworld below the Asháninka, and the Asháninka walk on the viracocha's sky as the spirits walk on the Asháninka's sky. *Viracocha* means "lake dweller" as lakes connect the different levels of the Asháninka world (Narby 1989, p.23). When the Asháninka asked Narby if they could go to his land, they were asking if it was physically possible, crossing the lakes to other levels, not financially as Narby had thought.

The Asháninka have a communal and spiritual view of land use and ownership, in addition to a high diversity of crops on which they depend (Narby 1989, p.27). However these are sometimes in conflict with their subscription to the ideas of the Law of Native Communities, which includes ownership of land as an object by a larger community, and with the Asháninka's urge to emulate colonists and engage in market economies with different ideas of ownership.

Land is owned collectively by the village, especially since the people farming it will eventually abandon it, but the plants the family plants there are owned by them (Narby 1989, p.279). Later, if this land is abandoned and someone else comes to clear the area around abandoned trees, those trees now belong to whomever cleared the area. This concept is very similar to the Malinké notion of land ownership. The Asháninka's urge to be part of the western market economy has caused some of them to subscribe to individual land ownership and to become more specialized in the their agriculture, which is normally diverse (Narby 1989, p.297). In Quirishari, for instance, people will use one

piece of land for fishing, gardening, hunting, raising animals, curing, wild gathering, and growing plants for sale in the market.

Healers such as *tabaqueros* who use tobacco or *ayahuasceros* who use ayahuasca have their soul transformed into animals such as jaguars that can travel to distant places while the healer's body stays in one place (Narby 1989, p.266). Tobacco and *tabaqueros* can have a protective effect on the landscape as well by virtue of the tobacco's strong smell (Narby 1989, p.267). The Adventists tried to teach the Asháninka that both tobacco and ayahuasca (believed to be the mother of tobacco by the Asháninka) were of the devil, but the Asháninka in Quirishari at least did not believe this, comparing the protective power of the plants to those of Jesus and that the plants have to be able to help one see the darkness as that is where the sickness lies (Narby 1989, p.268). The Asháninka believe that people in cities where there is less presence of spirits, must smoke so many cigarettes precisely for this protection the tobacco provides.

In the Perené, mestizo-Asháninka marriage is common, especially with Asháninka women and Spanish is usually spoken in a new mixed family. Weddings are held in the traditional manner: the wedding song could only be sung by men who had planted *maís* (corn). *Masato,* an alcoholic beverage made from the fermented root starch of yuca (*Manihot esculenta* Crantz [Euphorbiaceae]) , is offered in large wooden vessels (Keplinger, Laus et al. 1999).

3.3. Plant Knowledge Transfer

As an example of the transfer or acquisition of uses of introduced plant species, in Peru the Asháninka use the East Indian-native neem tree to treat diabetes (personal observation, 2003), a use that is found in India as well. When asked how this use came

about, Raúl Casanto Shingari, the chief of the Asháninka village of Paititi said that the neem tree was introduced to certain areas of Peru, such as the Amazon city of Pucallpa, when some Peruvians went to Costa Rica for an agriculture workshop, where they learned of the excellent pesticidal qualities of neem. Not having heard anything about its diabetes-treating qualities, one of the Asháninka who had diabetes tried a tea of it because of the bitter taste of the neem leaves and saw a rapid improvement in his condition. He then told his companions about this use, and its use spread around the community. If all of the facts of Raúl's story are correct, this would be a case of independently discovered use of an introduced plant, and can be included in the dataset. In Mali, neem is very common in large towns as a street tree and was introduced in 1950, the year of Malian independence, and is therefore called *mali yirini* or "plant of Mali", yet none of the 15 healers interviewed in Mali who were very familiar with the plant used neem to treat diabetes. In this case, even if neem had been used for diabetes or other diseases, because of the wide distribution of neem and much higher immigration to the area from the Indian subcontinent than to Peru, it would have been very difficult to determine if this was an introduced use, or an independently-discovered use of an introduced plant.

As explained by my collaborator and translator, Maramakan Kamissoko, the Malinké have an interesting view of the parasitic plant *gui* (*Tapinanthus* spp. [Loranthaceae]) that leads it to be one of the most revered plant medicines. The Malinké believe that the *gui* takes on and concentrates the medicinal qualities of the plant it grows on, and they place it in a hierarchy of plant part efficacy of fruits, leaves, bark, roots, and *gui*, going from least effective to most effective. So, for instance, if *toro* (*Ficus* spp. [Moraceae]) were

used for treating intestinal parasites, its roots would be used if available above other parts of the plant, but if a *gui* were found growing on a *toro* it would be considered the most effective at treating parasites and would be used preferentially. It is, however, not always easy to find *gui* growing on the appropriate plant and there are herbal healers who specialize solely in *gui* treatments. This concept of levels of efficacy also aligns nicely with the range of efficacies used in the relational efficacy system, and aligns partially with Balunas's (2006) work showing that below ground plant parts have a lower IC_{50} value (higher efficacy at the same concentration) than above ground parts. There seems to be no scientific work looking at efficacy of parasitic plants vs. their hosts to verify the Malinké's view of gui', but if the concept of bioamplification of compounds in predator and prey animals carries over to parasite-host relationships in plants, this would make sense. Moore (1979) claims that certain medicinal *Pedicularis* species have variable efficacy, perhaps from differing host plants and there is a similar preference for mistletoe (*Viscum album* L. [Viscaceae]) that grows on oak vs. other hosts in the anthroposophic medicine tradition from Europe (Ramm 2006) and a few similar historical records in European traditional medicine. Chemical studies show some evidence of parasite sequestration of host secondary compounds such as sesquiterpene lactones taken up by *Loranthus parasiticus* from its host *Coriaria japonica* (Okuda, Yoshida et al. 1987) and *Castilleja* species took up quinolizidine alkaloids from their *Lupinus* hosts and sequestered these repellent compounds in all tissues but the corolla and nectar, reducing herbivory but not pollination (Marvier 1998; Adler and Wink 2001). So although bioamplification of the host secondary compounds by parasite plants has not been shown, every other step in the process of differing secondary compound uptake by parasites

Figure 3.3. Covering a bark scar in Kita, Mali with dirt to prevent infection.

depending on hosts and ability of the parasite to use these sequestered compounds selectively has been shown, corroborating the Malinké idea that plant parasites are a more effective medicine than any part of their hosts.

In contrast, the Asháninka, although having a plant parasite in the same family *Marísaro* (*Phthirusa* spp. [Loranthaceae]) that is used medicinally, do not consider it a superior medicine to other plant based medicines in my observations or in the literature. This may be due to a lower prevalence of these plant parasites in the more diverse Amazon, a smaller range of hosts that the parasites can grow on (one collaborator said *Marísaro* grows mainly on *Persea americana, Citrus* spp., and *Psidium guayava* Raddi trees), or a less xerophytic habitat in the Amazon leading to less stress on the plants and less concentration of secondary compounds in the plants to avoid herbivory. They do however prefer *Marísaro* growing on certain *Citrus* hosts for treating certain disease and for other diseases, the host is not relevant, at least among the few healers I interviewed.

The Malinké seem also to have a more of a concern for harvest-induced stress on plants in their environment, making sure to take only root cuttings of plentiful species, and to seal wounds of trees from which bark is harvested by rubbing dirt in the cut area (see Figure 3.3). The Asháninka again do not seem to share this view, viewing almost all of the plants about which I asked them as being abundant and therefore one could harvest without concern for the health of the individual or population. Again this may be due to the lower physiological stress or human population density pressure of the Amazon environment.

Clearly, the Asháninka and Malinké share some key concepts about relations among people, plants, and animals, quick adoption of introduced species and use of ritualistic use of plants like *cola,* tobacco and *ayahuasca*, despite their vast separation in distance, habitat, and age of their cultures. The Asháninka's dependence on networks of people and their environment to advise the use of a particular plant, and the Malinké strong hierarchical village life yet still accepting strangers and exotic plants into their pharmacopoeia may stem from similar visions of the interconnectedness of these varied natural and social networks. They do however differ in their views and use of parasitic plants, plant conservation, separation of healer jobs, and diversity of the flora from which to draw medicinal plants. Perhaps it was their similarities of plant use or the overlap of the genera in the Asháninka and Malinké flora due to the biogeography of the two regions that led to the similarities in the two groups' medicinal plant use, as will become clear in subsequent chapters, but because of the clear lack of communication of plant use knowledge between the two groups, we can largely assume that most similarities are still

independent discoveries of effective medicinal plants that have undergone experimentation in each group's long history.

Chapter 4. Field Methods in the Peruvian Amazon and Malian Savanna

In order to achieve my goal to devise and verify a quantitative ethnobotanical scheme to pinpoint plants with a high potential of being biologically active against diseases such as malaria, asthma, and uterine fibroids, I needed to collect and accurately identify medicinal plants from several unrelated cultures. The larger the data set I gathered the better, although data sets from the literature and my previous work with the Q'eqchi and Itza Mayans in Guatemala and with Chinese, Dominican, Indian, and Western herbalists in New York can be incorporated as well. After learning the basics of fieldwork in New York and Guatemala, I selected and reviewed the ethnologies of the Asháninka and Malinké, cultures that are as unrelated as possible and therefore have not communicated medicinal plant uses to each other. With some understanding of these cultures, I set about finding contacts in Peru and Mali and with these two indigenous groups; learning basic tree climbing skills for collections, learning (or re-learning) Spanish, French, and a little Asháninka and Bamanakan; obtaining field collection permits, designing prior informed consent agreements; and getting Institutional Review Board (IRB) human subjects approval for my research. With all the appropriate permits and approvals, once I had traveled to each country I spent 2 months in each field site in Paititi, Peru, and Kita, Mali conducting interviews with healers, collecting and preserving herbarium vouchers and bulk samples of plants, and analyzing data on my collections using species-healer curves to make the best use of my time. In all, in Peru I conducted 9 interviews, collected 86

plant samples, 74 species, 73 genera, and 39 families, and in Mali I conducted 15 interviews, collected 90 plant samples, 80 species, 54 genera, and 41 families.

4.1. Pilot fieldwork

For beginning work on my thesis, I gathered data on the plants used by Indian, Chinese, European, and Latino herbalists around New York City to treat the related autoimmune diseases of eczema and diabetes. I interviewed 15 herbalists in Manhattan (see Table 4.1), often using the Mandarin Chinese, Spanish, and Hindi that I have learned in my travels, collecting the plants from their shops, and using the ethnobotanical library and the herbarium at the NYBG to identify the 130 species I had collected. As I did not have the tools to make complete dated phylogenies of these plants, I analyzed them using the methodology I had developed of looking for related plants used to treat related diseases in several unrelated cultures to determine their potential by merely looking for patterns of several plants in the same family or genus being used to treat both diseases in several of the cultures.

Herbalist	Location	Phone
Chinese		
L K Wong Herbalist	26 E Broadway	(212) 274-1823
N.Y. Tak Shing Hong Inc.	38 E Broadway	(212) 219-8313
Hong Kong Supermarket	109 E Broadway	(212) 227-3388
Ayurvedic		
Ayurvedic Health Center	204 W 96th St	(212) 280-1000
Indian & American Spice House	99 1st Ave	(212) 387-7812

Ayurveda Academy	22 W 34th St.	(212) 967-3967
Latin American		
Ramos Botanica	166 Rivington	(212) 260-8480
Botanica Sant a Barbara	4162 Broadway	(212) 795-9319
Botanica Las Mercedes	1252 St. Nicholas Ave.	(212) 928-2510
Botanica Reyes	340 Audobon	(212) 927-2133
El Paraiso Botanica	1311 St. Nicholas Ave.	(212) 928-6788
European/American		
Penny's General Store	97 1/2 E 7th st.	(212) 614-0716
Flower Power	406 E 9th	(212) 982-6664
Angelica Herb	147 1st Ave	(212) 677-1549

Table 4.1. Herbalists from four different cultures located in Manhattan

This points out several interesting families that are worthy of further research:

Apocynaceae for both diseases

Common Name	Scientific Name	Family	Order	Disease	Culture
Gurmar	Gymnema sylvestre	Apocynaceae	Gentianales	Diabetes	European
Gurmar	Gymnema sylvestre	Apocynaceae	Gentianales	Diabetes	Ayurvedic
Bai chian	Cynanchum stauntori	Apocynaceae	Gentianales	Eczema	Chinese

Table 4.2. Apocynaceae used to treat diabetes and eczema by European, Ayurvedic, and Chinese herbalists in New York City.

Although the gurmar used by the European herbalists is obviously a borrowed use and not an independent occurrence, this is an interesting grouping because the effectiveness of gurmar at lowering blood sugar levels has been backed up by scientific studies in rats (Chattopadhyay 1999). The gurmar was not as effective at lowering blood-sugar levels as Neem (*Azadirachta indica*) from Meliaceae, a family that had *A. indica* as its sole representative in this study.

Fabaceae for both diseases

Common Name	Scientific Name	Family	Disease	Culture
Bean	Phaseolus vulgaris	Fabaceae	Diabetes	European
Astragalus membranaceus	Astragalus membranaceus	Fabaceae	Diabetes	Chinese
Huang chi	Astragalus hoantchy	Fabaceae	Diabetes	Chinese
Licorice	Glycyrrhiza uralensis	Fabaceae	Diabetes	Chinese
Astragali	Astragalus hoantchy	Fabaceae	Diabetes	Chinese
Puerariae	Pueraria lobata	Fabaceae	Diabetes	Chinese
Clycyrrhizah	Glycyrrhiza uralensis	Fabaceae	Diabetes	Chinese
Guojian (Ge Geng)	Pueraria lobata	Fabaceae	Diabetes	Chinese
Glycyrrhiza	Glycyrrhiza uralensis	Fabaceae	Diabetes	Chinese
Fenugreek	Trigonella foenum-graecum	Fabaceae	Diabetes	Ayurvedic
Red Clover	Trifolium pratense	Fabaceae	Eczema	European
Balsam of Tolu	Myroxylon balsamum	Fabaceae	Eczema	European

Balsam of Peru	Myroxylon balsamum var. pareirae	Fabaceae	Eczema	European
Licorice	Glycyrrhiza	Fabaceae	Eczema	Chinese
Ku sheng	Sophora flavescens	Fabaceae	Eczema	Chinese
Licorice	Glycyrrhiza uralensis	Fabaceae	Eczema	Chinese
Mayrium	Clitoria ternatea	Fabaceae	Eczema	Ayurvedic
Sandal Surkh	Pterocarpus santalinus	Fabaceae	Eczema	Ayurvedic
Sheesham	Dalbergia sissoo	Fabaceae	Eczema	Ayurvedic

Table 4.3. Fabaceae used to treat diabetes and eczema by European, Ayurvedic, and Chinese herbalists in New York City.

Although this has the problems of large families standing out as found in previous family survey studies of medicinal plants (Moerman 1991), this family seems to very cross-cultural and used for both diseases. Hopefully its potential would be verified once dated cladograms can be found for this family.

One very interesting point is that powerful phytoestrogens have been found in the *Glycyrrhiza* genus from Fabaceae that are similar in chemical structure to the cortical steroids that are currently used to treat eczema (Evans 1996). This shows its potential for efficacy.

Apiaceae for eczema (5 species, 1 disease, 3 cultures)

Araliaceae for diabetes (2 species, 1 disease, 2 cultures)

Asteraceae for both diseases (12 species, 2 diseases, 3 cultures)

Ruscaceae for both diseases (2 species, 2 diseases, 1 culture)

Convolvulaceae for both diseases (2 species, 2 diseases, 1 culture)

Cucurbitaceae for both diseases (3 species, 2 diseases, 4 cultures)

Euphorbiaceae for eczema (2 species, 1 disease, 2 cultures)

Gentianaceae for both diseases (3 species, 2 diseases, 2 cultures)

Lauraceae for eczema (2 species, 1 disease, 2 cultures)

Meliaceae for both diseases (1 species, 2 diseases, 1 culture)

Myrtaceae for both diseases (3 species, 2 diseases, 4 cultures)

Poaceae for both diseases (3 species, 2 diseases, 1 culture)

Polygonaceae for eczema (2 species, 1 diseases, 2 cultures)

Rosaceae for both diseases (4 species, 2 diseases, 3 cultures)

Solanaceae for diabetes (2 species, 1 disease, 2 cultures)

Zingiberaceae for both diseases (3 species, 2 diseases, 1 culture)

4.2. Pilot fieldwork with the Maya in Guatemala

Continuing my research of different cultures, I traveled to the Petén region of Northern Guatemala in the summer of 20000 for a month to study the plants used by the Itzaj and Q'eqchi Mayans groups that have settled there. In addition to the Itzaj village of San Jose across lake Petén Itzaj from the tourist city Flores where there is a woman's herbal medicine garden collective, I went to the remote villages of Uaxactun and El Corozal where the people had not had much contact with foreigners, as these are the people that I believed could give me the best data on independent discovery of medicinal plants, without as much of the distorting effect of introduction of medicinal knowledge from the West. I was able to do much of this through the help of the Wildlife Conservation Society (WCS) office that I worked with in the Petén, as they help manage

the biodiversity and preservation of the Mayan Biosphere Reserve that occupies most of the Petén. I interviewed eight healers in these local communities about the plants they used to treat several diseases, a profile of their patients, and the origins of their knowledge. I was not able to bring back plant samples to the US due to the lack of time to obtain collecting permits, but I photographed these plants and identified most of them with the help of the WCS, a Mexican botanist Edilberto Ucan Ek' working there with the Itzaj, and several of the curators at the NYBG and the herbarium there. The data on these 77 plants used to treat eczema, diabetes, malaria, asthma, menopause symptoms and uterine fibroids in two Mayan cultures in Guatemala, were added to the data gathered in Peru and Mali to expand the cultural range of the data set.

4.3. Methodology

To accomplish this cross-cultural study, I traveled to the Southwest Amazon of Peru and Mali in western Africa to gather ethnobotanical data from indigenous groups on plants used to treat malaria, African sleeping sickness, Chagas disease, leishmaniasis, asthma, eczema, diabetes, and uterine fibroids. Field research was carried out in the Peruvian state (*departamento*) of Ucayali, where New York Botanical Garden (NYBG) researchers John Janovec, Miguel Alexiades, and James Graham provided contacts with people, from their work there. I spent six months learning the local languages and plant uses in this area. I had gotten in touch with the Austrian ethnopharmacologist Klaus Keplinger after reading his paper on *uña de gato* (*Uncaria tomentosa* (Willd. ex Roem. & Schult.) DC.) and the Asháninka he had worked with in Ucayali (Keplinger, Laus et al. 1999). He had put me in touch with the Asháninka Raúl Casanto Shingari, chief of the village of Paititi in the Yuruá district (*distrito*), who I was able to call from the US before

traveling to Peru and arrange a meeting when I arrived. In Mali, working with the *Département de Médecine Traditionnelle* (Department of Traditional Medicine, DMT) in the capital Bamako, and their connections with the *Association des Thérapeutes Traditionnels de Kita* (Association of Traditional Healers of Kita) in Kita, in the Western extent of Mali, I was able to interview fifteen Malinké healers during field work in 2004..

Gathering accurate ethnobotanical data involves first making contact with indigenous groups in Peru and Mali through my connections at the NYBG and CUNY. The more remote and more recently contacted a group is the better, as they will have less chance of introduced plant uses. Although the cultures selected must be as distant as possible, it is also necessary that they share some elements of the floras in their areas of the world. The areas compared need not have the exact same species, if they share some genera or families of plants it makes determining the plants relatedness easier. In comparing the flora of Peruvian Amazon and the dry savannas of Mali, we have found that 21% of their genera, and that 30% of the medicinal plant genera of the Mali savannas are also found in Peru (Adjanohoun et al., 1980; Boudet et al., 1986; Malgras, 1992). These are good numbers to make a more in-depth comparison of the medicinal plants of the two areas.

4.4. Diseases

A criterion for selecting diseases to study is to find diseases that are related and have the same underlying cause in the body. Using these criteria, I have selected malaria, African sleeping sickness, Chagas' disease, leishmaniasis, diabetes, eczema, asthma, and uterine fibroids. Diabetes, eczema, and asthma I have picked as all three are autoimmune diseases, with the latter two more closely associated in the "autoimmune triad". The third member of this autoimmune triad is hay fever, and the inheritance of these diseases can

jump between members of the triad, i.e. a child may inherit the disease in the form of eczema even though their mother may have asthma, and their grandfather may have hay fever. If one culture treats asthma with a certain plant and another distant culture treats eczema with the same plant, although these diseases seem superficially very different, they are considered closely related autoimmune diseases by Western medicine and therefore could be treated by the same plant chemicals acting on the underlying mechanism of the immune system. Thus, these two distant uses of the same plant for eczema and asthma can be considered similar uses, raising the potential of this plant.

Malaria, leishmaniasis, African sleeping sickness, and Chagas' disease are caused by a protozoal parasite infection, the latter three more specifically by a trypanosome (family Trypanosomatidae), and the latter two being in the same genus *Trypanosoma* (Benson et al., 2000; Federhen et al., 2000; Wheeler et al., 2000), thereby exhibiting different degrees of evolutionary proximity. Interestingly, the *Trypanosoma brucei* parasite that causes African sleeping sickness and the *Plasmodium* parasites that cause malaria have been linked with immunomodulatory effects and reducing the incidences of autoimmune diseases in areas where they are predominant in Africa (Greenwood, Herrick et al. 1970; Butcher 1991; Harnett 2005; Mansfield and Paulnock 2005; Ndungu, Urban et al. 2005), linking these seemingly disconnected parasitic diseases with the autoimmune diseases asthma, eczema, and diabetes via mechanisms in the human body.

Studying uterine fibroids allowed me to compare my work in Peru with ethnobotanical research that has been done on these diseases in Chile and among Dominican and Chinese groups in New York City by the Rosenthal Center for Complementary and Alternative Medicine at the Columbia-Presbyterian Medical Center

(CPMC) (Balick et al., 2000). In my work at the CPMC I have helped analyze the Chinese and Dominican plants used to treat uterine fibroids and I wanted to extend this important issue in women's health to other cultures and related diseases. Of the diseases I have mentioned, however, uterine fibroids was one of the more difficult to study as it has few outwardly apparent symptoms, and the fact that informants were often unwilling to discuss a women's reproductive health problem with me, a male researcher. Despite this difficulty, it was worth attempting to study this disease in order to add to the large cross-cultural databases already collected on this subject.

In all the field research I have conducted, cultural notions of disease have been difficult to deal with because of different symptomatic descriptions for what may be the same disease. What would be best would be to do direct observations of healers working with patients to see firsthand which plants they use for patients with different symptoms, and I tried to do this as much as possible when it was culturally acceptable, but some of the groups, so I had to be aware of cultural etiquette to observe the often private healer/patient relationship. If I could determine each groups disease epidemiology directly using simple blood or cheek swab tests, this would show beyond a doubt that people have the diseases I am studying, although not if they are treating these specific diseases. The difficulty of doing blood testing in another country with both the foreign countries' permit process, and CUNY's own human subjects review board would have made this nearly impossible to do as well.

Working with medical texts, doctors, translators, and healers of each culture, the symptoms of a disease in that culture and the name of the disease in the local language (Asháninka or Bambara) were determined to help resolve this issue. During interviews,

diseases were at first only described by their Western medical symptoms, not by name, and the collaborator was asked to name the disease and the plants used to treat it. If a particular collaborator did not give the name of the disease in their language based the stated symptoms, they would be interviewed again later about the same disease, but the second time it would be named in their local language and in the country's official language (Spanish or French) if they spoke that language. This dual description of each disease by symptoms and name will provide valuable information on whether more effective medicinal plants are found by describing symptoms or by naming diseases, once the efficacy of each plant has been determined.

Prior informed consent forms cleared with the City University of New York Institutional Review Board (CUNY IRB) on human subjects were signed with everyone interviewed that guaranteed immediate compensation for the healer's time, return of documentation of the results of the study to the community (Bletter 2006), that pharmaceuticals would not made from the medicinal plants described in the study, and that the names of the plants would not be revealed to anyone outside the study.

4.5. Disease symptoms

In order to conduct proper interviews with Asháninka and Malinké healers on the medicinal plants they use to treat the seven diseases I chose to study, the symptoms and epidemiology of each disease needed to be well understood. The symptoms had to be translated and well understood in the local language and medical system of the healers, i.e. describing diabetes patients as having urine that attracts ants (because of the high sugar content) is much more effective than describing these patients as having a high blood sugar levels or low insulin response. To this end, I give here a description of each

of the studied diseases using outwardly apparent symptoms as much as possible that I translated into French and Spanish and used as the basis for my disease interviews.

Type I diabetes (also known as insulin-dependent diabetes mellitus or juvenile onset diabetes) is defined as a chronic disease that stems from insufficient production of insulin by the pancreas, which leads to improper regulation of blood sugar levels (Isselbacher, 1980). The resulting excess of blood sugar can often lead to sugar in the urine, a symptom of this disease known for hundreds of years. This form of diabetes, as opposed to Type II or noninsulin-dependent diabetes, is more likely to occur in indigenous populations as it more often stems from genetic factors rather than lifestyle and diet. Type I diabetes is thought to sometimes be caused by auto-immunity– the body attack and destroying its own insulin-producing cells, the islets of Langerhans. Several researchers have expressed concern that indigenous groups would have no herbal remedies for Type 1 diabetes since it is so deadly if treated with anything but insulin (personal communication, Stephen King, 2007), but Type II diabetes not being an autoimmune means it does not fit easily into system of related diseases I have outlined here, not to mention that this concern was raised after I had finished my fieldwork, so I chose to stick with analyzing Type I diabetes. Type I diabetes presents the following physically apparent symptoms:

- Frequent urination
- Excessive thirst
- Hunger
- Fatigue
- Weight loss
- Blurry vision

- Nausea
- Vomiting
- Sugar in the urine

In an asthma attack, the lungs and throat become inflamed and there are attacks of wheezing, short breath, chest tightness, and coughing; this is another disease believed to autoimmune, although in a different group than diabetes (Isselbacher, 1980). Besides those in the definition, asthma is characterized by these apparent symptoms:

- Wheezing which begins and ends suddenly, and is more common at night and in the morning, in cold air, after exercise, and during heartburn
- Shortness of breath that is aggravated by exercise
- Laborious breathing
- Intercostal retractions (pulling of the skin between the ribs when breathing)

These symptoms are less common and/or appear only during extreme bouts of asthma:

- Extreme difficulty breathing
- Bluish color in the lips and face
- Severe anxiety
- Rapid pulse
- Sweating
- Decreased level of consciousness (severe drowsiness or confusion)
- Nasal flaring
- Chest pain
- Tightness in the chest

- Abnormal breathing pattern, with exhalation taking much longer than inhalation
- Breathing which temporarily stops
- Coughing up blood

Eczema is often found in people who have a family history of allergic reactions such as asthma and hay fever as well as eczema. These three diseases are often called the autoimmune triangle because of the body's overreaction against itself or external, seemingly benign irritants. Eczema is a chronic skin disease defined by itchy and scaly rashes (Isselbacher, 1980). There is often what is called "the itch-scratch cycle" in eczema where constant itching worsens rashes, making them itch more. Itching can be more common at night, when conscious control of itching is absent. Additional visible symptoms are:

- Dry, itchy or cracked skin, especially behind the ears, on the cheeks, arms and legs
- Lichenification- when the skin becomes thick and leather-like from excessive scratching
- Small, raised bumps called "papules" that can open and become scaly when scratched
- Small, rough bumps on the face arms and thighs called "keratosis pilaris"
- Dry, rectangular scales on skin called "ichthyosis," as in fish scales
- Increase in the creasing of the palms
- Hives ("urticaria") that can develop after exercise, hot baths, or exposure to allergens

- Inflamed skin around the lips
- An extra fold of skin developing under the eye, called an "atopic pleat" or "Dennie-Morgan fold" and a darkening of the eyelids, especially from hay fever attacks
- Increased susceptibility to skin infections such as warts or herpes simplex

Uterine fibroids (synonymous with benign tumors of the uterus, uterine leiomyoma, uterine fibromyomas, or leiomyomata) are benign tumors that develop in the uterine wall's muscle and connective tissue (Isselbacher, 1980). Although hard to diagnose from externally visible symptoms, and sometimes confused with ovarian tumors or pregnancy, uterine fibroids have the following symptoms:

- More frequent urination
- A feeling of swelling, gaseousness, or pressure in the lower abdomen
- Pelvic cramps associated with the menstrual cycle
- Menorrhagia- heavy menstrual bleeding with occasional blood clot inclusions, which can result in anemia
- Sudden and severe pain from twisting of fibroids

Leishmaniasis is a parasitic disease caused by the trypanosome *Leishmania donovani* (visceral leishmaniasis), *L. mexicana* (New World cutaneous leishmaniasis) , *L. braziliense* (New World mucocutaneous leishmaniasis) , or *L. tropica* (Old World cutaneous leishmaniasis), spread by the bite of the sand fly (*Phlebotomus argentipes*, *P. oreintalis*, *P. perniciosis*, or *Lutzomyia longipalpis; P. papatasi; L. olmeca* and *L. flaviscutella; L. umbritalis*, *L. trapidoi*, *L. verrucarim*, and *L. wellcomei* respectively)

(Cahill et al., 1989). Although each of these particular species of the leishmaniasis parasite is found in different parts of the world, their relations permit a perfect test for the related diseases section of my thesis, with a close relation between the parasites found in South America and Africa, and a slightly more distant relation with the *Trypanosoma cruzi* parasite that cause Chagas' disease found in the Americas and the *Trypanosoma brucei* parasite causing African sleeping sickness. All forms of leishmaniasis present with a skin ulcer forming at the site of the original lesion from the sand fly bite. Additional symptoms of mucocutaneous leishmaniasis include:

- A history of a sand fly bite
- Runny or stuffy nose
- Nose bleeds
- Ulcers developing into erosion of the mouth, tongue, gums, lips, nose, and nasal septum
- Difficulty breathing and swallowing

In the visceral form of leishmaniasis additional symptoms are:

- Persistent fever with a duration of several weeks that may cycle irregularly
- Night sweats
- Fatigue
- Weakness
- Appetite loss (anorexia)
- Weight loss
- Vague abdominal discomfort
- Vomiting in children
- Diarrhea in children

- Coughing in children
- Scaly, gray or dark skin
- Thinning hair

In the cutaneous form of leishmaniasis additional symptoms are:

- Small, flat or raised bumps on the skin
- The skin ulcer at the site of the original lesion heals very slowly over a matter of months
- Smaller satellite lesions may form around the ulcer

Chagas disease is another disease found in South and Central America caused by a trypanosome, in this case *Trypanosoma cruzi* and transmitted by the assassin bug (reduviid or cone-nosed bug, most commonly *Triatoma infestans*, but also *T. braziliense, T. dimidiata, T. sordida, Rhodnius prolixus,* and *Panstronsylus megistus*) when the bug defecates right next to the site where it has just bitten someone, and the person then wipes the parasite-infested feces into the bite, and hence into the bloodstream (Cahill et al., 1989). After the infection incubates over a week or two, the following symptoms appear:

- Swollen red area at site of previous insect bite
- Enlarged lymph nodes
- Swelling of one eye
- Fever
- Heartbeat irregular (arrhythmia) or rapid (tachycardia)
- Difficulty in swallowing

The related African sleeping sickness, which affects about 500 million people a year, is caused by the parasite *Trypanosoma brucei,* carried by tsetse flies (*Glossinia* spp .) which produces meninges, or inflammation of the brain covering (Cahill et al., 1989). The West African strain of the disease is caused by *T. brucei gambiense* and carried by the *G. palpalis* vector, whereas the East African strain is caused by *T. brucei rhodesiense* and carried by *G. morsitans*. In addition to the meninges, it produces these apparent symptoms:

- Swollen red painful nodule at site of inoculation
- Swollen lymph nodes all over the body
- Headache
- Fever
- Sweating
- Anxiety
- Insomnia at night
- Mood changes
- Drowsiness
- Uncontrollable urge to sleep

Malaria, caused by infection with the *Plasmodium* parasite, is one of the most destructive diseases of the tropics and developing countries, with about 200 million people getting the disease every year, and mortality rates of up to 10% in children under 5 (Cahill et al., 1989). Although easily stoppable if treated in a timely manner, access to the effective medicines is difficult in developing countries where the disease predominates, increasing mortality rates. The necessary drugs are rapidly becoming more

expensive, due to development of resistance to the cheaper drugs. People in resistant areas have had to advance up the list of more powerful drugs from chloroquine to mefloquine to doxycycline. About two weeks after the malarial parasite (*Plasmodium falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*) is passed into the host bloodstream by an *Anopheles* mosquito bite, the main symptoms of malaria are caused by the *Plasmodium* merozoite stage entering, developing in, and rupturing red blood cells. This leads to these apparent symptoms, with the cyclic fever being the most characteristic:

- Cyclic sequences of chills (~1 hour), fever (4-6 hours), and sweating (1-2 hours)
- Headache
- Nausea and vomiting
- Muscle pain
- Anemia, characterized by fatigue, chest pain, and shortness of breath
- Abdominal pain from enlarged spleen
- Bloody stools
- Jaundice
- Convulsions
- Coma

4.6. Prior Informed Consent Agreements and Human Subjects Approval

Every university study that involves humans or any other animal must be approved by the Institutional Review Board who reviews the entire research methodology that involves human subjects to ensure that it does not cause the subjects any undue stress and

that the benefits of the study outweigh the risks. The IRB and the ethics guidelines of the Society for Economic Botany (SEB, 1995) and the International Society of Ethnobiology (International Society of Ethnobiology 1998) require that researchers sign a Prior Informed Consent (PIC) agreement form with all human subjects before conducting research and interviews so that the interviewees know fully what the research is about, know their possible risks and benefits, and that they can terminate the research at any time if they feel uncomfortable or at risk without loss of compensation. The SEB and ISE guidelines further encourage researchers to protect the intellectual property of and include equitable benefit sharing for the people and communities they do research with in terms of returning results of research, documentation, capacity building, and giving a percentage of profits from commercial products derived from the research back to the community as money or in building resources such as schools, medical clinics, or protected parks and preserves.

To this end, I stated in my PIC that I would keep the information given to me by my collaborators confidential, that I would return the results of the research to them in the form of documentation, and that I would not develop the plants they discussed with me into pharmaceuticals. In both Kita and Paititi, the healers I talked to agreed that these were all very important points to have laid out before the interviews could proceed. Included here are the pertinent sections of my IRB application and the PIC forms in English, Spanish, and French that I signed with healers before interviewing them.

4.6.1. Risk and Benefit

There was minimal risk to all the subjects in this study as I was asking healers (health care providers) in the Peruvian and Malian communities about their normal activities in

terms of the plants they use as medicines, and there is no sensitive information requested. If they feel uncomfortable giving me any information they can choose not to be interviewed at any time, or ask for any audio recordings I have made to be erased. I will never be asking anyone to reveal a specific disease that they or someone else has, only what they use as a general treatment for a particular disease.

The benefits to the subjects and their community is the documentation of their medicinal plant knowledge which is often disappearing at a rapid rate as younger generations are often not interested in learning this information. The documentation I give back to the subjects and the community was laminated books of photos, descriptions, and medicinal uses of each plant about which I have interviewed them, laminated herbarium sheets (dry plant specimens), and any papers I write on the subject translated into their local language, all of which allows future generations to identify these plants and relearn this lost knowledge if they wish.

A more immediate benefit is that the outcome of my research pinpoints which of their many current plants used to treat each disease are the most effective treatment, so they can focus their efforts on that plant if they choose.

4.6.2. Confidentiality

The subjects' names are not recorded on audiotape and their recording is only noted with a code for their name in my notebook. The correspondence of subject names and codes was held only by me and did not be revealed to anyone.

Any publications, databases, or herbarium records (botanical libraries) stemming from this research did not include any subjects' names.

4.6.3. Possible Medical Conditions

I do not anticipate learning of any subject's specific medical conditions as I am solely asking them what medicinal plants they use in a general to treat certain conditions, not attached to any particular person. However, I provide subjects with information on local health clinics where they can go if they need treatment.

For interview subjects who cannot read the following prior informed consent agreements or do not speak French or Spanish, I have the agreement read and explained to them in their native language of Bamanakan or Asháninka, so they fully understand what they are signing.

THE GRADUATE CENTER
THE CITY UNIVERSITY OF NEW YORK

Ph.D. Program in Biology

The Graduate School and University Center
The City University of New York
365 Fifth Avenue
New York, NY 10016-4309
TEL 212.817.8100 FAX 212.817.1504

Medicinal Plant Research

I am Nat Bletter, a researcher from the biology/plant sciences department at the City University of New York and the New York Botanical Garden. I am doing a study to compare how people in different parts of the world use plants to treat the same illnesses in order to find out which plants might be the best for treating those diseases. I wanted to ask some questions about what plants you use to help treat illnesses and diseases.

With your permission I wanted to make an audio recording of the interview. However, you can listen to this recording and ask me to erase all or parts if you do not like it. I will also read back to you what you have told me in the interview once I have translated it, and you can ask me to correct or erase any part of the interview. Only I will listen to and have access to the audio recordings and translations, which was stored securely on my locked computer and backups. If you wanted to have the results of the study when I am finished I was happy to give you a copy.

The benefits of the study are that it will help us to learn which medicinal plants that you currently use might be best for treating diseases and illnesses. At the end of the study, I will give you a book, with photos, that you can use to teach your children about these plants, your local plant name, medicinal uses for each plant that you and the people in your community have told me about, and which of these plants I have found to be the best for each disease. You will also be given food or money equal to what you would normally receive for working the length of time of the interview. I did not tell anyone else which medicinal plants you use, unless you tell me it is OK to tell other people. I did not try to make drugs from these plants, but will use the plants as you use them.

I am asking all the healers in this community to answer these same questions. There are no known risks in taking part in this study, because your name and audio recordings did not be seen or heard by anyone but me. The interview takes about 2 hours to finish. I plan to interview about 20 people in your community.

Taking part in the study is voluntary. If you choose not to answer these questions, there was no penalty and it did not affect any services or other benefits you might receive from me. If you do fill out the survey, you may choose to not answer any question, but we ask you to answer as many questions as you can.

If you have questions about the survey, please contact me at the graduate center (CUNY), phone 212-677-0222, by email at nbletter@lehman.cuny.edu, or by mail at 455 FDR Dr., #707B, New York, NY 10002, U.S.A., or my advisor Doug Daly by phone at 718-817-8660, by email at ddaly@nybg.org, or by mail at the New York Botanical Garden, 200th St. & Southern Blvd., Bronx, NY 10458-5126, U.S.A.

If you have questions about your rights as a volunteer, please contact Hilry Fisher, telephone 212-817-7523, e-mail hfisher@gc.cuny.edu, or through mail at the Office of Research and Sponsored Programs, CUNY, 365 5th Ave., New York, NY 10016-4309, U.S.A.

Please take this cover sheet of explanation with you. I also have copies of the cover sheet and questions if you need them.

I have read this form and agree to participate in this study.

_____ _____

Name Date

I agree to be audiotaped: yes ___ no ___

_________________________________ _______________

Principal Investigator Date

Ph.D. Program in Biology

The Graduate School and University Center
The City University of New York
365 Fifth Avenue
New York, NY 10016-4309
TEL 212.817.8100 FAX 212.817.1504

Consent form in Spanish (for Peruvian subjects)

Estudio De la Investigación De las Plantas Medicinales

Me llamo Nat Bletter y estoy un investigador del departamento de Biología y Botánico de la Universidad de la Ciudad de Nueva York (CUNY) y el Jardín Botánico de Nueva York. Estoy haciendo un estudio para comparar cómo la gente en diversas partes del mundo utiliza las plantas para tratar las mismas enfermedades para descubrir que planta pudo ser el mejor para tratar esas enfermedades. Quisiera hacer algunas preguntas acerca de qué plantas usted utiliza para ayudar a tratar ciertas enfermedades. Trato aprender qué plantas usted utiliza y cómo cada planta se utiliza para ayudar a tratar varias enfermedades.

Con su permiso quisiera haré una grabación audio de la entrevista, pero usted puede escuchar esta grabación y pedir que borre todo o de partes si usted no se gusta. También le leeré qué usted me ha dicho en la entrevista después de la haya traducido, y usted puede preguntarme que corregir o borrar cualquier parte de esto si usted quisiera. Solamente yo escucharé y tendré acceso a las grabaciones audio y a las traducciones, que serán salvadas con seguridad en mi ordenador y salvaguardias bloqueados. Si usted quisiera tener los resultados del estudio cuando me acaban me placeré darle una copia.

Este estudio beneficiará a ustedes y a otros participantes porque usaré los resultados del estudio para encontrar qué plantas medicinales que usted usa actualmente pudo ser el mejor para cada enfermedad. En el final del estudio, le daré un libro con las fotos, su nombre local de la planta, las aplicaciones medicinales para cada planta usted y la gente en su comunidad me han dicho, y cuáles de estas plantas he descubierto para ser el mejor

para cada enfermedad. Usted pueda utilizar eso libro para enseñar a sus niños sobre estas plantas. También le darán el alimento o el dinero igual a lo que usted recibiría normalmente para trabajar la cantidad de tiempo que dura la entrevista. No diré a cualquier persona qué plantas medicinales usted utiliza, a menos que usted me diga que es aceptable de decir a la gente. No intentaré hacer drogas de estas plantas. Solo usaré las plantas como usted las utiliza.

Estoy pidiendo que todos los curanderos en esta comunidad contestaran a estas mismas preguntas. No hay riesgos sabidos en participar en este estudio, porque su nombre y grabaciones audio no serán considerados ni serán oídos por cualquier persona excepto mí. La entrevista lleva Dura cerca de 2 horas. Planeo entrevistarme con a cerca de 20 personas en su comunidad.

De participar en el estudio es voluntario. Si usted elige no contestar a estas preguntas, no habrá pena y no afectará ninguna servicios u otras ventajas que usted pueda recibir de mí. Si usted completa la encuesta, usted puede elegir no contestar a ninguna pregunta, pero pedimos que usted conteste a tantas preguntas como usted puede. Planeo entrevistarme con mas o menos 20 personas en su comunidad.

Si usted tiene preguntas sobre la encuesta, por favor entre en contacto conmigo en CUNY, teléfono 212-677-0222, por el email en nbletter@lehman.cuny.edu, o por correo en 455 FDR Dr., # 707B, New York, NY 10002, U.S.A., o mi consejero **Doug Daly** por el teléfono en 718-817-8660, por el email en ddaly@nybg.org, o por correo en New York Botanical Garden, 200th St. & Southern Blvd., Bronx, NY 10458-5126, U.S.A.

Si usted tiene preguntas sobre sus derechas como voluntario, entre en contacto por favor con **Hilry Fisher**, por el teléfono 212-817-7523, por el E-mail

hfisher@gc.cuny.edu, o por el correo a Office of Research and Sponsored Programs, CUNY, 365 5th Ave., New York, NY 10016-4309, U.S.A.

Tome por favor esta hoja de la explicación con usted. Yo también tengo copias de la hoja y las preguntas si usted las necesita.

He leído esta forma y acuerdo participar en este estudio.

_____ _____

Nombre Fecha

Acuerdo con la grabación audio: sí ___ no ___

_____ _____

Investigador Principal Fecha

Ph.D. Program in Biology

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The City University of New York
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Consent form in French (for Malian subjects)

Étude de Recherches de Plante Médicinale

Je suis Nat Bletter, un chercheur de en la service de biologie et de botanique à l'Université de Ville de New York (CUNY) et le Jardin Botanique de New York. Je vous demande de répondre à quelques questions au sujet de quelles plantes vous utilisez pour aider à traiter certaines maladies. Le chercheur veut savoir quelles plantes sont utilisées et chaque plante est utilisée pour traiter chaque maladie.

Avec votre permission, je ferai un enregistrement sonore de l'entrevue, mais vous pouvez écouter cet enregistrement et me demander de l'effacer tous ou des pièces si vous ne l'aimez pas. Je vous relirai aussi ce que vous m'avez dit dans l'entrevue après que je l'ai traduite, et vous pouvez me demander de corriger ou effacer toute partie de ceci que vous voudriez. Seulement j'écouterai et aurai accès aux enregistrements sonores et aux traductions, qui seront enregistrés solidement sur mon ordinateur et sauvegardes verrouillés.Si vous voudriez avoir les résultats de l'étude quand je suis terminé, je serai heureux de vous donner une copie.

Cette étude bénéficiera vous et d'autres participants parce que j'emploierai les résultats de l'étude pour trouver quelles plantes médicinales que vous utilisez pourrait être le meilleur pour chaque maladie. À la fin de l'étude, je vous donnerai un livre que vous pouvez utiliser pour enseigner vos enfants au sujet de ces plantes avec des photos, votre nom local de la plante, des utilisations médicinales pour chaque plante vous et les personnes dans votre communauté m'avez dit, et lesquelles de ces plantes j'ai découvert pour être le meilleur pour chaque maladie. Je vous donnerai également la nourriture ou

l'argent égal à ce que vous recevriez normalement pour travailler la durée de l'entrevue. Je ne dirai pas à n'importe qui autrement quelles plantes médicinales vous utilisez, à moins que vous me disiez vous voulez que je dise d'autres. Je n'essayerai pas de faire des drogues à partir de ces plantes. J'utiliserai seulement les plantes comme vous les utilisez.

Je demande à tous les guérisseurs dans cette communauté de répondre à ces mêmes questions. Il n'y a aucun risque connu en participant à cette étude, parce que votre nom et enregistrements sonores ne seront pas vus ou ne seront pas entendus par n'importe qui excepté moi. L'entrevue durera environ 2 heures à la finition. Je projette interviewer environ 20 personnes dans votre communauté.

Participer à l'étude est volontaire. Si vous choisissez de ne pas répondre à ces questions, il n'y aura aucune pénalité et elle n'affectera aucun service ou d'autres avantages que vous pourriez recevoir de moi. Si vous complétez l'étude, vous pouvez choisir de ne pas répondre à n'importe quelle question, mais nous vous demandons de répondre à autant de questions comme vous pouvez.

Si vous avez des questions au sujet de l'étude, s'il vous plaît entrez en contact avec moi à CUNY, par le téléphone 212-677-0222, par l'email à nbletter@lehman.cuny.edu, ou par la poste au 455 FDR Dr., # 707B, New York, NY 10002, ou mon conseiller **Doug Daly** par téléphone à 718-817-8660, par l'email à ddaly@nybg.org, ou par la poste au New York Botanical Garden, 200th St. & Southern Blvd., Bronx, NY 10458-5126, U.S.A.

Si vous avez des questions au sujet de vos droites en tant que volontaire, contactez s'il vous plaît **Hilry Fisher**, par téléphone 212-817-7523, par le E-mail hfisher@gc.cuny.edu,

ou par le courrier au Office of Research and Sponsored Programs, CUNY, 365 5th Ave., New York, NY 10016-4309, U.S.A.

Veuillez prendre cette feuille d'explication avec vous. Moi avons également des copies de la feuille et les questions si vous avez besoin d'elles.

J'ai lu cette forme et accepte de participer à cette étude.

_____ _____

Nom Date

J'accepte d'enregistrement sonore : oui ___ non ___

_____ _____

Investigateur Principale Date

4.7. Interviews

The Asháninka community of Paititi is located in the Southwest Amazon vegetation zone in the Ucayali Department of Peru, near the Brazilian border. The Asháninka who live in Paititi mostly speak the Yuruá Asháninka dialect, although some speak the Perené dialect as well, and there are one to two visiting teachers who are indigenous Shipibo, also from Ucayali Department. In the two years of fieldwork in Paititi (2003 and 2004) the population of the community fluctuated between 25-30 people during the two field seasons, comprising 6 families living in separate palm thatch and wood houses. The surrounding agricultural fields and rainforest are typical of the Southwest Amazon habitat (Daly, Silveira et al. 2007).

In Mali, working with the *Département de Médecine Traditionnelle* (Department of Traditional Medicine, DMT) in the capital Bamako, and their connections with the *Association des Thérapeutes Traditionnels de Kita* (Association of Traditional Healers of

Kita) in Kita, in the Western extent of Mali, I was able to interview fifteen Malinké healers during field work in 2004. The Malinké, one of the largest ethnic groups in Mali, with about 600,000 members, speak a combination of French, Bamanakan, and Malinké, and are generally Muslim, animist, or a combination thereof (Schaffer and Cooper 1980; Gordon 2005).

The field site of Kita, in the western end of Mali is in the Sudanese savanna area with some Guinean gallery forest vegetation type reaching up into the southern end of the town but with fewer of the baobab trees (*Adansonia digitata* L. [Malvaceae]) common in the eastern part of the country (Arbonnier 2002).

With a full set of diseases defined, and contacts in Peru and Mali from my NYBG and CUNY colleagues, my field research involved interviews with healers and patients, and gathering plants for identification. I found healers in the communities of Paititi and Kita by talking to community members and leaders, as well as working with cultural and conservation organizations in the country, such as the DMT in Bamako, Mali and Shinai Serjali and AIDESEP in Lima, Peru. Once in the communities, I used informal interviews with direct and indirect open questions (Alexiades, 1996) to find the people considered to be healers in the community asking such questions as

- If you or someone in your family is feeling sick, and you feel you cannot treat the illness yourself, are there other people that you go to for treatment?
- How often do you go to others for medical help?
- Are they members of your community or outsiders?
- Why do you trust this person with your health?
- Do they have a good reputation in this community?

- Have they treated you successfully in the past?
- Do they use plants during their treatment, or ask you to use plants in any way after the initial treatment?
- What do you usually give this person in exchange for their treatment?

I also asked each healer I interviewed if they knew of any other healers to whom I could talk. Although this is a form of "snowball sampling" which is not the best way to get a statistically rigorous random sample of a population (Alasuutari, 1998), the populations I worked with are small enough, and I was with them long enough, that I was able to find everyone considered a healer in the community, in essence a census of the population.

Once I determined the set of healers in each community I wanted to interview, I set about figuring out if any of their disease concepts match with the diseases I am studying through a set of open, direct questions in a semi-structured interview with each of the healers. So as not to bias them asking such pointless questions as “What plant do you treat diabetes with?”, I simply showed them pictures of (see -Figure 4.6) and described symptoms of each disease with the help of translators when necessary, to determine if they have a disease category in their culture that matches well with our Western disease categories. Specifically I asked each healer the following:

- What is your name, age?
- What is the name of your people?
- Where do you live?
- How long have you lived here?
- What is your position/occupation in the community?

- What type of healer do you call yourself?
- How long have you been a healer?
- Where and from whom did you learn to be a healer?

Then for each of the diseases I studied (malaria, leishmaniasis, Chagas' disease for Peru, African sleeping sickness for Mali, asthma, diabetes, eczema, and uterine fibroids), I asked the following questions after showing pictures of and describing symptoms of the disease:

- Have you ever seen or heard of someone having the symptoms I have described and shown you?
- Do you have a name for this type of illness?
- How do you define and diagnose this illness?
- How many people have you seen with this illness and over what time period?
- How many people have you treated for this illness and over what time period?
- If someone who has this disease is not treated, what happens to them?
- How do you treat this illness?
- Do you use plants?
- Do you use any plants as medicines that you have only appeared in your lands recently?
- Do you use any herbal remedies that you learned from people outside your group?
- Do you use other methods?

- If you use plants, what plants do you use to treat this illness?
- Do you have a name for this treatment?
- How do you prepare it?
- Are there special circumstances (time of day, season, mental state, climate, place) under which you prepare this medicine?
- How do you administer it?
- Are there special circumstances (time of day, season, mental state, climate, place) in which you administer this treatment?
- What are the amounts of this medicine the patient must use, how long, and how often should they use it?
- How long before you start to see the patient's condition improve while taking this medicine?
- Do you see the patient again while they are taking the medicine?
- How do you tell if this treatment is working to heal the patient?
- How does this medicine work to treat the patient?
- How effective is this medicine?
- Are their certain conditions of the patient (pregnancy, menstruating, child, man or woman, other illnesses) under which you cannot use this treatment on them?
- Where did you learn this treatment?
- How long have you been using it?
- Has your use of it, or its effectiveness changed over the time you have been using it?

For each plant the healers told me about or showed me, I asked the following additional questions:

- What do you call this plant?
- Where does this plant grow?
- Can you show me this plant now or some other time?
- Do encourage this plant's growth in any way?
- Where do you collect this plant?
- Are there special circumstances (time of day, season, climate, mental state, plant development stage, next to another plant, surroundings) in which you collect this plant?
- Are there any special procedures or tools you need to collect this plant?
- Do you only collect a certain amount of this plant from each place you find it, even if you need more?
- What do you do to ensure there was enough of this plant the next time you need it?
- What part of the plant do you use?
- Have you seen this plant with flowers or fruit?
- If so, under what conditions (season, climate, particular habitat)?
- Can the plant be stored for later use or must you use it immediately?
- If you store it, how and how long will it last?
- Where did you learn about this plants effectiveness for each illness you use it to treat?
- How long have you used this plant as a medicine?

- Has it been in your area as long as you can remember or did it appear recently?
- How far do you usually have to travel to collect it?
- Have you always had to travel that far?
- Has the plant gotten harder to find?
- Has its condition changed over the years?
- Is it all right if I collect some of the plant?
- May I take a photograph of this plant?
- Should I perform any special procedure before I collect this plant?

For each plant I was shown I gathered a voucher of the plants for identification and reference, taking a photograph of the plant and surroundings and recording the location via GPS, elevation, habitat, plant form, and phenological state. I followed the standard botanical practice of gathering four sets of each plant for distribution to my home herbarium, a major herbarium in the host country, a family specialist for identification, and the community from which it was gathered, so they have a way to train others in the community in plant identification. When possible, I gathered a bulk sample of the part of the plant that was used medicinally. These bulk samples where as large as possible without depleting the plant available to my collaborators, as much as I could possibly carry out on the plane from Paititi, or 1 kg, whichever was the least.

To preserve the plants in the humid climate of Paititi, I placed all the plant samples flattened between sheets of newspaper and liberally dowsed the stack of plants in rubbing alcohol. This stack was tightly sealed in several large plastic bags to keep the alcohol from evaporating. On leaving the field site, the plants were then dried over a kerosene

camp stove or in a standard electrical plant dryer at the USM herbarium in 2003 and at the Universidad National de Ucayali in Pucallpa in 2004. These plants were well preserved by the combination of alcohol and drying, although they did lose their color. In the dry climate of Mali, plants were placed in a plant press with newspaper and cardboard (both quite hard to find outside of the capital of Mali) and hung in an open window exposed to the sun. With the newspaper changed every day, plants were usually completely dry within three or four days.

I compensated each informant with money, food, or goods commensurate with what they would normally receive for this amount of time and what other anthropologists have given them. In Paititi where money is useless as there are no stores, I gave each interviewee a set of clothing for their family. In Mali, I gave each interviewee 15,000 CFA or about $30 US, for what was on average a four-hour interview. Upon completion of my work in each country, I compiled all the photos, vernacular names, and treatment regimens into a laminated book to give back to the people of Paititi and Kita so they have documentation of their knowledge to pass down to future generations if they like. For non-literate people in these communities, I constructed talking medicinal plant books, using cheap audio-recording chips powered by a solar panel on the back cover, that would say the name and uses of each plant as you turned the pages. These are explained further below.

I followed the standard ethics guidelines of the NYBG, CUNY, and the Society of Economic Botany regarding informed consent with my collaborators, confidentiality of their knowledge, and royalties on products derived from their plant knowledge. I also worked with host-country botanists and government officials to obtain proper permits to

export my plant samples from Peru and Mali and bring them back to the NYBG, once again utilizing the experience of the NYBG curators and CUNY professors who have collected in Peru and Africa as an aid in acquiring permits and for plant identification. In Peru it was necessary to obtain a collection permit from the government department of natural resources INRENA (Instituto Nacional de Recursos Naturales) prior to collecting any plants, and this permit required that I get written permission from the head of the community I was working with. Luckily, as the chief of Paititi, Raúl Casanto Shingari, was living in the city of Pucallpa at the time, this only required a few day trip to Pucallpa to meet with Raúl, discuss my research with him, and bring his signed permission letter back to Lima to deposit it with my collection permit application. This application however took two months to be processed, during which time I could not do any research with the Asháninka as the $1200 cost of Cessna plane flight with the Swiss American Mission into Paititi was prohibitive. After I had received the collection permit from INRENA and made collections in Paititi, and dried them, I deposited a duplicate of each collected plant with USM in Lima and sent a copy to Raúl in Pucallpa for the Asháninka to have a mini-herbarium. Once my duplicates had been deposited, I could apply for an export permit from INRENA with a list of the species collected and identified. This process took only three days and I was able to bring the plants by plane back to the US through the USDA inspection station in Miami airport.

In Mali, the permit situation was much simpler as the DMT I was working with was also a branch of the government and could issue me an export permit. A collection permit was not necessary there. As Mali has no official herbarium listed in the Index Herbariorum (Holmgren and Holmgren 1998), I deposited a duplicate of all the collected

plants at the informal herbarium at the DMT and put them in touch with the Holmgrens at NYBG to find out how to set up a registered herbarium. A set of the dried plants was also left with Maramakan Kamissoko of the Association de Thérapeutes Traditionnels in Kita as a mini-herbarium.

4.8. Collections

With the help of the collaborators, species described in the interviews were collected in quadruplicate when accessible to make into a small-scale reference herbarium for the communities in the study; for deposit in the study countries' main herbaria, Universidad Nacional Mayor de San Marcos (USM) in Peru and the *Département de Médecine Traditionnelle* in Mali; for sending to a family expert at other herbaria; and for deposit in my institutional herbarium (NY). Species were identified with the help of Gentry (1996), Arbonnier (2002), the aforementioned herbaria's collection, their staff, and several taxonomic experts.

Plants were preserved in rubbing alcohol in plastic bags in the Amazon to prevent the growth of mold until they could be taken out to a proper dryer in Pucallpa or Lima. In Mali, plants were easily dried on site due to the hot, dry climate, and merely needed to be hung in a window in a press, with daily changes of newspapers wick away the moisture.

4.9. Database

All collection data was entered in a Filemaker Pro 8 relational database on a Macbook computer with separate tables for collections, species, common name/species correspondences, literature references for each species, healers, disease interviews, plant interviews and assays done on each species, which connected to previous tables for healer

interviews. This relational database where multiple tables have relations to each other (a cell or field that is the same between multiple tables, e.g. the name of the healer in the table of all healers and their name in the disease interview table are linked), allows complex queries to be performed, such as, show all the species used in Peru to treat malaria but not diabetes that were described by a healer with more than ten years of experience treating people. The proper design of a database is essential to being able to extract valuable information from the data, and I had had experience with database design, good and bad, while working on the Chinese and Latino Urban Ethnobotany project at the Rosenthal Center for Complementary and Alternative Medicine at Columbia Presbyterian Hospital in 2000. Working with their multi-cultural data trained me well the pitfalls of database design, which is especially problematic with plant collections where there is the many-to-one (several names for one species) and one-to-many (one name could be several species) issue of plants' common names.

Without a clear database layout with one-to-multiple relations between each table, making these queries would be very difficult, so much time was spent clearly designing the correct tables and relationships to use. The most difficult relationship to handle properly in the database is that of common names and species as this is a many-to-many relationship, i.e. one common name can correspond to several species and one species can correspond to several common names, rather than the simpler one-to-one or one-to-many relations. Because of this, a separate common name/species table is created that has entry for every common name recorded, the species to which this common name corresponds, the language, and the healer who gave this name. This last bit of information is very important in being able to differentiate multiple species with the same

common name. If, for example, two different healers called the two different species *Uncaria tomentosa* (Willd. ex Roem. & Schult.) DC. and *Martynia annua* L. by the name *uña de gato,* then when the common name *uña de gato* appears in a remedy for diabetes for the healer who uses this name for *U. tomentosa*, if the common name/species table did not indicate the healer for each common name, the database would erroneously indicate that *M. annua* was used to treat diabetes as well through the connections of all the tables. This situation is avoided by indicating who uses each common name in the common name/species table. The database relationship layout with a common name/species table to resolve these name conflicts is shown in Figure 4.1.

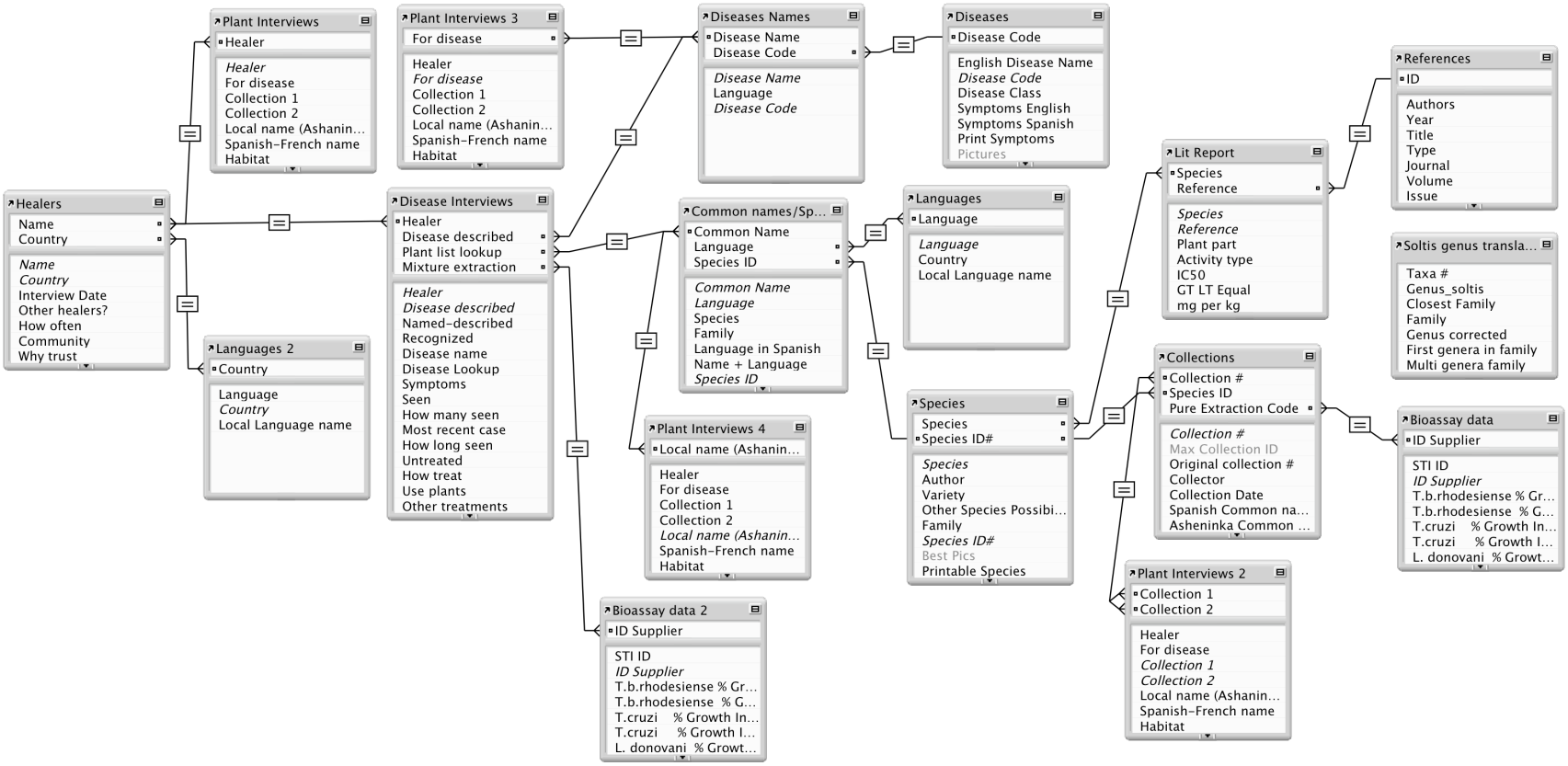

Figure 4.1. The relationship graph for the Filemaker relational database in which all the data from collections, identification, lab work, bioassays, literature review, and potential calculations are stored. Certain tables that need to be referenced multiple times, such as the bioassay data which applies to mixtures and single species collections, appear in the graph multiple times.

4.10. Species Accumulation Curves

The number of unique species that an ethnobotanist collects does not increase at a constant rate with the number of interviews conducted. Rather, the initial few interviews or collections will inevitably reveal the largest relative proportion of unique species, and, as more collections and interviews are performed in the same area, species will begin to be seen repeatedly, with fewer unique species found with subsequent collections and interviews. If the cumulative number of unique species is graphed versus the number of collection units, we get what is called a species accumulation or species-area curve and this curve can help us determine if the number of species is leveling off (reaching an asymptote) and therefore extensive additional collection effort will not produce any new species, or instead if the curve is continuing to increase at a near-constant rate and further collection effort will likely produce many new species. These curves can therefore help us budget limited time and resources to give maximal return. To make species accumulation curves smoother and easier to interpret a method called approximate randomization statistics is often used that reorders the collection order at random and recalculates the curve many thousands of times. The calculations needed to create these curves are quite difficult to perform in the field however, requiring specialized software such as EstimateS to compute the smoothed curves, leading to very few researchers actually employing these very useful field tools. By writing a "macro" or script program to calculate these smoothed species accumulation curves using approximate randomization statistics for the common spreadsheet program Microsoft Excel™ which is found on nearly every laptop computer brought into the field, we have made this extremely useful tool more accessible and we hope it will allow improved efficiency in

ethnobotanical, ecological, and floristic collections. This tool has been applied to medical ethnobotany data from the Asháninka indigenous community of Paititi in the Southwest Amazon area of Peru and the Malinké community of Kita in the sub-Saharan Sudanese savanna area of Western Mali and indicates that with the Malinké, a saturation of medicinal plant species has been reached, at least with the specific group interviewed. On the other hand, with the Asháninka, medicinal plant species saturation has not been reached despite the fact that the head of every household in the community has been interviewed.

4.10.1. Background

The calculation and application of species accumulation curves were introduced into ecology and floristic research 65 years ago (Fisher, Corbet et al. 1943), but their use during fieldwork when they can be most helpful in improving efficiency is still not very widespread, especially in ethnobotany research. Called by the various names of species accumulation curves, species-area curves, collector's curves, species-sample curves (Andrew and Hughes 2005), sampling to redundancy (Lyman and Ames 2004), rarefaction curves (when approximate randomization statistics are applied), species-informant curves (Castaneda and Stepp 2007), species-use curves (Balick and O'Brien 2004), and in this paper species-collaborator curves, this technique has been discussed a fair amount but rarely put into useful practice, especially during fieldwork. For example, only 6 of 361 (1.66%) articles in the journal Economic Botany for the years 2000-2007, 3 of 41 (7.32%) articles in the Journal of Ethnobiology (2005-2007), 1 of 102 (0.98%) articles in the Journal of Ethnobiology and Ethnomedicine (2005-2007), and 4 of 118 (3.39f%) articles in the journal Ethnobotany Research and Applications (2003-2007) for

which digital searchable versions of articles are available (14 of 622 or 2.25% in total) mention the use of species accumulation curves (Williams, Balkwill et al. 2000; Balick and O'Brien 2004; Lyman and Ames 2004; Shanley and Rosa 2004; Lepofsky and Lertzman 2005; Mayfield 2005; Amiguet, Arnason et al. 2006; Lozada, Ladio et al. 2006; Andel, Behari-Ramdas et al. 2007; Baco, Biaou et al. 2007; Bletter, Reynerston et al. 2007; Bussmann, Sharon et al. 2007; Castaneda and Stepp 2007; Silvaa, Tamashirob et al. 2007), and of those only six describe their use in the field rather than their calculation after the fact on returning from the field (Williams, Balkwill et al. 2000; Balick and O'Brien 2004; Lozada, Ladio et al. 2006; Andel, Behari-Ramdas et al. 2007; Bussmann, Sharon et al. 2007; Castaneda and Stepp 2007). When calculated after fieldwork is done, species accumulation curves can help determine if species saturation has not been reached in a particular site and whether researchers should return to the same field site and use the same collection techniques during the next field season rather than finding new sites with a new set of species or try new techniques that may reveal new species in different niches in the same area.

One difficulty of employing species accumulation curves in the midst of fieldwork is that their simplest calculation, graphing the cumulative number of unique taxa collected per sample in the order they were collected, produces anomalous and confusing results for two reasons. First, graphing the collections in their temporal order produces a jagged graph that does not give a good indication of where the asymptote of the curve may occur, so that if the last sample has a small number of unique taxa, it may seem that the curve still has leveled off in slope and no further collections are necessary, whereas putting a different sample last may indicate the opposite (Colwell, Mao et al. 2004;

Lepofsky and Lertzman 2005). This ordered calculation also produces no confidence intervals that might allow the comparison of species richness in similar collections from different areas (Lepofsky and Lertzman 2005). Second, basing the calculation on samples such as interviews, plots, or transects rather than the number of identified specimens (NISP) produces a plot of species density rather then species richness and can lead to errors when trying to compare species richness between collections with different NISP per sample.

To overcome these problems, the species accumulation curve should be calculated using approximate randomization statistics and based on NISP rather than samples. The approximate randomization statistics method essentially shuffles the order of sample or species collections many thousands of times, recalculating the curve for each shuffle, and then calculates an average of the curve from all of these shuffles along with confidence intervals. Basing the calculation on NISP rather than samples eliminates many of errors that arise from varying sample sizes, gives a better measure of species diversity, and allows comparison of collections from different contexts and sample sizes. The difficulty with performing these calculations is that they require complicated specialized software such as EstimateS (Colwell 1994 –2004) or Ecosim (Gotelli and Entsminger 2004) that researchers are unlikely to have on their computer in the field or to have learned the use of before coming to the field site.

4.10.2. Methods

To rectify this lack of simple to use and common programs to calculate species accumulation curves, I have written a short, easy-to-use program called a 'macro' for the nearly ubiquitous spreadsheet program Microsoft Excel that calculates and plots these

curves with confidence intervals using approximate randomization statistics based on NISP. This macro will run on any computer with Mac OS 9, Mac OS X, or Microsoft Windows, which covers almost all consumer computers except the rare Linux-based ones.

The macro is quite simple to use: the user merely enters a list of the species collected, with one collection unit (sample, plot, transect, interview) per column, enters the number of shuffles or iterations they would like at the top of the column after the final data column, and then clicks on the "Run" button which executes the macro. First the program calculates the total number of specimens collected (total NISP) and then for each iteration, it reorders the data randomly, calculates by searching for repeated names how many cumulative unique taxa have been collected for collections from one specimen to the total NISP, and records these cumulative unique taxa counts in the spreadsheet. Once these numbers have been recorded for every NISP and every iteration, the average and standard error (SE) are calculated for each NISP from one to total NISP, and these statistics are used to create a graph of cumulative unique taxa count vs. NISP with 95% confidence interval curves calculated by the average ± 1.96 * SE.

An exponential curve is fit to the data only if the iteration count given at the beginning is a negative number, as using regression on species accumulation curves can be quite error prone and should be used with great caution. I strongly discourage people from using curve fitting to predict asymptotes and estimated total potential collections, especially for publication, and this is merely provided as an extra planning tool that people may feel is missing otherwise. As several previous authors have stated (Balick and O'Brien 2004; Lepofsky and Lertzman 2005), species accumulation curves can appear to

have leveled off and reached an asymptote, but then after many more collections with no new taxa discovered, a single collection will suddenly uncover more new taxa, perhaps because a new niche, community, group, or habitat has been tapped, or because conditions have changed over the time between samples. Therefore, a curve that is leveling off should merely be used as guidance but not a guarantee that a exploring a new area may give a better return on collection effort.

It is advisable before running the macro with the full number of iterations to run first with only one iteration and check the sorted list of unique taxa names that the macro generates in the column to the right of the entered data and the iteration count to check for typos that may have led to two collections of the same taxa being counted as unique. For instance, with a scan through the alphabetized list of unique taxa, one might notice that there are both a " *Musa x paradisica*" and a " *Musa paradisica*" entry right next to each other which were meant to be the same thing, so the " *Musa paradisica*" entry in the sample collections list can be corrected to " *Musa x paradisica*" so the correct total unique taxa count is calculated. If Excel is used for data entry, it will suggest completions for cells after the first few letters are entered from similar entries that have already been made in the same column, so this can help somewhat with ensuring that taxa are always given the same exact name if all data is entered in one column. The macro has no way of knowing that two taxa that differ by only one or two letters are intended to be the same taxa as this would immensely complicate what is intended to be a fairly simple-to-use program. Therefore it is up to the user to ensure consistent spelling.

The macro runs in a reasonable amount of time, taking 10 minutes on a 2.4 MHz Pentium 4 PC with Windows XP, 70 minutes on a 1 GHz Macintosh G4 PowerMac, 91

minutes on a dual 2.0 GHz Intel MacBook on a dataset with 32 NISP, 15 total species, and 1000 iterations, all within the range of processor speeds that are found on current laptops which researchers may take into the field for recording data. The reason the macro took longer on the faster 2.0 GHz processor than the 1 GHz processor is most likely that the Microsoft Office 2004 version of Excel that is necessary to run the macro is not written natively for the Intel processor, and therefore the program must be interpreted or translated on the fly, slowing down the running of the macro. Unfortunately, although the program algorithm is easily parallelized for multiple processors (splitting independent segments of the program code to run concurrently on separate processors) as the calculation of one set of iterations do not depend on the results of another iteration, Excel only provides a mechanism for running multi-processor macros on Windows Excel 2007, which would greatly increase speed. Regardless, the macro is fast enough that, as long as researchers can perform a quick rough identification of the collected taxa, they can run the macro once a day on the day's collection data and use the result to plan their following day's collections.

This macro program, instructions for its use, and example data are available for download from http://www.lehman.cuny.edu/PlantPhD/speciesarea and should work with any version of Excel that can run Microsoft Visual Basic for Applications (VBA) macros, which includes as of this writing the Excel included in most versions of Office for Windows and Office 2001, Office X, and Office 2004 for Macintosh. However, it will not work with the Excel in Office 2008 for Macintosh, as the VBA on which the macro depends is no longer included in the Macintosh version of Office. This species accumulation macro is provided as open source software and can be modified and

improved for any non-commercial use, as long as the author is credited and new versions are submitted to him.

4.10.3. Analysis

When species accumulation or, in this case, species-collaborator curves were calculated using the described Excel macro program with collaborator interview order randomized after each interview in Paititi and Kita with newly collected plants, it aided immensely in the planning of fieldwork as it indicated that continuing interviews in the same community of Malinké healers in Kita would most likely not lead to the discovery of many more medicinal plants (see Figure 4.2), but that there were still probably many more Asháninka medicinal plants to be uncovered if healers in other nearby communities could be interviewed, even though every possible healer had been interviewed in Paititi. Figure 4.3 shows that even after 8 interviews, 99 NISP and 72 unique species, the species accumulation curve was still increasing rather steeply, so since I had interviewed every head of household in the town over two years, it would be in my best interest to find other people using medicinal plants in neighboring towns to find a more complete percentage of the Asháninka medicinal plant flora. Unfortunately, given the difficulty of travel in the Yuruá area where Paititi is located (only reachable by plane or several-day boat rides) and my limited resources, I was not able to visit nearby towns and see if the species accumulation curve would level off.

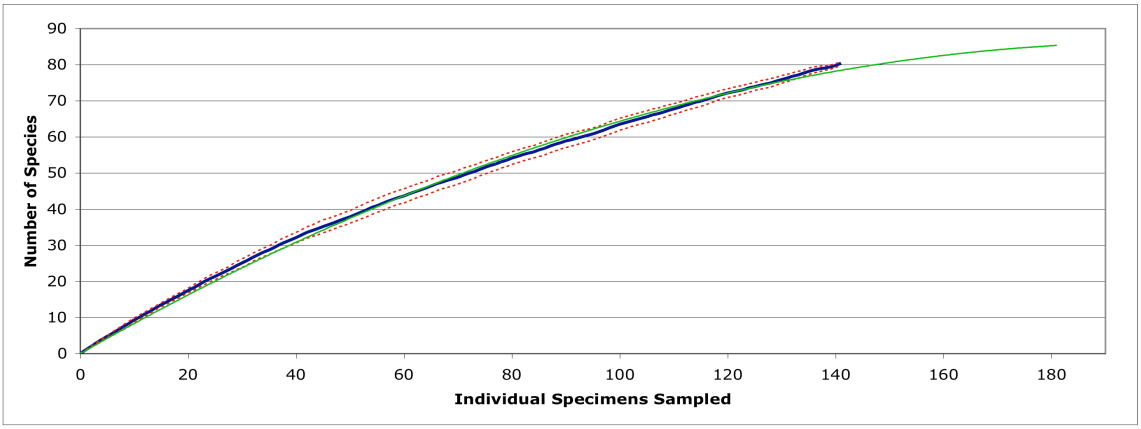

Figure 4.2. Kita species accumulation curve, computed via approximate randomization statistics on unique species described (dark solid line), 2.5 and 97.5 percentile bands (dashed lines), and a second-order polynomial regression fit to the curve (light solid line, $R^2 = 0.9983$).

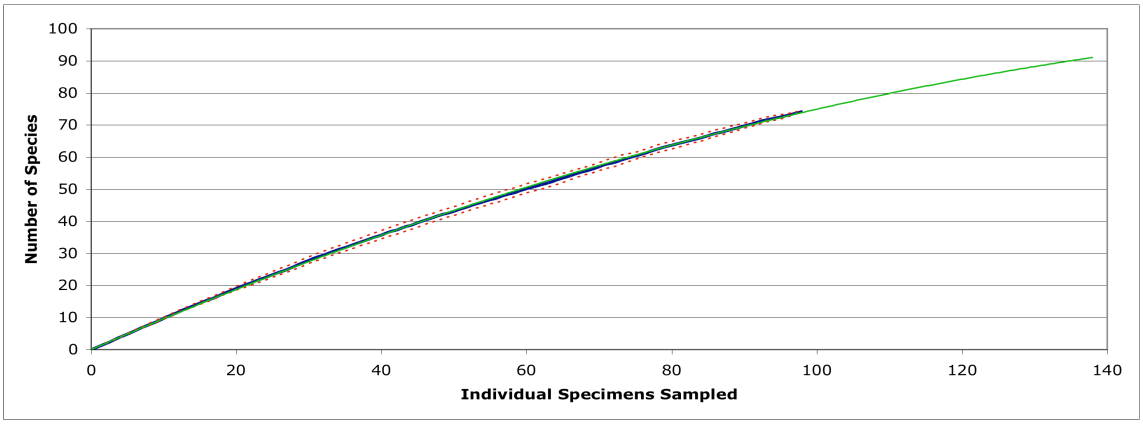

Figure 4.3. Paititi species accumulation curve, computed via approximate randomization statistics on unique species described (dark solid line), 2.5 and 97.5 percentile bands (dashed lines), and a second-order polynomial regression fit to the curve (light solid line, $R^2 = 0.9998$).

The situation in Kita was quite the opposite, with the slope of the species accumulation curve starting to level off after only fifteen interviews, 141 NISP, and 81

total number of unique species (see Figure 4.2). As is clear from Figure 1, the number of new unique species collected was quickly leveling off after 15 interviews and 90 collections in Kita, Mali done over 6 weeks of fieldwork. This leveling off of the smooth species accumulation curve calculated with the macro after each interview indicated that if I wanted to maximize the number of new species mentioned and collected, I would probably need to start interviewing a different group in Kita such as the Peul or Christian community that might have knowledge of a different set of plants. Alternatively, I could choose to move my interview and collection site to another habitat with other species, or decide that species saturation has been nearly reached and to finish fieldwork and return home with the data I had. I chose the latter action as my deadline in the field was nearing, but I feel I was only able to make this as a very informed choice because I had the species accumulation curve to make clear how research was progressing. Without this, I may have had to end fieldwork while worrying that I had not collected a significant proportion of the Malinké plants, or I may have continued interviews and collections, attempting to collect every last species and exhausting my fieldwork funds. In this case as well, the species accumulation data helped finish fieldwork in an efficient manner and left me with a clear conscience about my collection effort.

In fact, in a non-traditional application, species accumulation curves were used to determine if all the articles that might mention species accumulation curves with all its different names had been found in the literature. As only the years of the four journals that had digital searchable full text could be easily searched for the terms "species accumulation curves", "species-area curve", "rarefaction", "collector's curve", and "species-sample curve", I was not sure if previous, non-digital issues of these same

journals might have other articles mentioning one of these terms deep in their text. Since the meticulous reading of volumes of articles one by one was intractable, constructing an "article-issue curve" that graphs the number of accumulated articles that mention species accumulation curves in some way vs. the number of searched articles could resolve some of this doubt. As this curve shows (Figure 4.4) the number of articles mentioning species accumulation curves appears to still be increasing steadily from 14 articles after four journals, 19 volumes, 46 issues, and 622 articles have been searched. This assumes that each journal has the characteristics of an individual habitat, which has been shown to be somewhat valid, at least in terms of references to other articles (Ioannidis 2006). However, the focus on specific topics such as species accumulation curves will vary from year to year as new techniques are introduced and others fall out of favor, so one cannot make assumptions about the contents of journal volumes of many years previous based on the contents of the most recent few years of articles. Once again, if collections were moved to another "habitat" of other journals or much older volumes, other articles using species accumulation curves would most likely be found, but we can use this article-issue curve as guidance that the recent volumes of these specific journals have been well searched and the statistics are complete. It seems in this case that at least a few more non-digital volumes of these journals should be searched for references to species accumulation curves to see if the curve in Figure 4.4 levels off.

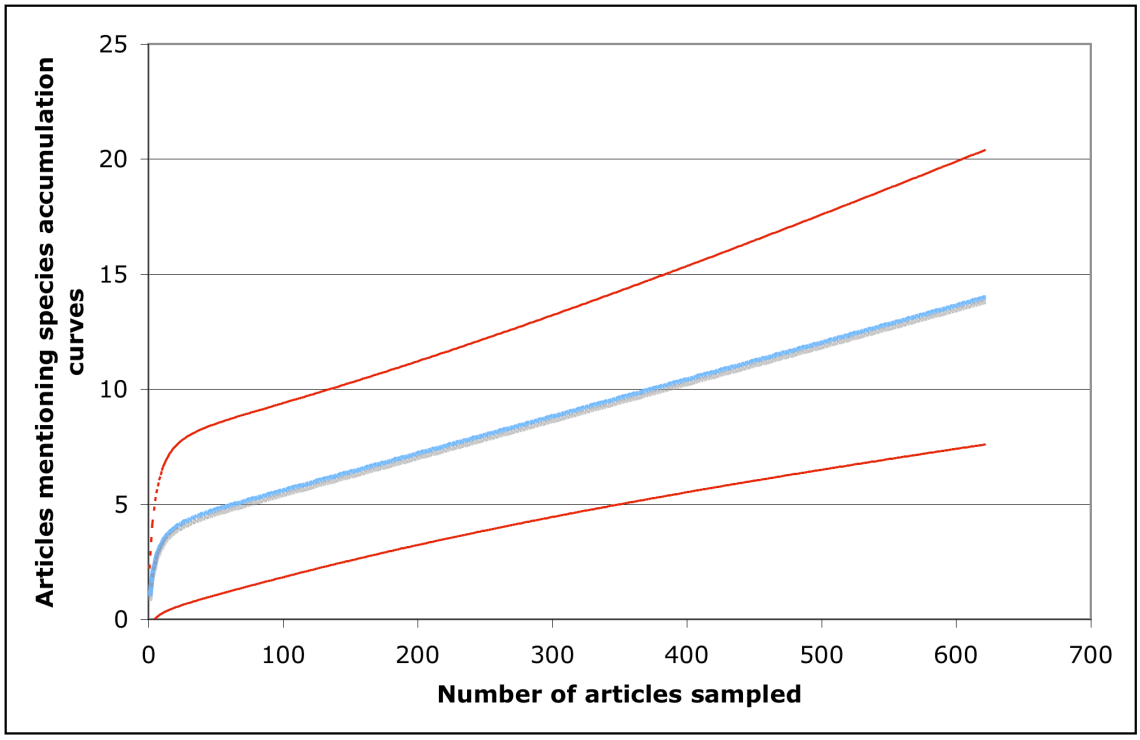

Figure 4.4. Article accumulation curve used for the ethnobotanical literature mentioning the use of species accumulation curves with 50 reshufflings of the sampling order to compute the average number of unique species described (thick central line) and 95% confidence intervals (thin side lines).

4.10.4. Future Work

We would like to extend the algorithms in this species accumulation curve macro to include additional statistical analyses that include more options for predictive curves, significance of current curve calculations, and greater ease-of-use for the researcher. Better statistics and simpler graphing and export of the results of the calculations should make this useful method more attractive to researchers and spread its adoption. As the code is released as open source software, it is hoped that other researchers with skills in

Excel macro programming will make these and other extensions to the code that will be released to the general community for improved application of species accumulation curves in field and other collection scenarios.

A tool for quickly and easily calculating species accumulation curves in the field for floristic, ecological, or ethnobotanical collections has been presented that is intended to encourage the widespread use of this helpful technique. Species accumulation curves are a useful tool to improve collection efficiency in any research that involves resource-limited collections when used appropriately. They are an even more valuable tool when they can be performed in the midst of collections, leading to better planning of fieldwork and collections, but this is only truly feasible if the calculation of the species accumulation curves is simple and can be done using ubiquitous software. The 'macro' program for Excel presented in this paper to calculate species accumulation curves attempts to fulfill both of these requirements of simplicity and ubiquity. The two cases of field collections of medicinal plants with the Asháninka in Peru and the Malinké in Mali where this macro was used to calculate species accumulation curves shows both ends of the spectrum: in Mali the species accumulation curve was leveling off and collections were ended, while in Peru the species accumulation curve was still increasing rapidly so more interview subjects were sought out. In both cases the quick calculation of species accumulation curves in the field allowed for better uses of time, money, and other limited field resources. Species accumulation curves or rarefaction curves can be applied to other types of limited collecting situations such as literature searches as well, with informative results such as in this paper where it was quite clear that no more articles mentioning species accumulation curves would be found even more recent volumes of ethnobotanical

journals were meticulously searched. The described simple macro should allow widespread adoption of the extremely valuable technique of species accumulation curves in ethnobotany and other limited collection-based studies, and we hope speed the necessary surveying of rapidly disappearing species, ecologies, and traditional ethnobotanical knowledge. Researchers must, however, understand the potential shortcomings of using species accumulation curves predictively. By distributing the code for this macro as open-source software, we also hope that the procedure will be improved upon and extended by other researchers.

4.11. Conclusion

The original data I have collected through these interviews comprises an accurate database which I can then analyze using my quantitative system and compare with data gathered on the same diseases from another area of the world. The use of quickly calculated species accumulation curves allowed me to use my time and resources in the field as efficiently as possible, and is a tool that I hope more ethnobotanists will adopt and improve upon. These tools have improved my short time in the field by allowing me to collect more efficiently and give back to the community in an effective way. Back in the U.S., I set about defining how these plants, cultures, and diseases that came up in field interviews are related. The next two chapters delineate how this taxonomic and phytochemical research of relations was carried out.

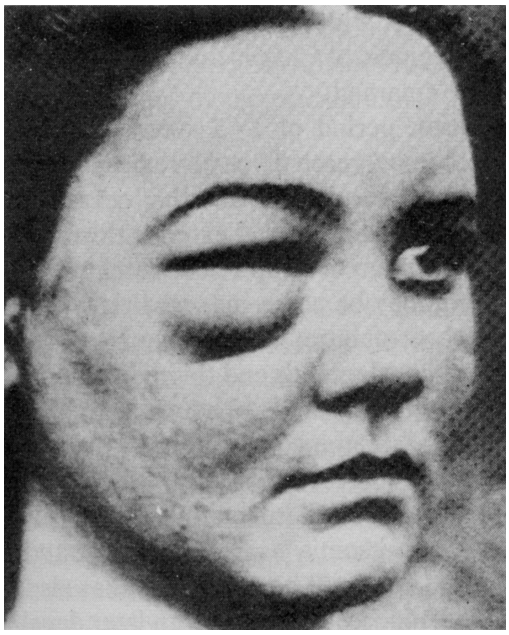

Figure 4.5. Outwardly apparent symptom of Chagas disease that was shown on a card to interviewed healers- edema of one eye.

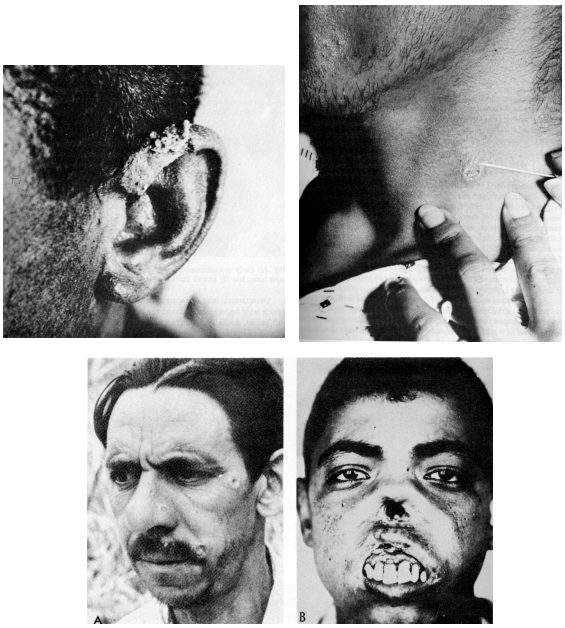

Figure 4.6. Outwardly apparent symptoms of cutaneous and mucocutaneous leishmaniasis that were showed on a card to interviewed healers. Clockwise from top left: lesion on ear, lesion on neck, erosion of the nose and lip developing from original ulcers.

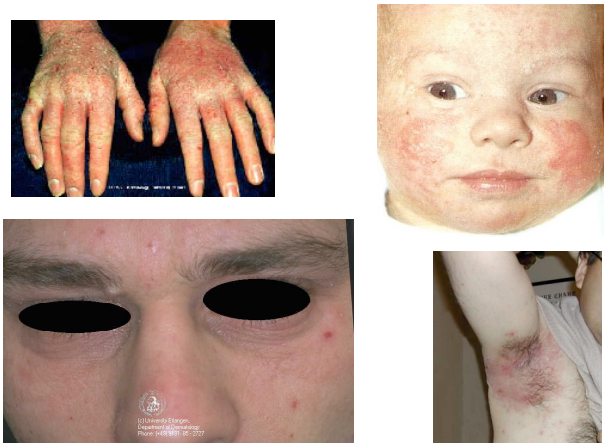

Figure 4.6. Outwardly apparent symptoms of eczema that were shown on a card to interviewed healers to describe the disease. Clockwise from top left: dry, red, scaly hands; dry, itchy, or cracked skin, especially on the cheeks, legs, arms, and behind the ears; an extra fold of skin developing under the eye, called an "atopic pleat" and a darkening of the eyelids; lichenification where the skin becomes thick and leather-like from excessive scratching.

Chapter 5. Laboratory Methods

The relational efficacy system is intended to reduce the amount of laboratory time needed to find new effective plant medicines, but in order to verify that the potential efficacy predicted by the system correlates with the actual efficacy of the plants, this efficacy must be determined independently via bioassays for the diseases the plants are used to treat. Therefore, crude extractions were done on a subset of the anti-parasitic plants for which I had bulk collections of the proper plant part, and these were put through a bioassay at the Swiss Tropical Institute (STI) for anti-plasmodial, anti-trypanosomal, and anti-leishmania activity. By determining if the ranking of plant species efficacy by the relational efficacy system correlates with the ranking from the EC_{50} values from the bioassays, we can say whether the relational efficacy system has predictive value, or if the mathematical model needs to be re-worked to better match the bioassay ranking.

5.1. Extractions

Essentially, after being ground to a powder in several stages, the dried plant material was then brought into the Lehman College Phytochemistry Laboratory for extraction. Each sample was labeled with a code to preserve confidentiality, weighed, prepared in accordance with the collaborators' directions, extracted in methanol, and dried down to a

powder; the final product was weighed and stored in a freezer until being sent off to the STI for bioassaying.

Laboratory notes were recorded in time-stamped fields in the Filemaker® sample database and backed up weekly to an external drive kept separate from the laptop used in the laboratory. Both the original and backup databases were password-protected to maintain confidentiality, in keeping with the research agreement signed with all collaborators. Laboratory notes were kept on the computer, as it would have been very difficult to keep track of the 176 plant samples, their code names, the mixtures, the mixtures' code names, and the extractions in a physical laboratory book, when constant cross-referencing was necessary. Every effort was made to keep printed notes in a physical laboratory book up to date with the online notes so that there was a clear physical record of the origins of all my samples in the laboratory for others to reference.

Extractions were done on raw dried individual plant parts and on mixtures and preparations that matched as closely as possible how the healers said they prepared the plant remedies. Thus, if a healer said (s)he mixed the bark of one plant with the burnt spines of another and then made an infusion, I would follow this procedure of burning the spines and making an infusion if I had collected both these plant parts in bulk. Different preparations (e.g., boiling, burning, mixing with honey or lemon juice, tincturing in water or shea butter) produce different chemical changes, and different compounds can be present in different parts of the same plant, thereby distorting the results if these preparation steps are not followed carefully (Balunas, Jones et al. 2006). Collection sites are key as well, and as mentioned in Chapter 4, I always asked my collaborators if there were special conditions for collecting each plant, e.g., should I collect only from one side

of a plant, if it is growing next to or on another plant, or from a certain slope? When it was possible to meet these special conditions for collection, I did so, but fortunately these cases were rare.

The first step with all samples was to grind them into a fine powder. Bark and root samples were first ground coarsely in the NYBG phytochemistry laboratory's Wiley Mill Model 4 grinder to about 3 mm pellets. Then these and other dried leaf and fruit samples were ground to a fine powder in a Braun® coffee grinder. These powders were then bagged, weighed, and given a code name, and the code and weight were entered in the database. This weight was essential for calculating mixture amounts where several plants were mixed together in a preparation, usually in equal weights, in which case the smallest weight of all the plants in a mixture was used as a basis for measure all the other plants in the mixture. If a plant was used in several mixtures, its total weight had to be divided evenly among all the mixtures in which it was found, including the pure sample. For example, if I had 40 g total of a powdered sample of one plant that was used in 3 mixtures, 10 g was allotted to each mixture and 10 g to the pure sample. If 10 g was the lowest weight in a certain mixture, all the other plants were used in 10 g quantities in that mixture. If one of the preparations was essentially equal to the pure sample (i.e., used alone as a powder and not cooked) this was not included in the calculation of dividing up the sample as the data from the pure sample could be used for the preparation efficacy.

After the material was ground and any mixtures prepared, the samples were sonicated in three times their volume of methanol (i.e. a 50 mL sample is covered with methanol to make 150 mL total) for 10 minutes in a VWR 50HT Sonicator and then let sit in a fume hood covered for at least 12 hours. All methanol and other solvents used in

these extractions were GR grade. The sample was then taken out of the fume hood and filtered through a Buchner funnel with #4 Whatman filter paper under vacuum into a filter flask. Once dried, the marc of the sample was placed in a labeled sealable plastic bag and stored in the freezer in case it was needed for later reference. The filtered liquid was placed in a round-bottomed flask with at least twice the volume of the liquid and six drops of butanol were added to the liquid to avoid bumping in the rotary evaporator. This round-bottom flask was placed on one of the laboratory's Büchi Rotavapor R-114 rotary evaporators and dried down under vacuum at 40° C until there was no visible liquid in the flask and no liquid falling into the waste container.

The dried sample was removed from the rotary evaporator and transferred to a 20 mL flask whose tare had been measured to four decimal places (100 μg) on a Mettler Toledo AG245 digital scale. A combination of methanol, acetone, hexane, and water, with further sonication and sometimes with heat up to 40° C were used to dissolve the dried sample in the round-bottomed flask into the solvent so it could be transferred to the 20 mL vial. Once no visible residue remained in the round-bottomed flask, the 20 mL vial was placed above dry heat from a hair dryer in a fume hood, and a positive flow of nitrogen was funneled into the open vial through a pipette head to avoid oxidation, until the liquid in the vial appeared to be no longer be reducing. In many samples, especially those with some sugars in them from fruit, bark, roots, or added honey in mixtures, a film would form in the vial that would prevent further evaporation of the liquid, so this film was broken with a pipette tip and the vial rotated to increase the evaporation surface area.

Samples that were dried under nitrogen until no more reduction in volume was visible were then placed in a -80° C freezer or in a -80° C ethanol bath of the lyophilizer for at least 3 hours until the sample was solid, to allow water sublimation.

Once solid, the samples were placed on the Virtis Freezemobile EL12 lyophilizer at 80° C and 50 milliTorr for 3 days until completely dry. After these dry samples were crushed into a powder and weighed, 10 mg was separated out to be sent to the STI for testing in their bioassays. All samples were then stored in a -30° freezer.

5.2. Assays

In vitro bioassays for all the parasitic diseases that were studied in this project – malaria (*Plasmodium falciparum*), Chagas disease (*Trypanosoma cruzi*), African sleeping sickness (*Trypanosoma brucei rhodesiense*), leishmaniasis (*Leishmania donovani*) – were conducted at STI, in Basel, Switzerland by Dr. Reto Brun and Marcel Kaiser. These procedures are as follows.

5.2.1. Plasmodium falciparum

Antiplasmodial activity was determined using the K1 strain of *P. falciparum* (resistant to chloroquine and pyrimethamine). A modification of the [3H]-hypoxanthine incorporation assay was used (Matile and Pink 1990). Briefly, infected human red blood cells in RPMI 1640 medium with 5% Albumax were exposed to serial drug dilutions in microtiter plates. After 48 hours of incubation at 37°C in a reduced oxygen atmosphere, 0.5 μCi 3H-hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto glass-fiber filters and washed with distilled water. The radioactivity was counted using a Betaplate™ liquid scintillation counter (Wallac,

Zurich, Switzerland). The results were recorded as counts per minute (CPM) per well at each drug concentration and expressed as percentage of the untreated controls. IC_{50} values were calculated from the sigmoidal inhibition curves. Assays were run in duplicate and repeated once.

5.2.2. *Trypanosoma brucei rhodesiense* and cytotoxicity

Minimum Essential Medium (50 μl) supplemented according to Baltz et al. (1990) with 2-mercaptoethanol and 15% heat-inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions were prepared covering a range from 90 to 0.123 μg/ml. Then 104 bloodstream forms of *Trypanosoma b. rhodesiense* STIB 900 in 50 μl were added to each well and the plate incubated at 37°C under a 5% CO_2 atmosphere for 72 hours. 10 μl of Alamar Blue (12.5 mg resazurin dissolved in 100 mL distilled water) were then added to each well and incubation continued for a further 2-4 hours. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analyzed using the software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA). Decrease of fluorescence (measuring inhibition) was expressed as percentage of the fluorescence of control cultures and plotted against the drug concentrations. The IC_{50} values were calculated from the sigmoidal inhibition curves. Cytotoxicity was assessed using the same assay and rat skeletal myoblasts (L-6 cells). The medium used for the L-6 cells was RPMI 1640 medium with 10% FBS and 2 mM L-glutamine.

5.2.3. Trypanosoma cruzi

Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells/well in 100 μl RPMI 1640 medium with 10% FBS and 2 mM L-glutamine as hosts for *T. cruzi* trypomastigotes. After 24 hours the medium was removed and replaced by 100 μl per well containing 5000 trypomastigote forms of *T. cruzi* Tulahuen strain C2C4 containing the ß-galactosidase (Lac Z) gene. 48 hours later, the medium was removed from the wells and replaced by 100 µL fresh medium with or without a serial drug dilution. Seven 3-fold dilutions were used covering a range from 90 µg/ml to 0.123 µg/ml. Each drug was tested in duplicate. After 96 hours of incubation, the plates were inspected under an inverted microscope to verify the growth of the controls and that there were no other organisms growing in them. Then the substrate CPRG/ Nonidet (50 µL) was added to all wells. A color reaction developed within 2-6 hours and could be read photometrically at 540 nm. Data were transferred into the graphics program Softmax Pro® (Molecular Devices), which calculated IC_{50} values.

5.2.4. *Leishmania donovani* axenic amastigote assay

Amastigotes of *Leishmania donovani* strain MHOM/ET/67/L82 were grown in axenic culture at 37°C in SM medium (Cunningham 1977) at pH 5.4 supplemented with 10% heat-inactivated fetal bovine serum under an atmosphere of 5% CO_2 in air. 100 μl of culture medium with 10^5 amastigotes from axenic culture with or without a serial drug dilution were seeded in 96-well microtiter plates. Seven 3-fold dilutions were used covering a range from 30 μg/ml to 0.041 μg/ml. Each drug was tested in duplicate and each assay was repeated at least once. After 72 hours of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile

conditions. 10µl of Alamar Blue (12.5 mg resazurin dissolved in 100 mL distilled water) were then added to each well and the plates incubated for another 2 hours. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analyzed using the software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA). Decrease of fluorescence (measuring inhibition) was expressed as percentage of the fluorescence of control cultures and plotted against the drug concentrations. The IC_{50} values were calculated from the sigmoidal inhibition curves.

5.2.5. *Leishmania donovani* macrophage assay

Mouse peritoneal macrophages (4 x 104 in 100 μl RPMI 1640 medium with 10% heat-inactivated FBS) were seeded into wells of Laboratory-tek 16-chamber slides. After 24 hrs, 1.2 x 10^5 *Leishmania donovani* amastigotes in 100 μl were added. The amastigotes were taken from an axenic amastigote culture grown at pH 5.4. Four hours later, the medium containing free amastigote forms was removed and replaced by fresh medium. The next day, the medium was replaced by medium containing different compound dilutions. Parasite growth in the presence of the drug was compared to control wells. After 96 hours of incubation, the medium was removed and the slides fixed with methanol for 10 min followed by a staining with a 10% Giemsa solution. The infection rates were determined by counting the infected and non-infected macrophages for the control cultures and for the ones exposed to the serial drug dilutions. The results were expressed as percent reduction in parasite burden compared to control wells, and the IC_{50} calculated by linear regression analysis.

5.3. Results

The results for these assays as reported by Marcel Kaiser are shown in Table 5.1, including the average of all the Peruvian and Malian samples, and the statistical significance of the difference between the two groups computed with ANOVA and t-test.

Table 5.1. Raw results data from STI for parasitic bioassays on Malian and Peruvian plant samples. Concentration 1 (Conc 1) is 9.6 μg/mL and concentration 2 (Conc 2) is 1.6 μg/mL, except where noted in the positive controls. * represents candidates for further study due to high growth inhibition. Significant differences in the t-test and ANOVA between the Mali and Peru averages are italicized.

		T. brucei rhodesiense		*T. cruzi*		*L. donovani*		*P. falciparum*	
Country	Sample	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)
Mali	brnk2	5.2	5.0	0.2	10.3	27.9	25.3	9.6	4.0
Mali	bsbl2	5.6	0.0	16.8	1.3	35.1	18.5	28.0	18.2
Mali	btb2	10.7	3.3	14.3	10.3	33.2	17.1	47.2	18.0
Mali	btl2	0.0	0.0	16.8	20.8	29.7	15.6	10.2	10.7

		T. brucei rhodesiense		*T. cruzi*		*L. donovani*		*P. falciparum*	
Country	Sample	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)
Mali	dla2	0.0	0.0	10.3	22.6	25.7	14.5	16.7	6.0
Mali	dn2	0.0	0.0	25.5	0.0	22.9	4.5	5.5	6.9
Mali	dskm2	45.5	12.7	0.0	16.1	24.8	22.8	19.7	0.8
Mali	ghr2	0.0	3.1	11.8	0.0	30.4	22.3	13.5	7.2
Mali	gnu2	0.0	2.2	0.0	0.0	31.7	22.0	21.4	3.8
Mali	kbt2	0.0	0.0	11.1	7.1	38.6	26.6	14.5	1.0
Mali	klkl2	0.0	1.6	26.3	31.7	32.3	28.1	16.4	2.4
Mali	klkr2	7.9	5.0	6.0	2.0	10.9	11.9	25.1	10.6
Mali	kndl2	47.9	10.9	0.0	0.0	32.1	2.7	27.6	15.9
Mali	knm2	16.2	9.4	0.0	0.0	27.9	9.2	29.1	11.1
Mali	knss1	13.0	11.3	6.7	0.0	29.3	7.0	17.2	9.2
Mali	knss2	14.7	7.2	16.5	0.0	31.5	3.8	35.3	2.6

		T. brucei rhodesiense		*T. cruzi*		*L. donovani*		*P. falciparum*	
Country	Sample	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)
Mali	ksf2	44.9	11.7	0.0	0.0	31.3	8.6	27.9	2.9
Mali	Imn2	11.1	3.0	28.4	0.9	38.5	13.6	19.8	6.4
Mali	Imr2	19.4	13.1	16.8	0.0	43.5	10.2	54.3	12.7
Mali	Lng2	20.1	9.6	11.4	0.0	5.3	0.0	28.8	13.7
Mali	nkl1	19.9	6.1	0.0	0.5	0.0	0.0	*80.7	5.2
Mali	nkl2	13.7	0.0	5.8	5.1	2.4	0.0	*93.0	21.1
Mali	nma2	0.0	0.0	0.0	0.0	14.3	0.4	0.0	0.0
Mali	nr2	0.0	0.0	0.0	0.0	16.6	0.0	12.1	0.1
Mali	ntmb2	0.0	0.0	0.0	18.5	17.0	6.2	11.8	0.0
Mali	par2	21.1	5.9	4.3	2.0	3.9	0.0	6.3	0.3
Mali	sg1	4.0	0.0	0.0	0.0	16.6	3.9	39.8	0.0
Mali	sg2	11.2	0.0	0.0	0.0	11.1	9.7	*88.3	7.2

		T. brucei rhodesiense		*T. cruzi*		*L. donovani*		*P. falciparum*	
Country	Sample	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)
Mali	slfn1	0.0	0.0	0.0	18.1	22.2	8.4	0.0	0.0
Mali	slfn2	0.0	0.0	27.3	0.0	22.7	5.9	11.2	0.0
Mali	slns2	0.0	0.0	0.0	21.1	26.3	13.5	7.1	0.0
Mali	smya1	0.0	0.0	40.3	28.0	24.7	10.7	0.0	0.0
Mali	smya2	0.0	0.0	35.7	10.0	19.9	15.4	2.3	0.0
Mali	sndj2	11.4	5.7	0.0	0.0	8.1	2.2	9.7	11.2
Mali	sndj4	0.0	0.0	0.0	15.0	25.7	14.9	0.0	1.7
Mali	sndj6	4.9	0.0	7.0	11.2	23.2	12.1	8.4	3.1
Mali	srt2	0.0	0.0	0.5	21.5	29.8	13.3	0.0	4.3
Mali	ssb2	0.0	0.0	14.6	12.7	27.6	10.9	25.3	7.8
Mali	ssL2	0.0	0.0	20.8	20.0	33.1	11.8	12.4	5.9
Mali	tkn2	0.0	0.0	36.8	31.1	31.8	10.9	7.5	5.2

		T. brucei rhodesiense		*T. cruzi*		*L. donovani*		*P. falciparum*	
Country	Sample	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)
Mali	tra2	41.4	0.0	12.7	8.9	21.4	4.9	35.1	7.4
Mali	trb2	14.8	4.6	0.0	4.7	18.9	3.6	32.2	1.7
Mali	zdr2	65.0	6.4	0.0	2.8	35.2	11.7	76.5	5.6
	Mali Average	10.9	3.2	9.9	8.2	24.1	10.6	23.9	5.9
Peru	#1	3.9	0.0	35.0	0.0	33.9	13.9	5.9	2.1
Peru	#6	0.0	0.0	26.3	10.0	33.2	3.6	6.2	2.3
Peru	2ab	*93.7	10.8	13.6	6.0	31.6	31.3	12.9	0.0
Peru	c1a, c1b	43.4	0.0	9.6	10.7	44.4	29.7	8.1	8.1
Peru	e1	22.4	16.4	0.0	0.0	32.8	13.5	*84.4	34.5
Peru	g1	2.8	0.7	15.4	0.0	33.0	23.9	11.9	0.0
Peru	o1	0.8	0.0	22.3	16.6	34.5	10.5	1.7	0.0

		T. brucei rhodesiense		*T. cruzi*		*L. donovani*		*P. falciparum*	
Country	Sample	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)
Peru	p1	0.0	0.0	33.0	21.1	17.4	4.9	6.8	0.0
Peru	p2	2.6	0.0	19.2	18.1	21.0	0.0	15.6	0.0
	Peru Average	18.8	3.1	19.4	9.2	31.3	14.6	17.1	5.2
t-test unequal variance Mali/Peru average difference p-value		0.4836		*0.0409*		*0.0324*		0.4759	
ANOVA average difference p-value		0.2680		*0.0306*		0.0530		0.4397	
Standard	Mearsoprol	98.2 at 0.015 μg/mL	48.3 at 0.003 μg/mL						

		T. brucei rhodesiense		*T. cruzi*		*L. donovani*		*P. falciparum*	
Country	Sample	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)	% Growth Inhibition (Conc 1)	% Growth Inhibition (Conc 2)
Standard	Benznidazole			89.6 at 1.2 μg/mL	57.6 at 0.2 μg/mL				
Standard	Miltefosin					92.5 at 1.32 μg/mL	53.5 at 0.22 μg/mL		
Standard	Artemisinin							100.0 at 0.018 μg/mL	64.4 at 0.003 μg/mL

Table 5.2. IC_{50} values and solubility for the best sample candidates (those that had greater than 80% parasite inhibition), with positive controls for comparison.

Sample	*T. brucei* rhod. IC_{50} (μg/mL)	*P. falciparum* K1 IC_{50} (μg/mL)	Cytotoxicity L6 IC_{50} (μg/mL)	Solubility
Melarsoprol	0.003			
Benznidazole				
Miltefosine				
Chloroquine		0.052		
Podophyllotoxin			0.008	
2ab	10.29	no data	>90	insoluble in DMSO
e1	no data	2.94	>90	
nkl1	no data	4.29	>90	
nkl2	no data	2.72	>90	
sg2	no data	3.41	>90	

Among the best candidates indicated by these data, preparation *2ab* was the only one from the Asháninka, perhaps reflecting the greater emphasis of their remedies on autoimmune diseases, especially diabetes, rather than the parasitic diseases more recently introduced there. More interestingly among the five best candidates in Table 5.2, *e1* is actually an animal rather than a plant product extract. It is difficult to speculate as to why one of the Asháninka's most effective antiparasitic remedies is animal-based, except that

it may relate to the fact that introduced diseases such as malaria reached the interior of the Amazon transferred by animal carriers (Diamond 2003; Frankel 2005). Perhaps the Asháninka are taking advantage of defenses that these animal species have evolved against the parasites as the animals have been exposed to the parasites for a longer time than the Asháninka,

From the averages of the percent inhibition of each parasite for the grouped Malian and Peruvian plants, and from the p-values of the ANOVA tests of the differences in these averages shown in Table 5.1, it can be seen that Peruvian plants have a higher average inhibition (i.e., they are more effective) of all the parasites except *Plasmodium*, but this difference is significant only for the Chagas and leishmaniasis assays. Perhaps this latter result is due to the fact that Chagas disease is endemic to South America and leishmaniasis, although found on both continents, had not been seen in Mali in any of my collaborators' memory, so the Malinké had no experience with treating either of these diseases. However, if this reasoning holds, it is strange that the Asháninka mixture *2ab* was more effective at inhibiting the African sleeping sickness parasite *T. brucei rhodesiense* than any of the Malinké preparations, when African sleeping sickness does not exist in South America at all. This could again be due to the Malinké's lack of experience with treating African sleeping sickness, as few of my collaborators remembered ever seeing someone with this disease given its near eradication in the region in the past few decades. Alternatively, one might attribute the efficacy of an Asháninka's remedy against a disease they do not experience to the power of the relational efficacy method, as African sleeping sickness' parasite *T. brucei rhodesiense* is closely related to diseases with which the Asháninka do have experience (congeneric

with the Chagas parasite *T. cruzi* and confamilial with the leishmaniasis parasite *L. donovani*, Trypanosomatidae); still, *2ab*'s efficacy against these two other parasites is relatively not very high (61st percentile inhibition for *T. cruzi* and 67th percentile for *L. donovani*).

Another interesting comparison is that of the efficacy of plants found by naming a disease rather than describing symptoms. When the efficacy of plants used to treat leishmaniasis found by naming the disease (*uta* in Spanish) vs. by describing the disease symptoms (open lesions at the site of a bug bite) were compared by ANOVA, the named-disease species had a greater average inhibition percentage in bioassays than the described-disease species, with $p = 0.0610$. Though not quite significant, this is very close and the opposite of the general notion that better remedies will be found by describing diseases than naming them to avoid language difficulties and issues different disease concepts. It will be interesting to see if this effect remains as more species are bioassayed and if this is analyzed in other areas of the world.

5.4. Conclusion

I had hoped to do bioassays on more plant samples and mixtures and more disease categories, but the difficulty of getting bulk samples out of Paititi and the expense and time required for further autoimmune disease bioassays prevented this. If one of the duplicate voucher specimens brought back to the US for sending to taxonomic experts is not needed and can provide sufficient material for testing, perhaps these can be processed in the future to provide more significant statistics and support for the relational efficacy method. The 40-150 g dried bulk samples from Mali often provided more than a hundred times the amount of final dried extract than the few milligrams needed for the bioassays,

so a 5-10 g sample from a herbarium sheet should be sufficient for a few bioassays. Even with data that were obtained from 52 simple and mixture plant extracts, there are already some interesting candidates with good efficacy of 80% or more inhibition and some sign that using related plants from unrelated cultures to treat related diseases may lead to more effective plant medicines, given that the Asháninka seem to have an effective remedy for a disease they have not experienced themselves. Next, the relation numbers must be passed through the relational efficacy system to see if it predicts ranking of the medicinal plants similar to that obtained from the bioassays.

Chapter 6. Systematic Methods

In order to properly identify all plant material, collections were identified as specifically as possible in the field, using field guides such as Arbonnier (2002) for Mali and Gentry (1996) for Peru, later with experts in the host country herbaria (USM in Peru, Bamako [*Département de Médecine Traditionnelle*, no *Index Herbariorum* designation] in Mali), and finally back at the NY herbarium with the help of Douglas Daly and other experts in the flora of these two areas at the NYBG and other institutions. After identification, the utmost care was taken to keep this potentially sensitive intellectual property (IP) confidential, and new systems were worked out to help deal with IP-containing ethnobotanical vouchers when they are deposited in herbaria where they are viewable by at least the greater scientific public, if not the general public, as discussed in Section 6.4. The phylogenetic distances (or conversely relatedness) among all the plants, diseases, and cultures being considered had to be determined through a mix of molecular, morphological, and chemotaxonomic data and, in the case of diseases, plant-based disease taxonomy techniques.

6.1. Identification

As it is generally best to identify plants when more characters are apparent in the fresh sample, such as smell or texture, an effort was made to identify plant samples as soon as they were collected and an appropriate field guide could be consulted. Using Gentry's book (1996) in Peru, I could readily identify a few families, while others had distinguishing characteristics such as spines, odors, trinervy, latex, or liana cross sections,

but otherwise plants were left unidentified until they could be shown to an expert back at the herbarium in Lima (USM) or at NY. Having over 50% of the samples fertile was a boon to identification. In Mali, the Arbonnier (2002) field guide was excellent for field identification as it has a vegetative key, spine key (due to the vast number of *Acacia* species in West Africa), flower key, and fruit key, meaning that almost all collections could be identified to species in the field; the few that could not turned out not to be in the book. This was especially important since NY does not have a good reference collection of West African plants against which to compare, so Malian plants would have been difficult to identify with only the NY collection.

Smell is an underutilized sense in the identification of plants, and therefore a great effort was made to fully describe the scent of bark slashes and, if present, the reproductive parts of the plant. Scent is of course a character that is not well preserved in dried herbarium specimens, and we generally lack an adequate vocabulary to describe scents, but the latter problem could be ameliorated by training botanists in the proper description of smells, just as we are trained in the description of flower and fruit types. Wine tasting has a well-developed vocabulary for scent description from which we can draw ideas and vocabulary (Brochet and Dubourdieu 2001). Using all five senses can definitely improve recall of facts, as it strengthens the number of neuronal connections associated with any memory (Christie 2000). Perhaps this will help botanists to identify many plant varieties on mere sight or "on smell" of the bark slash as do many indigenous groups who deal regularly with these plants (Schultes, Raffauf et al. 1992). Although we cannot generally have the dream of taking an high-performance liquid chromatograph (HPLC) or gas-chromatograph/mass spectrometer (GC/MS) machine into the field with

us to analyze chemistry, we do have one of the most attuned chemical sensors in our nose with us at all times.

At USM in Lima, more collections were identified with the help of botanists Asunción Cano and Joaquina Alban. At the DMT herbarium in Bamako, several species that were not in Arbonnier's book were identified with the help of Rokia Sanogo and Seidou Dambelé, especially to help distinguish the many species of *Combretum*, which are not easily separable and some of which have been synonymized.

At NY, plants that could not identified before were identified at least to family with the help of Doug Daly, and then further identified with specialists Michael Nee, Wayt Thomas, and Andrew Henderson, supplemented by others at the Missouri (MO) and Kew (K) herbaria. Those that could not be identified by specialists to species were initially narrowed down to several possible genera with Doug Daly's help and using monographs on the families. Then we found all the collections in these genera from the appropriate geographic area in the NY herbarium and tried to match them with my collections. In this manner, we were able to identify to species all but a few of the 176 total collections. The remaining unidentified specimens were all sterile collections of small herbs that were therefore not in any of the field guides for the area. The few from Peru that remained unidentified I collected again on my second visit to Paititi in 2004; in one case this helped with identification, but in another it did not as it was again a sterile collection.

All identifications were entered in the Filemaker relational database with separate tables for collections, species, common name/species correspondences, literature references for each species, and assays done on each species, which connected to previous tables for healer interviews. Queries were made from this database to the

NAPRALERT natural products Web database (http://napralert.org/) in an automated fashion using Applescript I developed that allowed for the download of the list of journal articles, compounds, and bioassay and laboratory studies associated with each plant species in general or with treating each of the seven diseases being considered, giving a backup efficacy measure to the bioassays that were done on my specific collections at the Swiss Tropical Institute. This automation was performed by having Applescripts form the appropriate Web query string for each species, which was then passed to the NAPRALERT Web interface, and the results were parsed into data usable in the database.

This database should allow powerful queries to be created such as, "What Fabaceae species are used by more than three healers to treat diabetes that have bioassay IC_{50} values less than 1 mg?", "how many of the collections from Peru have therapeutic uses confirmed by more than two journal articles?," or "what genera are used in both Peru and Mali for multiple parasitic diseases?" Unfortunately, NAPRALERT downloads its data in a Portable Document Format (PDF), rather than a table that could be integrated immediately. Instead, the data is listed in plain text in an inconsistent matter, making automatic parsing of the data nearly impossible without checking on each one. This would be intractable with the 255 species files downloaded from NAPRALERT, and I am still looking for a solution for this vexing problem.

6.2. Taxonomy and dated phylogenies of plants, diseases, and cultures

Using dated phylogenies that are as accurate as possible is key to the success of the relational efficacy system. This involved much literature research and I had hoped that these phylogenies, especially those of the angiosperms, would be more clearly delineated

at the time of this writing than they currently are, but these phylogenies will be in flux for some time to come, as molecular clock techniques are improved and more plant fossils are found and identified. Therefore, we must be able to manage the variable nature of this data.

At the suggestion of Damon Little and Lúcia Lohmann, I attempted several other ways of deriving the distance relations of the diseases and plant species. First and simplest was to use the patristic distance in calculating the distance matrix (Schuh 2000), which requires a tree on which to base it, because it uses the amount of change that separates two taxa as measured along the branches between the taxa in the calculation. The divergence node distance of Webb (Webb 2000) is another simplified way of determining distances that gets around the shortcoming that patristic distances do not work for polytomies. This would have to be calculated by hand however, since there is no program to easily calculate it for the 332 families in Soltis' Angiosperm Phylogeny Group phylogeny (Soltis, Soltis et al. 2000), aside from the fact that it would not represent the secondary molecular phylogeny very well given different evolution rates on different branches. Most of these measures based on gene sequence data give distance as opposed to relatedness measures.

One problem with using the more readily available distance measures between plants, diseases, and cultures vs. relatedness or similarity measures is that it is unclear what value to use for the distance of the same plant, disease, and culture. The most obvious value of zero creates problems since if we take the basic equation for plant potential

$$P_{s,d,c} = \frac{1}{N_s N_d N_c} \sum_{s',d',c'} \frac{R_{s,s'} R_{d,d'}}{R_{c,c'}} \qquad \text{Eq. 12}$$

and we convert it to distance measures most simply by using $D_{x,x'} = 1/R_{x,x'}$, we get

$$P_{s,d,c} = \frac{1}{N_s N_d N_c} \sum_{s',d',c'} \frac{D_{c,c'}}{D_{s,s'} D_{d,d'}} \qquad \text{Eq. 13}$$

so if either $D_{s,s'}$ or $D_{c,c'}$ are representing the same plant species or culture, this will create a divide by zero error. We can use either a very small value for $D_{x,x'}$ of the same unit, try to convert this value to relatedness with

$$R_{x,x'} = \begin{cases} 1 \; if \; D_{x,x'} = 0 \\ \left(\frac{1}{D_{x,x'}} - 1 \right) \frac{1}{D_{min}} \; otherwise \end{cases} \qquad \text{Eq. 14}$$

yielding values between zero and one, or use the formula $R_{x,x'} = 1 - D_{x,x'}$ to yield values between zero and one. For the sake of simplicity, the first option was used with a $D_{x,x}$ of 0.001. Since this is a non-linear relationship, it would be interesting to see which of these three options yields the best correlation with bioassay data.

6.2.1. Disease "phylogeny"

The network of relations of diseases cannot be properly called a phylogeny if we are considering a collection of diseases such as in this project that are parasitic, genetic, and environmental, as these diseases do not have true evolutionary relationships, so instead it should be called a dendrogram. To generate the disease dendrogram using the plant-based disease taxonomy (PBDT) described in Section 2.5 for the apparently unrelated categories of parasitic diseases, autoimmune diseases, and uterine fibroids, a list of all the laboratory-tested and verified (i.e., some efficacy against the disease was found in bioassays) plants and plant-derived compounds used for each disease was downloaded from the NAPRALERT database (www.napralert.org), James Duke's USDA Phytochemical and Ethnobotanical Database (http://www.ars-grin.gov/duke/), and for

fibroids from PubMed (http://www.ncbi.nlm.nih.gov/pubmed). To use as an outgroup in the systematic software, information on plants used to treat depression were downloaded as well, since this disease seems to have no relation to parasitic or autoimmune diseases. The following pharmacological terms were used respectively for each disease to derive plant and plant compound lists from these databases: depression (antidepressant), malaria (antimalarial and antiplasmodial), leishmaniasis (antileishmaniasis), Chagas' disease (antitrypanosomal), African sleeping sickness (antitrypanosomal), diabetes (antihyperglycemic and antidiabetic), eczema (antieczema and antieczemic), asthma (antiasthmatic), and uterine fibroids (antifibrotic). Antitrypanosomal activity was not divided out in either database between anti-*T. brucei* (sleeping sickness) and anti-*T. cruzi* (Chagas) activity, so each referenced article needed to be checked to determine which species the article was testing. Fibroids returned very few verified plants (2) and compounds (1), so PubMed was used to flesh out the list of plants used to treat this disease with few visible symptoms. These lists come from NAPRALERT as Portable Document Format (PDF) files, from which the text was extracted into Rich Text Format (RTF) files via batch processing in Adobe Acrobat Professional. The species and chemical names were then extracted from these RTF files with Microsoft Word using a set of global search and replace operations, and then converted to tables using the Text to Table tool, all automated via Applescript scripts controlling Word. These tables were then combined using Microsoft Excel into a table of all the species (rows) with documented activity against each disease (columns) and this was saved as a NEXUS format text file for import into TNT (an excerpt of which is shown in Table 6.1, and the complete table can be downloaded in tab-delimited text format from

http://nat.myphotos.cc/disease_species.txt or in Excel format at http://nat.myphotos.cc/disease_species.xls). Species that had multiple citations of being used for the same disease, were still counted as a '1' in the data matrix, since the table merely records presence or absence of a plant being used for a disease, not how many studies have reported it used for that disease. Species and plant compounds used to treat diseases were weighted exactly the same. The full data matrix initially included 9 disease "taxa" with the outgroup, and 2277 treating plant species "characters", but PAUP, which was used to convert the file from a transpose data matrix (characters as rows instead of columns) to a normal matrix, could not load such a large file. To remove this barrier, all uninformative characters (those present for only one taxa, i.e. autapomorphies) were removed, leaving 398 informative characters. Similarly, there were 1195 total and 415 informative genera, 250 total and 136 informative families, and 2963 total and 414 informative compounds.

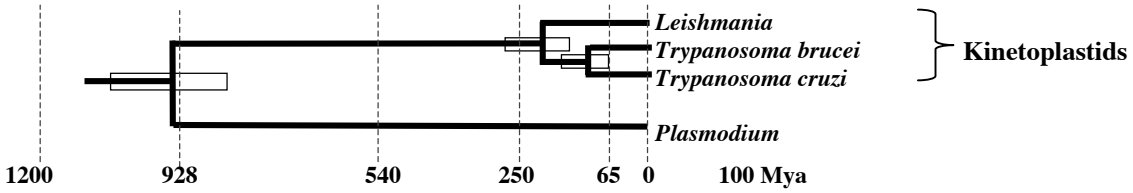

Figure 6.1. Adapted from (Douzery et al., 2004) Fig. 1. "Divergence time estimates (Mya) among eukaryotes, based on a Bayesian relaxed molecular clock applied to 30,399 amino acid positions. The topology is the highest-likelihood one, with branch lengths proportional to the absolute ages."

Table 6.1. An excerpt of the table of plants tested in labs to work against the eight studied diseases and the outgroup depression, derived from NAPRALERT, PubMed, and Jim Duke's USDA Phytochemical and Ethnobotanical Database.

Species	sleeping sickness	Chagas	leishmaniasis	malaria	diabetes	asthma	Eczema	fibroids	depression
Abuta pahni	0	1	1	0	0	0	0	0	0
Abuta rufescens	0	1	1	0	0	0	0	0	0
Acacia nilotica	1	0	0	1	1	0	0	0	0
Acacia species	0	1	1	0	0	0	0	0	0
Acaena ovalifolia	0	1	1	0	0	0	0	0	0
Acalypha benensis	0	1	1	0	0	0	0	0	0
Acalypha stricta	0	1	1	0	0	0	0	0	0
Acanthospermum australe	0	1	0	1	0	0	0	0	0
Acanthospermum hispidum	0	1	1	1	0	0	0	0	0
Achillea fragrantissima	0	0	1	1	0	0	0	0	0
Achyranthes aspera	0	0	0	1	1	1	0	0	0
Achyranthes rubrofusca	0	0	0	1	1	0	0	0	0
Achyrocline alata	0	1	1	1	0	0	0	0	0
Achyrocline flaccida	0	1	1	0	0	0	0	0	0
Achyrocline polycephala	0	1	1	0	0	0	0	0	0

Achyrocline ramosissimosa	0	1	1	0	0	0	0	0	0
Achyrocline satureioides	0	1	0	1	1	0	0	0	0
Adhatoda vasica	0	0	0	1	0	1	0	0	0
Aegle marmelos	0	0	1	1	1	0	0	0	0
Aerva lanata	0	0	0	1	1	0	0	0	0
Ageratina azangaroensis	0	1	1	0	0	0	0	0	0
Ageratina pentlandiana	0	1	1	0	0	0	0	0	0
Ageratum conyzoides	1	1	0	1	0	0	0	0	0
Agrocybe species	0	1	1	0	0	0	0	0	0
Ailanthus altissima	0	0	1	1	0	0	0	0	0
Albizia gummifera	1	0	0	1	0	0	0	0	0
Albizia lebbek	0	1	0	0	0	1	0	0	0
Alchornea cordifolia	1	0	0	1	0	0	0	0	0
Allium cepa	0	0	1	0	1	1	0	0	0
Allium sativum	0	1	1	0	1	0	0	0	0

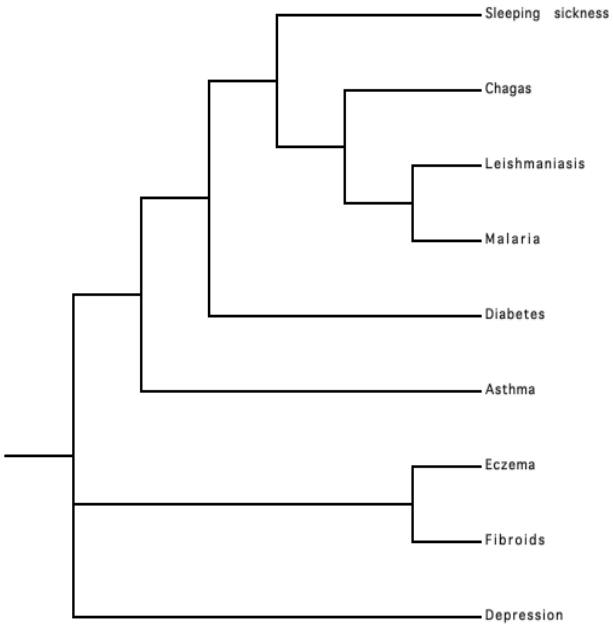

Figure 6.2. Dendrogram of disease created by the parsimony technique with PAUP with depression as an outgroup on disease treating genera and compounds data.

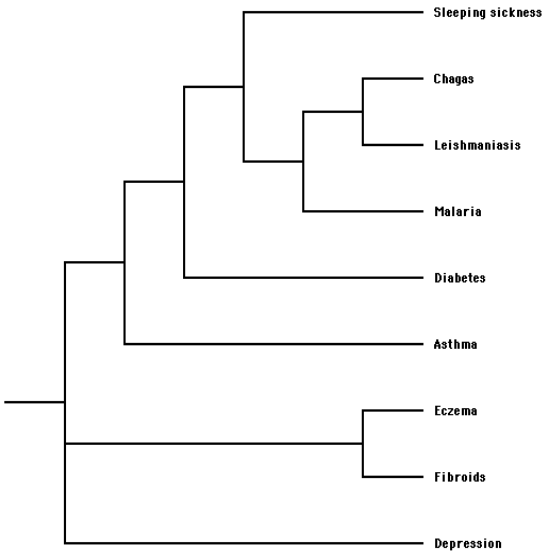

Figure 6.3. Dendrogram of disease created by the neighbor-joining cluster technique with PAUP with no constraints on disease treating species and compound data.

PAUP version 4.0b10 (paup.csit.fsu.edu) was then used to derive a dendrogram of the diseases using parsimony (Schuh 2000) and the Unweighted Pair Group Method with Arithmetic mean (UPGMA) and neighbor joining clustering algorithms, at the suggestion of Paola Pedraza, due to the fact that the parsimony algorithm assumes that characters make transitions from state to state, which is not true of disease-treating plants as characters. PAUP was run with the following settings: MaxTrees = 100, AddSeq = random, NumTrees=1000, NumReps (for bootstrap)=200.

The outgroup in systematic analysis must be sufficiently different from all the other taxa being analyzed to not fall within the dendrogram accidentally, yet similar enough to them to have at least some characters in common with the taxa being analyzed (Schuh

2000), something difficult to determine for the clearly unrelated diseases of fibroids, parasites, and autoimmune diseases. We also need to pick a disease or therapeutic category that has many laboratory-proven plants used to treat it listed in NAPRALERT to get good resolution on the dendogram, so depression was selected as the outgroup since it has little inflammatory or immune etiology, as compared to the parasitic and auto-immune diseases. The results in Figure 6.2 when the unrelated antidepressant pharmacological category was used as an outgroup in the parsimony algorithm, show that there are some issues with this method since it puts the trypanosome disease leishmaniasis closer to the parasitic disease malaria than to the other trypanosome diseases, contrary to what we would expect. So, instead I used neighbor-joining techniques to compute the trees and their inherent distance matrices.

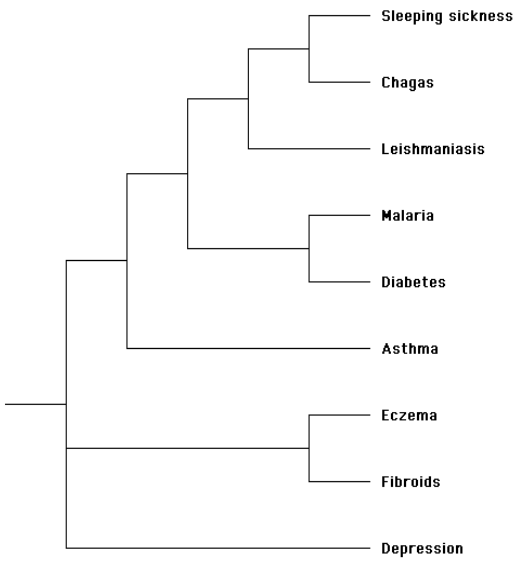

Figure 6.4. Dendrogram of disease created by the neighbor-joining cluster technique with PAUP with the constraint of the phylogeny of the parasitic disease from (Douzery, Snell et al. 2004).

Trees and distance matrices were created using disease-treating compounds and the genera of the disease-treating plants to ensure better overlap between the different disease categories, in cases where there were no exact species in common between categories. For instance with fibroids, only two species and one compound were found in NAPRALERT as laboratory-verified treatments. This yielded 415 informative genus

characters and 414 informative compound characters of the nine diseases. As this thesis was being completed, it was suggested (John Hall and Damon Little, personal communication, 2008) to use techniques such as independent contrasts (Ackerly and Reich 1999) that could include the phylogeny of the plant species involved in the calculation of the disease tree, but this is prohibitive given that the phylogeny of the hundreds of species used in the PBDT would need to be determined. However, this idea can be incorporated in future research.

The dendorgram produced by the neighbor-joining algorithm is shown in Figure 6.3. As a way of calibrating the PBDT-derived dendrograms, the subtree of parasitic diseases (Figure 6.1) was compared to traditional systematics-derived trees for this clade determined from molecular techniques (Douzery, Snell et al. 2004). The tree topology and branch lengths were found to be somewhat similar supports the general validity of the PBDT technique for other diseases. This makes sense, as PBDT is using disease treatments as a proxy for shared biochemistry and shared biochemistry, which is derived from shared genetics and evolution. However, there were some differences with the known parasite phylogeny so as the neighbor-joining algorithm allows parts of the tree to be constrained by known trees, the tree for the parasites in Figure 6. was used to constrain the neighbor-joining algorithm in PAUP producing the tree shown in Figure 6.4.

Given that the combination of genera and compounds used to treat the diseases concerned seemed to give the unconstrained dendrogram that best matched the known parasite phylogeny, and that it had the most informative characters, especially for diseases poor in disease-treating taxa such as fibroids, this data set was chosen to determine the distance matrix for diseases using PAUP's SaveDist command, as given

below in Table 6.2. The data for the genera and compounds used to treat each disease were combined into one data matrix that was read into PAUP, which then output a p-distance of the disease taxa for input into the relational efficacy system.

This matrix has several problems in that it shows leishmaniasis as more similar to malaria than to Chagas and sleeping sickness, the two parasites in the same Trypanosomatidae family as leishmaniasis, and it shows fibroids, eczema, and asthma as more similar to sleeping sickness than Chagas. Therefore, the relational efficacy potentials were calculated using two disease distance matrix alternatives: this PBDT distance matrix and one based only on the parasitic diseases derived from the patristic amino-acid sequence distance from Rodriguez-Ezpeleta, et al. (2007). The results for these two methods of distance measurement were compared to see which one correlated better with the independent measure of plant efficacy. Distances for the parasitic diseases derived from the time to the most recent common ancestor (see Table 6.4) as delineated in the eukaryote phylogeny in Douzery et al. (2004) could be used as a distance measure as well, but this ignores the difficulties common in molecular-clock approaches of different mutation rates affecting genes, proteins, and disease etiology, so the amino-acid sequence distance for the parasitic diseases is a better immediate measure of how the disease is being treated by plant compounds in the human body than the time on parasite phylogeny branches.

Table 6.2. Mean distance measures (or 1/relatedness) derived for the diseases from running the PAUP distance matrix command run on the genera and compounds used to treat the diseases.

	fibroids	eczema	asthma	diabetes	malaria	leishmaniasis	Chagas
sleeping sickness	0.261	0.272	0.298	0.522	0.599	0.530	0.450
Chagas	0.431	0.445	0.468	0.627	0.671	0.400	
leishmaniasis	0.564	0.573	0.590	0.741	0.551		
malaria	0.741	0.733	0.720	0.521			
diabetes	0.375	0.378	0.351				
asthma	0.082	0.096					
eczema	0.039						

Table 6.3. The normalized patristic amino-acid distance of the parasites derived from amino acid sequence data from Rodriguez-Ezpeleta et al. (2007).

	Sleeping sickness	**Chagas**	**malaria**	**leishmaniasis**
sleeping sickness	0	0.114	0.995	0.196
Chagas	0.114	0	0.969	0.180
malaria	0.995	0.969	0	1.000
leishmaniasis	0.196	0.180	1.000	0

Table 6.4. The normalized time to most-recent-common ancestor distance of the parasites derived from dated eukaryote phylogeny data from Douzery et al. (2004).

	Sleeping sickness	Chagas	malaria	leishmaniasis
sleeping sickness	0	0.114	1.000	0.222
Chagas	0.114	0	0.000	0.222
malaria	1.000	1.000	0	1.000
leishmaniasis	0.222	0.222	1.000	0

A logistic regression for each of the 56 pairwise comparisons of diseases using the genus and compound treatments as factors and the correlation coefficient as the distance measure, multidimensional scaling with Manhattan distances, and multivariate correlation coefficients were suggested as other ways to calculate the disease distances, but due to the difficulty of producing these and multitude of distance measures I already had to test in the system, these were left for future work.

As mentioned in Chapter 2, another method of measuring disease relations is via the genetic drug-disease connectivity map (Lamb, Crawford et al. 2006) and disease network (Goh, Cusick et al. 2007) which use human genome project data to connect diseases via the genes, proteins, and drugs that are involved in each disease. This would likely work only for the diseases with a genetic basis or predisposition (uterine fibroids, eczema, asthma, and diabetes) and a measure of relatedness would have to be derived from the connectivity network. The relatedness values from this system can again be compared to those derived from the disease-treating plant systematics above as a way to test and calibrate this method. Of the eight diseases I studied, the data from this research

unfortunately only includes the genetic disorders asthma and diabetes, and malaria susceptibility and resistance. In addition to this incomplete coverage, though this system creates a network there is not a clear method from the paper for deriving a measured distance between disease pairs.

6.2.2. Plant phylogeny

There is the possibility of using the newly emerging supertree techniques (Sanderson, Purvis et al. 1998) to combine phylogenies from individual families or genera with the larger angiosperm phylogeny if there is some overlap in the characters given that different genes or morphological characters are often used within each taxa. This would allow, for instance, the nesting of the Bignoniaceae from Lohmann (2006) into Soltis' angiosperm phylogeny to determined more refined distance measures with Bignoniaceae species in my data set. These techniques are so new that they are not yet very robust and there is still much to be hashed out about the methods, so they seem best left for future work.

This does bring up the interesting question of which gene or morphological characters to use to measure species distances that would best represent the plants' secondary chemistry similarities with which we are directly concerned. Chemotaxonomic characters would obviously best represent the distances we are concerned with, but exact phytochemistry is often not determined for many plant species, given the difficulties of extractions and isolation, along with the move to molecular systematics. Morphological characters that are more closely linked to plant chemistry, such as nectaries or trichomes, would be the best to use, though the determination of these characters may suffer the same fate as chemotaxonomy with the rise of gene sequencing. Of the gene sequence

characters to use, it seems nuclear genes would be better to use than chloroplast or mitochondrial genes, since these are more directly related to the genes producing the secondary compounds in the plant that are acting medicinally in the human body. So if these are available, the nuclear molecular sequences seem to be most accessible and appropriate. Soltis' three-gene phylogeny uses 18S rDNA, rbcL, and atpB sequences, of which only 18s rDNA is a nuclear gene, but finding any phylogenies with such good coverage and more nuclear genes will be nigh impossible at the moment.

A script extracting information from the National Institute of Health's National Library of Medicine Taxonomy Web site (http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html), based on the Angiosperm Phylogeny Group phylogeny, was used to clean up and translate the genera used in Soltis's APG tree file to subfamilies and families for cross-referencing with the families of the species in my database. In the confusing cases where there were several genera representing a family in Soltis' data, none of which corresponded to a genus from my database in that family, if the family had clearly defined subfamilies such as in Fabaceae and Asteraceae, I would use the subfamily of Soltis' genera and of the genus from my data to find a match from the distance matrix. Failing a match based on exact genus or subfamilies, I would randomly pick a genus from Soltis' multiple genera to represent the family in the distance matrix. Distances were determined by reading Soltis' gene data matrix and tree file into PAUP and having it save a distance matrix with the "uncorrected distance often referred to as the p-distance or dissimilarity (D) distance" measure for the entire dataset into a file. A section of the 567-genera, 332-family Soltis plant distance matrix is shown in Table 6.5.

Table 6.5. A section of plant distance matrix made using the p-distance on Soltis' 567 genera molecular data.

Genus	Abatia	Abelia	Acer	Acharia	Acorus	Actinidia	Aechmea	Aesculus
Abatia	0	0.051	0.057	0.042	0.076	0.056	0.071	0.058
Abelia	0.051	0	0.050	0.050	0.069	0.038	0.069	0.054
Acer	0.057	0.050	0	0.053	0.074	0.053	0.074	0.016
Acharia	0.042	0.050	0.053	0	0.075	0.056	0.070	0.055
Acorus	0.076	0.069	0.074	0.075	0	0.072	0.063	0.075
Actinidia	0.056	0.038	0.053	0.056	0.072	0	0.074	0.053
Aechmea	0.071	0.069	0.074	0.070	0.063	0.074	0	0.076
Aesculus	0.058	0.054	0.016	0.055	0.075	0.053	0.076	0

6.2.3. Cultural phylogeny

In order to derive the phylogeny and distance matrix for the cultures being considered there are luckily several possibilities: glottochronologies based on languages (Cavalli-Sforza, Menozzi et al. 1994), and several gene-based phylogenies that stem from Cavalli-Sforza's work and the Human Genographic Project (Wells 2006). Simple measures such as geographic distance between two cultures are clearly difficult to use as a proxy of medicinal-plant-knowledge transfer since geographic barriers such as oceans and mountains impede cultural transfer but are not factored into simple geographic distances. Glottochronology which is based on similar words between languages (cognates) to date language divergences (Cavalli-Sforza, Menozzi et al. 1994) would be a good proxy for knowledge transfer since it represents the exchange of information through language if

not for the fact that linguists do not consider to be reliable past 5,000 years ago (Foster 1996) which is much younger than the likely date of divergence of the Malinké and Asháninka we are considering. Glottochronology would probably be usable for dating divergences of closer groups such as the Asháninka and Maya.

So it seems the genetic cultural distance is currently the most reliable measure, and several of the more recent calculations of this have good coverage of the groups that interest us. The update to Cavalli-Sforza's work in Ramachandran, et al. (2005) and Li, et al. (2008) based on gene sequences of 53 worldwide groups are a great aid in determining cultural distances. Though both of these sequencing projects covered what they call the *Mandenka*, another name for the Malinké, neither one covered the Asáhninka specifically, so the geographically nearest group, the Karitiana of Bolivia, were used to represent the Asháninka. If we want to include the other groups such as the Maya, Chinese, Dominican, Indian, and European herbal traditions with whom I have studied in Guatemala and New York City, corresponding groups in Li et al.'s data near these groups must be found. For these I have selected the French for Europe, Sindhi for India, Han for China, Colombian for the Dominican Republic, and Maya for the Guatemalan Itzaj Maya. Using the French to represent European medicine, though seemingly arbitrary, makes little difference since the smallest distance with the French, that of the Sindhi in India, at 0.02295563 shows little difference with the distance between the Sindhi and the Russians (0.02142575) and the Sindhi and the Italians (0.02348291), differences of only about 5%, unlikely to affect the overall calculations. The choice of the Colombia to represent the Dominicans is a bit harder to defend, but this is the closest non-

Mesoamerican culture to the Caribbean in Li's study, so there is no other good choice. The pairwise distance matrix for these groups is shown in Table 6.6.

Table 6.6. A section of the cultural distance matrix derived from Li et al.'s (2008) protein gene sequence data.

Li	French	Mandenka	Sindhi	Han	Colombian	Karitiana
Mandenka	0.16293223					
Sindhi	0.02295563	0.13811186				
Han	0.11821877	0.20571508	0.08516563			
Colombian	0.16309355	0.26324222	0.1370715	0.1317164		
Karitiana	0.2179459	0.32480059	0.19225195	0.18185524	0.11137485	
Maya	0.12531661	0.23238266	0.10413671	0.1008648	0.03393518	0.08711611

6.3. Translation and conglomeration of data

To transfer trees derived from PAUP or downloaded from other sources into the database to make the final relational efficacy calculations, the distance matrix tables in text or excel format were read into Excel and an Applescript communicated between this data in Excel and the Filemaker database that has the information on which species are used in which cultures to treat which diseases. These data are kept in separate programs since the plant-treatment data is best kept in a relational database like Filemaker, but this program is not appropriate for the 500-by-500 plant-genera distance matrix, while the latest 2008 version of Excel can handle large tables like this (older versions had a 256-column limit). The Applescript program calculates the potential efficacy for each species by looking up the relatedness value of each species, disease and culture and summing these values across the entire database.

6.4. Herbarium specimen confidentiality

In ethnobotany, many professional and academic organizations have codes of ethics or informed consent agreements with collaborators that include guidelines for confidentiality of uses and species names that can restrict access to data or specimens. At the same time, researchers must collect and deposit herbarium vouchers with informative labels in order to maintain scientific rigor and credibility. This paper explores possible solutions to these seemingly incompatible goals. Few guidelines exist for how to keep the data associated with herbarium samples and vouchers confidential for the long term. Here we explore the issues involved and assess several measures that can be implemented in different combinations to better safeguard a traditional community's knowledge, i.e., their intellectual property. Options include keeping ethnobotanical vouchers separate from the rest of an herbarium, omitting location and collector information from voucher labels, ensuring that online databases are secure, restricting electronic access to certain fields in databases, and formulating long-term agreements with herbaria. We examine the pros and cons of each combination of measures in an effort to advance science while maintaining knowledge confidentiality.

6.4.1. Background

Currently, most pharmaceutical companies are not searching for herbal remedies among records of indigenous plant uses, preferring instead to seek novel compounds and activities in the laboratory via combinatorial chemistry and rational drug design, which happen to be free of the complications that attend international programs involving the Convention on Biological Diversity (Newman, Cragg et al. 2003). Interest in

bioprospecting for medicinal plants from indigenous knowledge is cyclical, however, and pharmaceutical companies already show signs of returning to natural products (Laird and Wynberg 2005) and to their previous agreements with botanical gardens for assaying every species new to science that is collected as well as surveying herbaria for plant use information (Raskin and Ripoll 2004).

This recent resurgence of interest in and discussion of bioprospecting and protection of intellectual property rights (IPRs) can have both positive and negative effects on the future of ethnobotanical research. On the one hand, increased interest in natural products and ethnobotany-oriented biodiversity prospecting may lead to increased support for research in these areas as well as benefits for traditional communities; unfortunately, it may also result in attempts at illegal short-cuts by the ethically compromised, or eventually in reduced commercial and academic interest in ethnobotanically guided bioprospecting because of the difficulties associated with IPR issues.

In fact, some researchers, because of the sensitive issue of biopiracy or at their collaborators' request, have chosen to completely avoid the topic of herbal medicine in their research even when it is sorely needed in their research areas and communities to preserve traditional knowledge and aid community health (e.g., personal communications Julie Velasquez Runk 2006, Christiane Ehringhaus 2005, Marina Campos 2006).

If we can provide better IPR safeguards for collaborators in the context of herbarium collections, this should help open doors for a resurgence in ethnobotanical research; moreover, it will help prepare us for increased commercial pressure in this age of ever-broadening Internet access to data. Honoring collaborators' IPR is already a requirement of Prior Informed Consent (PIC) forms signed by researchers and their collaborators,

research agreements with host countries, and many of the ethics codes of professional societies to which researchers belong and journals in which they publish (Society for Economic Botany 1995; International Society of Ethnobiology 1998; Ethnobotany Research and Applications 2006; Guterman 2006) . Regardless, some current practices leave herbaria exposed to several opportunities for what has been termed biopiracy, tilting the balance towards negative effects on ethnobotany research. To avoid this, herbarium policies on the storage and access to ethnobotanical voucher specimens and their associated data need to be evaluated and revised as necessary to honor the confidentiality sections of our consent agreements with collaborators as a legal contract.

In drafting prior informed consent agreements for ethnobotanical research with the Asháninka of Southeastern Peru and the Malinké of Western Mali, we concluded that new policies and protocols for depositing herbarium vouchers are necessary to protect the intellectual property of these groups while still advancing botanical science. Several authors and researchers have called for devoting more attention to the issues of intellectual property rights (IPR) when forming research agreements, collecting plants, depositing plants, and publishing species names (Posey and Dutfield 1996; Bannister and Barrett 2001; Laird 2002; Guterman 2006; Lewis 2006).

One approach to protecting traditional botanical knowledge, the practice of not publishing species names of plants for which a particular use has not been previously published, is becoming more accepted, if not widespread (Milliken and Albert 1996; Milliken 1997; Alexiades 1999; Lewis, Lamas et al. 1999; Laird 2002; Lawrence, Phillips et al. 2005; Lewis and Elvin-Lewis 2006). This represents one measure for protecting traditional knowledge, but there is still the worry of what can happen to plant

specimens, their identities, and attached information once they are out of researchers' hands and deposited in a herbarium as voucher specimens, as well as the fate of the botanical and use information. Hereafter we refer to researchers who deposit sensitive specimens in an herbarium as "depositors" to distinguish them from visiting researchers (or "visitors") who are viewing those specimens.

As scientists, we want this knowledge to still be studied, preserved, and returned to the communities, while advancing systematics, floristics, conservation, and ethnobotany in general. Meanwhile, having seen the surge of bioprospecting in the 1980's and 1990's and the backlash in the past decade from communities and non-governmental organizations fearing biopiracy, we also want to ensure the protection of indigenous knowledge and IPR protection related to indigenous knowledge, and make certain that if an outside party benefits from this knowledge, the communities will be made aware of this and benefit as well.

One could attempt to draw parallels to clinical trials and medical research in which researchers reveal only patient data that is pertinent to the study or allow other researchers to analyze anonymous tissue samples, without revealing patient names nor even enough data that the patients' identity can be inferred (Section 2504.10.5 of the US Privacy Act, Executive Office Of The President - Office Of Administration 1980; International Statistical Institute 1985; Section 4.2.b of The Data Protection (Subject Access Modification) (Health) Order 1987, Her Majesty's Stationery Office 1987). Similarly, in ethnobotanical research we often do not want to reveal the species name or even enough information about the species that its exact identity can be inferred, but rather just the overall statistics of use. The differences between the patient in the medical

study and the species (represented by the voucher) in the ethnobotanical study are that in the latter (1) to maintain rigor, we need to be able to re-confirm the identification (the "diagnosis"), and (2) in many if not most instances, we need the specimen and the non-ethnobotanical data for other purposes, such as floristics or conservation. Our task is to decouple as much as possible the name and use data so each can be studied separately without losing the link altogether, as if we wanted to do medical research on a set of anonymous subjects and at the same time do a separate study on the genealogy of these same subjects using their names, without the data from the two studies being connected.

6.5. The voucher in ethnobotany

The conflicts and contradictions surrounding ethnobotanical vouchers stem from the fact that the voucher must serve multiple purposes. It is what separates science from hearsay, the proof that a particular species served a particular purpose in a particular time and place, and it is a principal component of modern ethnobotany that makes reproducible results possible, a cornerstone of what gives ethnobotany rigor and credibility as a scientific pursuit. As science, ethnobotanical results are not and should not be published nor their conclusions disseminated without the existence of vouchers to give them substance.

The voucher also documents the flora of a particular place and time, and many if not most ethnobotanical studies are conducted in places where the cultures as well as the flora are both threatened and poorly documented, so it essential that the voucher be accurately identified and integrated into floristic efforts.

In a broader sense, the ethnobotanical voucher represents a precious piece of human patrimony, an empirical discovery sometimes of life-saving value that may never

be repeated and so should not be lost to posterity. In practical terms, and in the present and the near future, however, the voucher also represents the intellectual property of the culture that linked that species to a particular purpose, so both ethically and legally we are obliged to devise protocols and mechanisms for appropriate confidentiality for both the identity of the voucher and the data associated with it.

6.5.1. Loss of confidentiality

This information can conceivably get into the wrong hands in several ways through publications, the physical specimen, or electronic databases. In response, there is a spectrum of controls and safeguards to choose from that affect physical access to specimens and electronic access to data; options include tighter monitoring of who consults the herbarium, physical segregation of ethnobotanical specimens, and data fields that are blocked or tied to pop-up windows that display the associated research agreements for the specimen. Moreover, in addition to addressing current circumstances, the measures implemented must consider the situation ten years or more from now and anticipate changes in herbarium staff or databases and in the status of the group whose IPRs are being protected.

In the herbarium, IPRs can be compromised under several scenarios. If use data are recorded on the label, the visitor may reveal them intentionally or inadvertently in the course of routine data transcription. Even if the use data do not appear on the label, the ease of obtaining information about where depositors have done field work and their subjects of study makes it a trivial matter to make inferences about uses, especially if the ethnobotanist's study was narrowly focused.

These scenarios risk violation of the confidentiality of the collaborators' plant knowledge stated in the depositor's research agreement, and they necessarily raise the issue of who has access to the herbarium. Some but not all major herbaria, currently ask visitors the purpose of their visit and allow access to researchers who work for a sister institution or a conservation-oriented organization, and often to amateurs, as long as their stated purpose is scientific and non-commercial. Visitors considered legitimate are not explicitly monitored nor is their recorded data on specimens screened, so opportunities for foul play do exist, however minimal.

The groundswell toward databasing herbaria and making all the data available on-line is intended to potentiate biodiversity research and consequently conservation arguments, and to give investigators in developing countries equal access to data, but this powerful advance brings far greater threats to IPRs than traditional access to herbarium specimens. In the absence of restrictions, anyone can search through herbarium databases for plants collected in a certain area or by a certain collector that are known to have particular uses and profit from these uses without compensation to the person or communities who originally held this knowledge. In this context, the ease of obtaining information about where depositors have done field work and their research topics is far more dangerous.

6.5.2. Possible solutions

Herbaria can and should institute one or more of a number of policies to protect the confidentiality of ethnobotanical data, and these can involve the following:

Controlling physical access to the herbarium. As noted above, most if not all herbaria require prior notification from visitors, including institutional affiliation and

purpose of the visit. On arrival, they are asked to sign in, and in many herbaria they are given a set of instructions and regulations, and herbarium administrators could ensure that these prohibit transcription or imaging of ethnobotanical data. They should also question any visitors or institutions with which they are not familiar. Some herbaria require that the request for the visit and the statement of purpose come from an official at the visitor's home institution. All herbaria should adopt this policy. Most herbaria tend to scrutinize more carefully requests to study CITES-listed groups such as orchids and bromeliads, so they could apply the same caution across the board. To extend this same protection to ethnobotanical specimens, visiting researchers should be asked to sign a simple agreement stating they will honor the protections and IPR described in PIC and research agreements attached to specimens that they are examining.

Monitoring herbarium use. This is usually not feasible, unless herbarium administrators or staff are willing to make occasional spot checks on visitors, partly out of courtesy but also as a means of monitoring.

Ensuring that there is a "haystack" of non-ethnobotanical specimens from the same geographic area or taxonomic groups in the herbarium to hide the "needle" of ethnobotanical collections. If the ethnobotanical collections in question are from geographic areas, taxonomic groups or collectors not represented in the herbarium by many collections, as noted above, an ethically compromised person might be able to infer uses or properties based on knowledge easily acquired about the nature of a known project or expedition. Herbaria could therefore monitor ethnobotanical collections that are rare in one of these ways, and they may take additional measures restricting physical access to the relevant specimens, as discussed below .

Placing advisories on herbarium sheets with ethnobotanical data, or omitting ethnobotanical data from labels but not from controlled-access databases. For specimens whose labels contain sensitive information, it would be easy to glue an advisory onto at least new accessions with confidential data, along with the standard labels, informing the user of the existence of legal agreements with the data sources as well as restrictions on data transcription or imaging. This may make the specimen stand out, however, when we are often trying to hide it in a haystack of many specimens. Writing the information directly on the herbarium sheet, if it will fit as opposed to on a label, may be a better option, as it will not make it stand out from other specimens as much. Alternatively, the ethnobotanical data associated with such specimens could be omitted from the hard-copy label and stored in administrator-access-only fields in the database.

Blocking fields in databases. Many herbaria that have databased collections have structured their databases such that outsiders cannot get access to use information for ethnobotanical collections and locality information for CITES-listed species, and this restriction is maintained as long as the species are so listed. It is a trivial matter to impose the same controls on other fields such as the collector or project. Again, less straightforward is setting time limits on the blocks.

Putting pop-up windows in databases containing advisories or contracts. This is a painless and easy measure to implement, and entire PICs or links to full contracts can be made to appear in a pop-up window when the record for a IPR-sensitive specimen is consulted on-line.

Blurring data on labels of on-line images of specimens. This is a measure already being implemented in several herbaria to obscure locality data for CITES-listed taxa, and again the same can easily be done for use data.

Placing ethnobotanical specimens in locked cabinets. This is a rather extreme measure that some herbaria are legally obliged to take for species that yield controlled substances, and some opt for locking cabinets containing highly threatened taxa, but at least the latter can be examined on special request. The problem with locking up ethnobotanical collections is that they usually represent various taxonomic groups and a visiting (or resident) botanist will not know to look for them, so they are lost to science if not posterity. Another challenge would be how to know whether or when to finally unlock them, because while CITES lists are updated and easy to consult, different ethnobotanical specimens could be associated with any number of projects with different access instructions and time limits.

Instructions for herbarium administrators. For herbarium administrators to implement any or all of the measures just discussed, the ethnobotanist must provide them with the necessary materials and information, i.e., a paper or digital copy of the PIC and any contracts, permits and authorizations associated with the project, and specific instructions on the time frame during which any given measure must be operative. A legal agreement signed by both the depositor and the herbarium may facilitate both parties honoring the confidentially of deposited specimens, if this level of formality is desired.

A problem that can arise with all these restrictions on specimen access is that the specimens are forgotten in their inaccessible state and then the collector passes away, the

group they were collected from dies off, the species becomes extinct, the herbarium administration changes, or the herbarium moves. If the access restrictions on these specimens are never lifted, it would lead to the valuable scientific knowledge of these species, uses, and groups being lost forever. To avoid such potential loss, agreements with an herbarium must have mechanisms for eventual release of restricted information and ways to ensure that agreements carry over through changes in the herbarium staff, structure, databasing, or location.

Measures can be put in a depositor's will to ensure that if (s)he passes away, the information will be released, but this is harder to do with the unfortunate but equally possible demise of an indigenous group. An online herbarium database could therefore develop a mechanism for periodic (e.g. yearly) notifications to inform the herbarium staff to contact those with ethnobotanical collections registered in the herbarium and verify the status of the agreements and indigenous groups or traditional communities connected to the ethnobotanical collections, so that the use and location information on these specimens can be released for general access if the agreements expire or the groups become extinct.

This measure can be complicated by the fact that specimens might be covered by international agreements such as the CBD that apply to the entire country and therefore the use could be considered the IP of that country and not just the group who recounted the plant use. In this case, the use information might only be released for general access once the depositor's will allows it, and the CBD lapses or the country changes their policies. Admittedly this is a lot of information to track for herbaria, most of which are underfunded and understaffed. Therefore, the onus should be on the depositor to make it

as easy as possible for the herbarium staff to ensure the confidentiality of their specimen information while at the same time making it accessible to further scientific research when the time comes.

6.6. Herbarium agreement

In discussions with our local herbarium administrators and other botanists, we have developed a draft contract which is listed in Appendix B that can be signed by both the herbarium and the depositor that ensures continued confidentiality of deposited voucher specimens. This gives us enduring trust in the fate of our deposited samples, even when we or the herbarium staff leave the institution, by making it a legal document, signed by the herbarium administrator and the depositor. Without this, a simple word-of-mouth promise from herbarium staff to keep vouchers confidential will last only until that person leaves the herbarium.

The main features of this draft herbarium confidentiality agreement are as follows:

All pertinent vouchers marked by a "Do not database" stamp, to avoid the vouchers being databased in the future when the genus or family is revised, and collection information becoming easily accessible in a digital format.

No digital access to use data, until the depositor's will allows it, the collaborators allow it, research agreements are revised, or a pre-set time limit expires.

Links in the database to the physical specimen (barcodes, collector number) are blocked and only become unblocked by the aforementioned events with other data. This unblocking triggers a notification to other herbaria where duplicates of this specimen are deposited of any changes that may have occurred in the identification of the specimen.

No herbarium visitors allowed to take tissue samples from herbarium specimens that fall under this agreement for genetic, chemical, or molecular analysis.

Depositors should make the required procedures as simple as possible to follow and minimize the workload of the herbarium staff needed to uphold the agreement.

Depositors must allow legitimate, non-commercial use of their specimens for research in systematics, floristics, and ecology to continue with minimal hindrance, while upholding their PIC and research agreements

IPR-sensitive sheets indicated in the agreement should be kept in a separate area with restricted and monitored access.

To reduce the work of the herbarium staff, depositors should strive to deposit specimens in herbaria that already have a large collection from their research area so that floristicians can still access the key information. Information in the database linking the duplicates of these specimens in the depositor's home herbarium to other herbaria will be blocked until all other data for the specimen is unblocked.

For collections from geographic areas represented by few specimens, keep vouchers under restricted access until the number of general collections from that region reaches sufficient size that the ethnobotanical vouchers make up only a small proportion, i.e. making a haystack to hide the needle.

This last measure is necessary to camouflage collections from geographic areas poorly represented in the herbarium, so that the collections' use can not be easily deduced. Thus, these collections are not released into the general herbarium until they are dispersed in a collection of sufficient size (2000 in this case) from the same geographic area, making the proverbial needle in the haystack, if not impossible, much more difficult

to find. The size of the haystack collections could be determined by balancing the effort required to make the extra collections with the desired low odds of finding an ethnobotanical collection among the general collection, e.g. the haystack must be twenty times the number of ethnobotanical collections from the area to make the odds 1 in 20 of finding an ethnobotanical collection, as long as this number is no greater than 5000.

In light of these requirements, we examined several possible strategies that a depositor may use to protect the ethnobotanical information on their herbarium collections, with the potential pros and cons of each scheme. Each of the five herbarium scenarios and their features are described in Table 1 with further discussion of each scenario and its fit to different situations below.

Table 6.7. Five scenarios for depositing collections in herbaria with varying degrees of confidentiality protection and scientific openness, with the pros and cons of each scenario.

Features	Scenario A	Scenario B	Scenario C	Scenario D	Scenario E
	Deposit collections as standard herbarium specimens with all field data, including uses fully visible, and provide full digital access	Deposit collections in an herbarium with database fields for uses and exact location locked, pop-up windows with PICs and research agreements for appropriate specimens, and modified screening of visitors where researchers are introduced by their home institution and they sign a visiting herbarium agreement that forbids publishing use data	Deposit collections in an herbarium with the collectors' names, uses, and exact location removed from physical label and hidden as fields in the database; otherwise full digital access; no herbarium confidentiality agreement; and depositor makes general botanical collections from the same area to create a haystack to hide the ethnobotanical data	Deposit collections in an herbarium with the collectors' names, uses, and exact location removed from physical label and hidden as fields in the database; otherwise full digital access; herbarium confidentiality agreement in effect	Keep collections fully in a restricted-access or personal herbarium with collectors' names, uses, exact location all appearing on specimen label; database access at discretion of depositor; herbarium confidentiality agreement in effect if in a restricted-access herbarium; and complete field data given out only under depositor's supervision for species identification verification or according to depositor's will
Pros:					

Features	Scenario A	Scenario B	Scenario C	Scenario D	Scenario E
Location preserved for conservation and biogeography purposes	✓				
Specimens can be used for taxonomic and floristic purposes	✓	✓ (geographic information is sacrificed)	✓ (geographic information is sacrificed)	✓ (if allowed by herbarium agreement)	✓ (if allowed by herbarium agreement and depositor)
Specimens properly preserved in herbarium environment.	✓	✓	✓	✓	
Use information is preserved if lost by depositor or collaborator (misplacement, death, cultural erosion)	✓	✓			✓ (if allowed by herbarium agreement and depositor)
All field data accessible, so species identifications can be verified by other researchers	✓				

Features	Scenario A	Scenario B	Scenario C	Scenario D	Scenario E
Collaborators' IPR , informed consent form, and agreement with host country are honored		✓ (partially)	✓ (partially)	✓	✓
Collections and uses not easily discovered through database or herbarium sheets			✓	✓	✓
If hardcopy of herbarium label is lost, digital version survives, and vice versa	✓	✓			
Making general botanical collections of the geographic area ensures that the depositor is much more informed on the general flora of the area, and is better able to identify future collections			✓		

Features	Scenario A	Scenario B	Scenario C	Scenario D	Scenario E
Guarantee of label information not being databased if family is revised or monographed				✓	✓
Depositors have control over what information will be revealed to visitors				✓ (partial- depends on herbarium staff)	✓
Cons:					
Collections easily located and uses easily discovered through database or herbarium sheets	✓				
Possibility that plants will be easily picked out in the herbarium and their collector and use inferred if collections are from a geographic area from which the herbarium has few other collections	✓	✓			

Features	Scenario A	Scenario B	Scenario C	Scenario D	Scenario E
Plants not properly preserved in herbarium environment					✓
Collaborators' IPR and in some cases informed consent form and agreement with host country are violated, and depositors may not be allowed to continue work in collaborators' country or homes	✓				
Location data cannot be recovered for conservation or floristic purposes		✓	✓	✓	✓
Difficult to use specimens for taxonomic research				✓	✓

Features	Scenario A	Scenario B	Scenario C	Scenario D	Scenario E
A potential conservation risk if high demand for the resource is generated, as collectors can easily find the plant in the field	✓	✓			
Use information is lost if depositors lose it and/or collaborators forget information or die , or depositors lose information, retire, or leave academia		✓	✓	✓ (unless specified otherwise in depositor's will or herbarium agreement)	✓ (unless specified otherwise in depositor's will)
Field data not accessible, so species identifications cannot be verified by other researchers		✓	✓	✓	✓

Features	Scenario A	Scenario B	Scenario C	Scenario D	Scenario E
Families may be databased or monographed, no guarantee that collections' label information will not be re-entered and then plants can be easily connected to depositors' project and uses, especially if that geographic region is rarely collected.	✓	✓	✓		
If hardcopy of herbarium label is lost, digital version inaccessible, and vice versa		✓	✓	✓	
Creating haystack collections can occupy a large percentage of the depositor's valuable time, money, and export luggage weight, which is especially difficult in remote locations.			✓		

Features	Scenario A	Scenario B	Scenario C	Scenario D	Scenario E
Depositors must have full trust in herbarium staff to not reveal any information to visitors	✓	✓	✓		

Scenario A: Deposit collections as standard herbarium specimens with all field data, including uses fully visible, and provide full digital access. This is the traditional method of treating voucher specimens that is currently used in most major herbaria worldwide. This scenario has the most support for furthering research in ecology, floristics, taxonomy, and biogeography, but has the worst features for safeguarding IPR or honoring many ethnobotanical research agreements that require confidentiality. This is suitable and acceptable only for collections with a small percentage of ethnobotanical specimens containing IP, and collections from geographic areas where many other non-ethnobotanical specimens have already been deposited in the depositors' herbaria. Even so, there are no safeguards against violation of confidentiality or consequent barriers to future work on that site.

Scenario B: Deposit IP-sensitive collections in an herbarium with database fields for uses and exact location locked, pop-up windows with PICs and research agreements for appropriate specimens, and modified screening of visitors where researchers are introduced by their home institution and they sign a visiting herbarium agreement that forbids publishing use data. This is the minimum set of policies that herbaria can enact to ensure ethnobotanical collections are protected. Some of these policies are being used in a few herbaria with sensitive collections such as specimens of ethnobotanical or CITES-listed species. The implementation of these policies in some herbaria shows that herbaria staff are concerned with protecting sensitive information, but perhaps are unaware of how ethnobotanical information and collections could be abused. It affords some protection to the confidential information of uses and the possibility of connecting species names with

collectors and uses, but especially in cases where there are few other collections in the herbarium from the same geographic area, the use(s) could be easily inferred from the project or collector. More stringent screening of herbarium visitors serves to keep out potentially non-legitimate visitors, and there will at least be legal recourse if uses from physical labels are published, breaking PICs.

Scenario C: Deposit collections in an herbarium with the collectors' names, uses, and exact location removed from physical label and hidden as fields in the database; otherwise full digital access; no herbarium confidentiality agreement; and depositor makes general botanical collections from the same area to create a haystack to hide the ethnobotanical data. This scenario provides some protection from inferring uses of collections from rarely collected areas, but it is not something we have seen put into practice because of the necessary extra work of making many general (non-ethnobotanical) collections while in the field. Often ethnobotanical field work is severely time- and resource-limited, so having to create a haystack of many general collections to sufficiently hide a modest ethnobotanical collection of a hundred specimens and then carry them out of the field site on foot or in small boats, cars, or planes, may make this extra collection prohibitively expensive. In cases where ethnobotanical collections may be part of a larger general botanical project in an area, this scenario may be much more feasible as general collections will be made regardless.

Scenario D: Deposit collections in an herbarium with the collectors' names, uses, and exact location removed from physical label and hidden as fields in the database; otherwise full digital access; herbarium confidentiality agreement in effect. This scenario, the same as Scenario C with the addition of the agreement, has many of the same

protections as the previous scenario of projects in rarely collected areas, but requires perhaps less work on the depositors' part. This is because the haystack collections to hide the ethnobotanical collections can be allowed to grow over time without extra collection effort and the collections will be under restricted access, protected and not viewable, until the pre-set time given in the herbarium agreement. This scenario does however create much more work, new practices, and responsibility for the herbarium staff, when herbaria are already a severely underfunded and understaffed area of many botanical gardens (Cholewa 1997; Barkworth 2002). These changes may have to be introduced slowly to the herbarium to ease the transition and we hope to continue discussions with our and other herbarium staff to come to a satisfactory compromise of extra work and IPR protection. These measures should be implemented only for new accessions, as herbaria cannot be expected or asked to implement them retroactively.

Scenario E: Keep collections fully in a restricted-access or personal herbarium with collectors' names, uses, and exact location all appearing on specimen label; database access at discretion of depositor; herbarium confidentiality agreement in effect if in a restricted-access herbarium; and complete field data given out only under depositor's supervision for species identification verification or according to depositor's will. This is the most protectionist of all the scenarios mentioned, allowing the depositor full control of which parts of which specimens and which data are seen by whom. Although this extreme protectionism may put the health of the collections at risk if specimens are not kept in climate-controlled conditions, as it is often hard to maintain proper conditions for plant specimen preservation in a space not designed for it, this is how many of the largest herbaria of the world (including NY, BP, and OS) started – with an ardent researcher's

personal herbarium (Parnell, Szujko-Lacza et al. 1987; Holmgren and Holmgren 1998, http://sciweb.nybg.org/science2/libr/finding_guide/scweb5.asp). The fact that specimens that spent some time in researchers' personal herbaria are still in use shows that, if cared for properly, these specimens can last the tests of time and use. This scenario is most likely only feasible for someone who has the space, time and stability to set aside at home or in their laboratory to maintain the personal herbarium, and they must be sure to include instructions in their last will and testament on the destiny of the herbarium and the information contained therein so all this valuable information does not disappear with them. Depending on restrictions imposed by the depositor, this scenario could severely restrict taxonomic, biogeographic, and floristic work on these specimens, remaining inaccessible for years if not decades.

6.7. Discussion

In our own work, we have utilized several of these scenarios, from straight depositing of ethnobotanical vouchers in unprotected herbaria without listing uses to keeping specimens completely out of unprotected herbaria until a confidentiality agreement can be reached with the herbaria and still keeping collector, use, and location information off of labels and locked in databases to which only the depositor and herbarium staff have access. We believe that ethnobotanical collections can still be of value to scientific research even with some of these restrictions.

The schemes we have surveyed show differing degrees of efficacy in protecting collaborators' IPR, but botanists are usually required to leave duplicates of specimens in a herbarium of the field site or the host country. If this is not required, it is at least common practice and courtesy (Alexiades 1996). Implementing an agreement as

discussed above in a host country with a different legal system and fewer human and informatics resources in the herbarium may be quite an onerous task. One may unfairly hope that the herbarium will not be put online or databased in the near future, or hope that people wishing to find the intellectual property attached to the specimens will not bother to search these distant herbaria. This hope may work in some cases, but again, it is the depositors' duty to help protect the intellectual property they have promised to protect in their research agreements and PICs, and to do their best to explain these issues to the host country herbarium staff, and bring up potential conflicts that might not currently be under discussion. This is especially true if the country is a signatory to the Convention on Biological Diversity as revealing the uses of these specimens may violate sections of the Convention. If use data is left off of specimen labels and host country herbaria do not have herbarium computer databases, this could present a problem as the host country will not have any record of uses exactly where this record is most needed. However, the host country could be given access to the use data, if these herbaria are willing to sign herbarium agreements that restrict access to ethnobotanical specimens.

6.8. Future Research

One way to have it both ways for information that one wants to keep secret yet still use is to borrow methods from computer science and public key cryptography called "zero-knowledge proofs" or "selective disclosure" (Feige, Fiat et al. 1987), where digital signatures can prove that you have certain knowledge without revealing it by performing operations on the secret information that someone without the information could not possibly perform. One can make the analogy of these proofs that the depositor, "Bob", wants to prove to another researcher, "Sue", that he has a key that unlocks a door without

revealing to Sue what that key looks like. To do this, Bob puts behind a wall a locked door that can only be opened by the key, and he can only get to one side or the other of the wall by unlocking the door with the key. Sue then asks Bob to come out a random side of the wall, making sure to lock the door behind him. If she repeats this request many times, she can prove to herself beyond a reasonable probability, that Bob has the key to the door without ever seeing the door. The same procedure can be done with the "key" of the taxonomic identity of a particular plant that Bob is trying to prove to Sue he knows. With this selective disclosure, herbarium staff who follow the herbarium confidentiality agreement or computer programs with the correct data could perform calculations on the restricted data (e.g. 10 times the number of petals plus 40 times the average leaf length divided by the number of ovaries), so that someone viewing the results of many of these calculations would know with high certainty that this plant is one of the species it is purported to be. As with lock-and-key controlled physical specimens, there is a danger that with the death of the depositor, a change to an incompatible computer system, or a change in herbarium staff that some of this inaccessible information will be lost to science. Points in the depositor's will can alleviate some of the risk from the depositor's passing, but the other two risks require more research and assurances that information is passed on along with changes in the system.

We could also borrow ideas from double blind medical experiments where herbarium staff with no knowledge of uses or the depositor's project present to visiting researchers or those researchers not involved with the collections being examined the true ethnobotanical collections hidden among many false collections which act as the placebos of medical experiments. In this case, if there were no haystack collections to

hide rarely collected areas and the locations of all collections presented are masked or blurred on the herbarium sheet, the "false" or placebo collections gathered from the collections in the same herbarium from nearby geographic areas would act as a sort of haystack collection. Clearly, these are just nascent procedures that need to be better described as part of future research, but ethnobotany is not the first science to have to manage the practical use of confidential information, so we should build on the work of these other sciences.

6.9. Conclusion

We have delineated a set of scenarios with different methods for depositing ethnobotanical voucher specimens in herbaria to try to reconcile the seemingly opposing goals of protecting intellectual property and furthering scientific research and openness. Clearly none of these scenarios is perfect for every researcher or situation, and each researcher must choose, preferably with his or her collaborators and the herbaria involved, a scenario that best meets their shared needs. There must be a continuing dialogue among all groups of stakeholders who use herbarium specimens and data – ethnobotanical, conservation, systematics, and floristic researchers; collaborators; host country regulation organizations; herbaria staff; scientific journals; and funding agencies – to determine the best set of scenarios and methods to merge scientific research and IP protection. We hope to have advanced the dialogue with this work and look forward to comments and additional ideas from the larger ethnobotanical community, researchers and collaborators.

Chapter 7. Integration of Data and Analysis

Now that all the information on medicinal plants of the Asháninka and Malinké has been collected and the relationships of the plants, diseases, and cultures have been determined, the data can finally be synthesized to estimate the potential disease-treating efficacy for each plant species, and this potential can be compared to independent ratings of efficacy derived from bioassays on the plant extracts. Dealing with uncertainty of species identifications is an integral part of ethnobotanical collections, where plants are often collected non-fertile or merely as roots in a market, and I have attempted to design the relational efficacy system to take these uncertainties into account, giving error ranges to the final potential efficacy that are still useful for ranking the species. Given the large amounts of data and computation necessary for this system, every attempt has been made to make the processing algorithm as efficient as possible and allow it to take advantage of multiprocessor computer systems by dividing out the processing into independent parts when the appropriate programs can handle multiprocessing.

A prerequisite to this hypothesis, the idea that two distant cultures will focus on similar taxa for their medicinal plants and therefore the medicinal flora of these cultures will prove to be more similar than their general flora, is tested using approximate randomization statistics on cross-cultural field data of the plants used by the Malinké of Mali and the Asháninka of Peru to treat the diseases malaria, African sleeping sickness, Chagas' disease, leishmaniasis, diabetes, eczema, asthma, and uterine fibroids. In this case, the similarity of the medicinal floras is found to be significantly greater than the

similarity of the general floras, but only when the diseases in question are grouped into the categories of parasitic and autoimmune diseases

7.1. A comparative case study using families and genera

The same data used to test the main hypothesis of this research of the Asháninka and the Malinké and the eight diseases were used to test the prerequisite hypothesis that the medicinal flora of two distant cultures are significantly more similar with each other than the general flora of the two cultures areas are similar.

7.1.1. Cultures

In choosing cultures for this study, the more remote and more recently contacted a group is the better, as they will have less chance of introduced plant uses. Although the cultures selected must be as distant as possible, it is also necessary that they share some elements of their floras. The areas compared need not have the exact same species, but if they share some genera or families it will make determining the plants' relatedness values easier. In comparing the flora of Peruvian Amazon and the dry savannas of Mali, Doug Daly and I have found that 21% of their genera overall and 30% of the medicinal plant genera of the Mali savannas are also found in Southwest Amazon area of Peru (Adjanohoun, Aké Assi et al. 1980; Boudet and Lebrun 1986; Malgras 1992; Daly, Foster et al. 1996). Therefore, although at first glance it might seem ludicrous to try to compare the medicinal floras of such divergent habitats as a rainforest and a savanna, this flora overlap percentage is high enough to make a more in-depth comparison of the medicinal plants of the two areas. The fact that the cultures of the Peruvian Amazon and the Malian savannas are so distantly related that they are very unlikely to have communicated

medicinal plant uses to each other also raises the probability that any related plants used by both of them to treat related diseases are independent discoveries, which strengthens the quantitative model. Lewis et al. (1988) have suggested the same idea that use of similar medicinal plants by nearby Jívaro communities in the Peruvian Amazon corroborates those uses and the medicinal efficacy of the plants.

7.1.2. Diseases

Testing the model requires finding diseases that occur in Peru and/or Mali and are related and have the same underlying cause in the body. Using these criteria, malaria, African sleeping sickness, Chagas' disease, leishmaniasis, diabetes, eczema, asthma, and uterine fibroids were selected. Diabetes, eczema, and asthma were picked as all three are autoimmune diseases, with the latter two more closely associated in the "autoimmune triad." The third member of this autoimmune triad is hay fever, which was not included in this study because it is not thought to be common in the indigenous groups selected. If one culture treats asthma with a certain plant and another distant culture treats eczema with the same plant, although these diseases seem superficially very different, they are considered closely related autoimmune diseases by Western medicine (Isselbacher 1980)and therefore could be treated by the same plant chemicals acting on the underlying mechanism of the immune system. Thus, these two distant uses of the same plant for eczema and asthma can be considered similar uses, raising the estimate of the efficacy of this plant.

Malaria, leishmaniasis, African sleeping sickness, and Chagas' disease are all caused by protozoan parasite infection, the latter three more specifically by a trypanosome (family Trypanosomatidae), and the latter two in the same genus *Trypanosoma* (Benson,

Karsch-Mizrachi et al. 2000; Federhen, Harrison et al. 2000; Wheeler, Chappey et al. 2000), thereby exhibiting different degrees of evolutionary proximity. Studying uterine fibroids allows comparison of my work in Peru with ethnobotanical research that has been done on this disease in Chile and among Dominican and Chinese groups in New York City by the Rosenthal Center for Complementary and Alternative Medicine at the Columbia-Presbyterian Medical Center (CPMC) (Balick, Kronenberg et al. 2000). Of the diseases mentioned, however, uterine fibroids is the most difficult to study solely via interviews, as it has few outwardly apparent symptoms.

7.1.3. Methodology

To accomplish this cross-cultural study, ethnobotanical data was gathered in structured interviews and plant collections with healers of the indigenous Asháninka of Paititi village in Ucayali, Peru and the Malinké of Kita in 2003 and 2004, focusing on plants used to treat malaria, African sleeping sickness, Chagas' disease, leishmaniasis, asthma, eczema, diabetes, and uterine fibroids. The families and genera of the general flora have been determined for Kita, Mali from Arbonnier (2002) and for Paititi, Peru from Daly and Silvera (2007) which covers the state of Acre, Brazil, which is also in the Southwest Amazon floristic zone where Paititi is found. However, because of the prior informed consent agreements with my collaborators, neither species, genus, nor family names are given, as has become fairly common practice in recent medical ethnobotanical research (Milliken and Albert 1996; Milliken 1997; Alexiades 1999; Lewis, Lamas et al. 1999; Laird 2002; Lawrence, Phillips et al. 2005; Lewis 2006). This thesis shows that despite not revealing plant names, there is interesting work that can be published with this data that advances the science of ethnobotany. A summary of the field collections and overlap

percentages (the number of each taxa that were found in both field sites divided by the number of total taxa found when the taxa from both field sites are combined, or intersection of the taxa / union of the taxa) is given in Table 7.1.

7.1.4. Analysis

These original data of medicinal plant use in Peru and Mali comprise an accurate database of shared plant uses that can be analyzed using the described quantitative system and compared with data gathered on the same diseases from other areas of the world. Further literature research on the collected plant species as well as chemical analysis will be necessary to measure the relatedness of the plants, cultures, and diseases involved in the study using dated phylogenies and to calibrate the quantitative system using well-studied medicinal plants.

Part of the hypothesis of this work is that the medicinal floras of different cultures are taxonomically more similar than the general floras of the geographic areas where the cultures are located. This hypothesis is relatively easy to test using contingency tables of the overlapping medicinal and general floras of the two cultures, the odds-ratio or Jaccard similarity index of these tables, and approximate randomization statistic techniques, a technique that recalculates statistics thousands of times while resampling from collected data (Manly 2006). Approximate randomization or approximate randomization statistics techniques are in the same family of numerical approaches to statistical analysis that sample without replacement as Monte Carlo methods, essentially reshuffling the labels or experimental group on each collected datum. Monte Carlo methods differ, however, in that they create new data based on theoretical probability distributions of the system under study.

Contingency tables are used in statistical comparisons of counts of occurrences of outcomes in several populations with different experimental groups, most often in two by two tables. The odds ratio (OR) statistic is calculated as

$$\frac{N_{\textit{present in Peru, present in Mali}}\,N_{\textit{absent in Peru, absent in Mali}}}{N_{\textit{present in Peru, absent in Mali}}\,N_{\textit{absent in Peru, present in Mali}}} \qquad \text{Eq. 15}$$

where each *N* is the count from one of the four central squares of the contingency table, which would be

$$\frac{57 \bullet 266}{136 \bullet 6} = 18.581$$

in the case of Table 7.2a, comparing the families of the general flora of Peru and Mali, in the context of the 465 flowering plant families in the APG system. In these tables, the OR explains that an outcome is a certain amount more likely for one experimental group versus another, e.g. that if a family is present in the Peruvian Amazon flora it is 18.581 times more likely to be present than absent in the Malian savanna flora in the case shown in Table 2. The Jaccard similarity index is a measure of the overlap of the two sets and is calculated by the intersection of the two sets divided by the union of the two sets, i.e.

$$\frac{N_{\textit{present in Peru , present in Mali}}}{N_{\textit{present in Peru , present in Mali}} + N_{\textit{present in Peru , absent in Mali}} + N_{\textit{absent in Peru , present in Mali}}} \qquad \text{Eq. 16}$$

It should be noted that the Jaccard similarity calculation does not use the number of taxa absent from both sets (e.g. 266 in Table 2), while the odds ratio calculation does.

The null hypothesis H_o here that we wish to reject is that just by chance the two cultures have wound up with similar medicinal floras merely by selecting from similar general floras, i.e., the odds-ratio or Jaccard similarity index of the medicinal flora is no

greater than the odds-ratio or Jaccard index of the general flora than chance would allow. By testing the null hypothesis that medicinal floras have the same or lower similarity than the general floras, approximate randomization statistics will here allow the calculation of the statistical significance of the difference in the similarity of the medicinal and general taxa contingency tables, a significance whose calculation is not well defined using standard exact statistical techniques. To perform this approximate randomization without replacement, the numbers in the two contingency tables' categories are reshuffled thousands of times, keeping the row totals the same and recalculating the similarity difference between the tables for each reshuffle. The significance p then is computed as N_g/N_t where N_g is the number of reshuffles where the medicinal floras' similarity is higher than the similarity of the general floras and N_t is the total number of reshuffles.

To calculate these similarities, contingency tables were created of the families and genera found in the medicinal and general flora of the Southwest Amazon area of Peru and in Mali, using Angiosperm Phylogeny Group (Stevens 2001) designations and total worldwide counts for families and genera. Approximate randomization statistics were calculated 10,000 times using a 500 MHz Apple PowerBook Pismo running Resampling Stats version 4.0 (Simon 1997), with each table comparison run taking several minutes to complete. The resulting list of contingency tables, ORs, similarity values, and significance for families and genera for each disease and disease category as well as the general flora are given in Tables 7.2-21.

When these contingency tables comparing the general flora of Mali and the Southwest Amazon area of Peru with the medicinal plants of the Asháninka and Malinké are examined, it is clear that there is significant similarity within the general flora and the

medicinal flora from the G test, and that the medicinal flora has a significantly higher similarity between the two areas than the general floras' similarity. It can be seen from these tables that in all cases where the medicinal flora similarity or OR is greater than those of the general flora (numbers in italics), it is statistically significant. This allows us to accept our prerequisite hypothesis H_1, but if we look more deeply into the disease categories and the difference between the contingency tables using the genus and family, the results become more complicated and less consistent. There seem to be more significant results of higher similarity in medicinal plants for individual diseases and categories than in the general flora when we analyze the presence/absence contingency tables of genera rather than families, as shown in Table 7.2 and the summary in Table 7.3. There is also variation in significance when looking at different disease ranks, i.e., individual disease vs. disease categories such as parasitic or autoimmune diseases.

Table 7.4 shows the distribution of plant taxa used to treat different diseases and categories in Peru, Mali, and in both cultures. In the combined medicinal flora of Peru and Mali the largest percentage of plant taxa are used to treat autoimmune diseases (20.60% of families present, 5.91% of genera present), and within in this category, diabetes has the highest representation (16.08% of families present, 3.23% of genera present). Parasitic diseases are the second-highest-represented category (17.59% of families present, 4.57% of genera present), with malaria best represented within this category (20.60% of families present, 5.91% of genera present). For the Peruvian medicinal flora, this same pattern of the predominance of autoimmune diseases and diabetes within that continues, but within the second-highest-represented category of parasitic diseases, leishmaniasis predominates rather than malaria (5.70% of families

present, 1.14% of genera present). This may be due to leishmaniasis being native to South America or at least present in South America much longer than malaria, a relatively recent introduction (Noyes, Morrison et al. 2000). In Mali, autoimmune diseases are still the predominant category (41.27% of families present, 17.78% of genera present), but within this category, eczema rather than diabetes is the best represented (5.70% of families present, 1.14% of genera present), most likely because of the drier environmental conditions that often bring on eczema and fewer of the high-starch foods such as yuca (*Manihot esculenta* Crantz [Euphorbiaceae]) that can exacerbate diabetes. The parasitic disease category in Mali is again the second best represented, and within that malaria is best represented (30.16% of families present, 14.22% of genera present) as opposed to leishmaniasis in Peru, most likely due to malaria's origins in Africa (Joy, Feng et al. 2003). It should be noted that these differences are statistically significant only when considering the combined medicinal floras of Peru and Mali and the disease categories, not individual diseases.

Unfortunately, I cannot include further analysis such as family or genera distributions as there are several families with only one or a few species present in both Peru and Mali, so merely naming the family would reveal the species used for a particular disease and break my confidentiality agreement not to publish previously unpublished species uses.

This confirmation of the hypothesis with disease categories and certain taxa ranks but with inconsistencies when analyzing individual diseases or other taxa ranks shows that we need to move away from analyzing plant and disease data in these somewhat subjective groupings as they will give us unverifiable results depending on what level the data is analyzed (e.g. species, genus, or family; individual disease or disease category)

and which published groupings we used (e.g. the old Malvaceae *sensu stricto* or the new Malvaceae *sensu lato* which includes the old Malvaceae, Sterculiaceae, Tiliaceae, and Bombacaceae (Stevens 2001)). Instead, we need to put into practice a system that uses more universal notions of groupings that are not quite so subjective and often changing. Using phylogenies to measure evolutionary distance, phytochemistry to gauge the similarity of disease-treating mechanisms and the compounds in different plants, and cultural genomic phylogenies can give us more robust information about the relations of plants, diseases, and cultures that should give us more consistent results. It is on these systems that the following theoretical quantitative cross-cultural synthesis technique called "relational efficacy" is based.

Table 7.1. A summary of the collections of the medicinal plants of the Asháninka of Peru and the Malinké of Mali, and their overlap percentages.

	Collections	Species	Genera	Families
Peru	86	74	73	39
Mali	90	80	54	41
Both	n/a	3	11	25
Total	176	152	116	55
Overlap (Both/Total)		2%	10%	46%

Table 7.2. The overlap of general and medicinal families and genera found in the Southwest Amazon area of Peru and in Mali, as contingency tables, with the significance of each table, and for all but Table 7.2a and Table 7.2b (the general flora comparison), whether the odds ratio and Jaccard similarity is significantly greater than the odds ratio and Jaccard similarity for the general flora. Odds ratios and Jaccard similarities that are greater than the corresponding values for the general flora (Table 7.2a and Table 7.2b) are italicized.

Families

A. General flora family comparison

		Peru		
		present	absent	
Mali	present	57	6	63
	absent	136	266	402
		193	272	465

Odds ratio: 18.581 p value: 1.6849×10^{-18}

Jaccard similarity: 0.286

Genera

B. General flora genus comparison

		Peru		
		present	absent	
Mali	present	93	132	225
	absent	1045	11996	13041
		1138	12128	13266

Odds ratio: 8.088 p value: 3.2759×10^{-41}

Jaccard similarity: 0.073

C. All medicinal family comparison

D. All medicinal genus comparison

		Peru		
		present	absent	
Mali	present	25	14	39
	absent	16	410	426
		41	424	465

Odds ratio: 45.759 p value: 2.291×10^{-21}

p value (OR > general OR): 0.057994

Jaccard similarity: 0.455 p (Jaccard > general): 0.0001

		Peru		
		present	absent	
Mali	present	11	43	54
	absent	62	13150	13212
		73	13193	13266

Odds ratio: 54.257 p value: 3.4275×10^{-15}

p value (OR > general OR): 0.027597

Jaccard similarity: 0.095 p (Jaccard > general): 0.0001

E. Parasitic family comparison

	Peru	
	present	absent

F. Parasitic genus comparison

	Peru	
	present	absent

Mali	present	13	10	23
	absent	11	431	442
		24	441	465

Odds ratio: 50.936 p value: 1.5238×10^{-13}

p value (OR > general OR): 0.091491

Jaccard similarity: 0.382 p (Jaccard > general): 0.0001

Mali	present	7	31	38
	absent	20	13208	13228
		27	13239	13266

Odds ratio: 149.123 p value: 4.2180×10^{-13}

p value (OR > general OR): 0.011299

Jaccard similarity: 0.121 p (Jaccard > general): 0.0001

G. Trypanosomal family comparison

		Peru		
		present	absent	
Mali	present	7	6	13
	absent	8	444	452

H. Trypanosomal genus comparison

		Peru		
		present	absent	
Mali	present	3	12	15
	absent	15	13236	13251

	15	450	465

	18	13248	13266

Odds ratio:	64.750	p value:	5.0237×10^{-09}
	p value (OR > general OR):		0.10409
Jaccard similarity:	0.333	p (Jaccard > general):	0.0001

Odds ratio:	220.600	p value:	5.2761×10^{-07}
	p value (OR > general OR):		0.018798
Jaccard similarity:	0.100	p (Jaccard > general):	0.0001

I. Malaria family comparison

		Peru		
		present	absent	
Mali	present	4	15	19
	absent	5	441	446
		9	456	465

J. Malaria genus comparison

		Peru		
		present	absent	
Mali	present	2	30	32
	absent	7	13227	13234
		9	13257	13266

Odds ratio:	23.520	p value:	0.00014609	Odds ratio:	125.971	p value:	0.00012384
	p value (OR > general OR):		0.31347		p value (OR > general OR):		0.022198
Jaccard similarity:	0.167	p (Jaccard > general):	1	Jaccard similarity:	0.051	p (Jaccard > general):	1

K. Autoimmune family comparison

		Peru		
		present	absent	
Mali	present	13	13	26
	absent	15	424	439
		28	437	465

Odds ratio:	28.267	p value: 2.1699×10^{-11}
	p value (OR > general OR):	0.27887

L. Autoimmune genus comparison

		Peru		
		present	absent	
Mali	present	4	36	40
	absent	35	13191	13226
		39	13227	13266

Odds ratio:	41.876	p value: 4.0460×10^{-06}
	p value (OR > general OR):	0.10629

Jaccard similarity:	0.317	p (Jaccard > general):	0.0001

M. Eczema family comparison

		Peru		
		present	absent	
Mali	present	4	13	17
	absent	5	443	448
		9	456	465

Odds ratio:	27.262	p value:	8.7749×10^{-05}
	p value (OR > general OR):		0.28537

Jaccard similarity:	0.182	p (Jaccard > general):	1

Jaccard similarity:	0.053	p (Jaccard > general):	1

N. Eczema genus comparison

		Peru		
		present	absent	
Mali	present	1	19	20
	absent	9	13237	13246
		10	13256	13266

Odds ratio:	77.409	p value:	0.010375
	p value (OR > general OR):		0.014399

Jaccard similarity:	0.034	p (Jaccard > general):	1

O. Diabetes family comparison

		Peru		
		present	absent	
Mali	present	2	13	15
	absent	17	433	450
		19	446	465

Odds ratio: 3.919 p value: 0.13742

p value (OR > general OR): 1

Jaccard similarity: 0.063 p (Jaccard > general): 1

P. Diabetes genus comparison

		Peru		
		present	absent	
Mali	present	0	15	15
	absent	22	13229	13251
		22	13244	13266

Odds ratio: 0.000 p value: 1

p value (OR > general OR): 1

Jaccard similarity: 0.000 p (Jaccard > general): 1

Q. Asthma family comparison

		Peru		
		present	absent	
Mali	present	4	9	13
	absent	8	444	452
		12	453	465

Odds ratio:	24.667	p value:	0.00010722
	p value (OR > general OR):		0.29017
Jaccard similarity:	0.190	p (Jaccard > general):	1

R. Asthma genus comparison

		Peru		
		present	absent	
Mali	present	1	16	17
	absent	11	13238	13249
		12	13254	13266

Odds ratio:	75.216	p value:	0.010655
	p value (OR > general OR):		0.014599
Jaccard similarity:	0.036	p (Jaccard > general):	1

S. Fibroids family comparison

T. Fibroids genus comparison

		Peru		
		present	absent	
Mali	present	6	14	20
	absent	6	439	445
		12	453	465

Odds ratio: 31.357 p value: 1.2956×10^{-06}

p value (OR > general OR): 0.33487

Jaccard similarity: 0.231 p (Jaccard > general): 1

		Peru		
		present	absent	
Mali	present	3	23	26
	absent	10	13230	13240
		13	13253	13266

Odds ratio: 172.565 p value: 1.0923×10^{-06}

p value (OR > general OR): 0.024098

Jaccard similarity: 0.083 p (Jaccard > general): 0.0001

Table 7.3. A summary of the significance of the Jaccard similarity (Sim.) and odds ratio (OR) for the families and genera of plants used to treat each disease category between the Asháninka and Malinké being higher than the general flora similarity and odds ratio between the Asháninka and Malinké (° : $p < 0.1$, • : $p < 0.05$, •••• : $p < 0.0001$, NS: not significant, $p > 0.1$)

	Families		Genera	
Diseases	Sim.	OR	Sim.	OR
All studied	••••	°	••••	•
Parasitic	••••	°	••••	•
Trypanosomal	••••	NS	••••	•
Malaria	NS	NS	NS	•
Autoimmune	••••	NS	NS	NS
Eczema	NS	NS	NS	•
Diabetes	NS	NS	NS	NS
Asthma	NS	NS	NS	•
Uterine fibroids	NS	NS	••••	•

Table 7.4. The number of plant families and genera used to treat each disease and disease category in Peru, Mali, and the combined medicinal flora of the two areas. Those distributions with a significant difference based on a log-likelihood ratio test (G-test) are marked with a footnote, all other pairwise comparisons of categories or diseases are not significantly different.

	Mali				Peru				Combined			
	Families		Genera		Families		Genera		Families		Genera	
Disease	n	percent of present	n	percent of present	n	percent of present	n	percent of present	n	percent of present	n	percent of present
Parasitic	23	36.51%	38	16.89%	24	12.44%	27	2.37%	35[b]	17.59%	58[c d]	4.57%
Malaria	19	30.16%	32	14.22%	9	4.66%	9	0.79%	25	12.56%	39	3.07%
Trypanosomes[a]	13	20.63%	15	6.67%	15	7.77%	18	1.58%	22	11.06%	30	2.36%
Chagas	0	0.00%	0	0.00%	4	2.07%	5	0.44%	4	2.01%	13	1.02%
Leishmaniasis	0	0.00%	0	0.00%	11	5.70%	13	1.14%	11	5.53%	5	0.39%
Autoimmune	26	41.27%	40	17.78%	28	14.51%	39	3.43%	41	20.60%	75[c]	5.91%
Diabetes	15	23.81%	19	8.44%	19	9.84%	22	1.93%	32	16.08%	41	3.23%
Asthma	13	20.63%	17	7.56%	12	6.22%	12	1.05%	22	11.06%	28	2.20%

Eczema	17	26.98%	20	8.89%	9	4.66%	10	0.88%	23	11.56%	29	2.28%
Fibroids	20	31.75%	26	11.56%	12	6.22%	13	1.14%	26[b]	13.07%	36[d]	2.83%

[a] Trypanosomes includes Chagas disease, leishmaniasis, and African sleeping sickness.

[b] The combined plant families of Peru and Mali used to treat uterine fibroids and parasitic diseases had a significant difference with p = 0.01911

[c] The combined plant genera of Peru and Mali used to treat autoimmune and parasitic diseases had a significant difference with p = 0.03987

[d] The combined plant genera of Peru and Mali used to treat uterine fibroids and parasitic diseases had a significant difference with p = 0.04909

7.2. Programming

The calculations for determining the potential efficacy for each plant species were performed using a combination of Applescript, Excel, and Filemaker Pro. Although Applescript is a fairly slow, interpreted (i.e. not compiled) language that does not allow for parallel processing, this was not so much of an issue given the speed of today's computers, and any loss of time was made up for was made up for by having the infrastructure in place for reading in and manipulating large tables of data.

The tables of distance matrices for species, diseases, and cultures were placed in a spreadsheet file along with the list of species used for each disease. Each species could have multiple possible identifications if its identification was indeterminate. The distance matrix for plant species had to be based solely on the plant families derived from Soltis' (Soltis, Soltis et al. 2000) molecular data and the APG classification since finer resolution phylogenies from which taxa relations could be measured could not be found in the literature. If these measurable phylogenies are published in the future in a format compatible with Soltis' molecular data and in a format where the raw data can be obtained (i.e. not just an image of a tree in a paper, but a standard format Nexus or Hennig file), these can still be integrated into the data. It is a bit shocking to me and several systematists I consulted with (Doug Daly, Paola Pedraza, Damon Little, personal communication, 2008) how few of the published plant phylogenies are readily available as raw data from the authors or online systematic repositories such as TreeBASE (http://www.treebase.org/treebase/), Tree of Life (http://www.tolweb.org), or the Encyclopedia of Life (http://www.eol.org/), since these are experimental results that should be verified by peers and having to meticulously transcribe the data from images in

papers makes this verification prohibitively difficult. I urge systematists to see their results as a tool to be used by other biologists and not just ends in themselves.

Each of the distance matrices for diseases based on parasite phylogeny and PBDT were tested individually to get separate sets of results. The distance matrix for the cultures were derived from Li, et al.'s (2008) molecular data for the Mandinke [sic], Maya, and Karitiana groups, these being the closest ones to the Malinké, Asháninka, and Maya that I'm considering.

These matrices were placed in an Excel spreadsheet and along with the Filemaker database of all the species used to treat each disease in each culture and a list of the species found in each mixture, this was used by the Applescript program to go through each species and sum up the equation

$$P_{s,d,c} = \frac{1}{N_s N_d N_c} \sum_{s',d',c'} \frac{D_{c,c'}}{D_{s,s'} D_{d,d'}} \qquad \text{Eq. 17}$$

for every other species s', disease d', and culture c' by looking up the distance value for each pair s and s', d and d', and c and c' in the respective distance matrix table. During each run, the potential of each species was summed over cultures and diseases to get an overall potential of that species to treat any of the diseases considered.

The Applescript program code to calculate the potential efficacy is as follows:

```
property diseases : {"leishmaniasis", "chagas", "malaria", "sleeping sickness", "diabetes", "asthma", "eczema",
"fibroids"}
property cultures : {"peru", "mali"}
property plantsheet : "P dist"
property diseasesheet : "D dist"
property culturesheet : "C dist"
property plantMin : 1.0E-3
property diseaseMin : 1.0E-3
```

```
property cultureMin : 1.0E-3
-- precompute the row and column for each culture on the pairwise culture distance matrix to save time in the loop
set clist to {}
repeat with ccult in cultures
	set col to 1
	tell application "Microsoft Excel"
		set val to (value of cell 1 of column col of worksheet culturesheet)
		repeat until val is ""
			if val is (ccult as text) then
				exit repeat
			end if
			set col to col + 1
			set val to (value of cell 1 of column col of worksheet culturesheet)
		end repeat
		if val is "" then
			display dialog "Culture " & ccult & " was not found on worksheet " & culturesheet
			return
		end if
		copy col to end of clist
	end tell
end repeat
-- precompute the row and column for each disease on the pairwise culture distance matrix to save time in the loop
set dlist to {}
repeat with disease in diseases
	set col to 1
	tell application "Microsoft Excel"
		set val to (value of cell 1 of column col of worksheet diseasesheet)
		repeat until val is ""
			if val is (disease as text) then
				exit repeat
			end if
```

```
                set col to col + 1
                set val to (value of cell 1 of column col of worksheet diseasesheet)
            end repeat
            if val is "" then
                display dialog "disease " & disease & " was not found on worksheet " & diseasesheet
                return
            end if
            copy col to end of dlist
        end tell
    end repeat
    tell application "FileMaker Pro Advanced"
        set rslt to ""
        set nCultures to count of (cultures)
        set nDiseases to count of diseases
        tell layout "Species sans Pics"
            show every record
            set nSpecies to count of records

            -- Species
            repeat with cSpeciesRec from 144 to nSpecies
                set speciesRec to (a reference to record cSpeciesRec)
                set spInd to (cell "Soltis index" of speciesRec) as integer
                if spInd is "" then exit repeat -- this species is not in the distance matrix
                set species to cell "Species" of speciesRec

                -- Disease
                repeat with cDisease from 1 to nDiseases
                    set disInd to item cDisease of dlist as integer
                    set disease to item cDisease of diseases
                    -- for each species we need to cycle through the cultures to add up the result
                    set speciesPotCSum to 0
```

```
-- Culture
repeat with cCulture from 1 to nCultures
	set cultInd to item cCulture of clist as integer
	set culture to item cCulture of cultures

	set speciesPot to 0

	-- Disease prime
	repeat with cDiseaseP from 1 to nDiseases
		set disPInd to item cDiseaseP of dlist as integer
		set diseaseP to item cDiseaseP of diseases
		if diseaseP = disease then
			set disDist to diseaseMin
		else
			tell application "Microsoft Excel"
				set disDist to (value of cell disInd of column disPInd of worksheet diseasesheet)
			end tell
		end if

		-- Culture prime
		repeat with cCultureP from 1 to nCultures
			set cultPInd to item cCultureP of clist as integer
			set cultureP to item cCultureP of cultures

			try -- is this diseaseP treated in this cultureP? If not, we can save checking all the species for it
				set found to (records whose cell "Healers::Country" = cultureP and cell "Disease interviews::Disease described" = diseaseP)
				if cultureP = culture then
```

```
                                        set cultDist to cultureMin
                                    else
                                        tell application "Microsoft
Excel"
                                            set cultDist to
(value of cell cultInd of column cultPInd of worksheet culturesheet)
                                        end tell
                                    end if


                                    -- Species prime, start counting from
here
                                    repeat with cSpeciesRecP from 1 to
nSpecies
                                        set speciesRecP to (a
reference to record cSpeciesRecP)
                                        set speciesP to cell "Species"
of speciesRecP


                                        try -- is this speciesP used in
this cultureP to treat this diseaseP?
                                            set found to
(records whose cell "Species" = speciesP and cell "Healers::Country" = cultureP and cell "Disease interviews::Disease
described" = diseaseP)
                                            --set rslt to rslt &
species & "\n"
                                            if speciesP =
species then
                                                set
specDist to plantMin
                                            else
```

```
                                                                                set
spPInd to (cell "Soltis index" of speciesRecP) as integer
                                                                                if spPInd
is "" then exit repeat -- this species is not in the distance matrix
                                                                                tell
application "Microsoft Excel"

            set specDist to (value of cell spInd of column spPInd of worksheet plantsheet)
                                                                                end tell
                                                                            end if

                                                                            set speciesPot to
speciesPot + cultDist / (disDist * specDist)
                                                                            log speciesPot
                                                                        end try
                                                                    end repeat -- cSpeciesRecP
                                                                end try
                                                            end repeat -- cultureP
                                                        end repeat -- diseaseP
                                                        set speciesPotCSum to speciesPotCSum + speciesPot
                                                        log "speciesPotCSum = " & speciesPotCSum


                                                    end repeat -- culture

                                                    -- normalize by species disease potential Psd by number of cultures, and
Nc*Nd*Ns
                                                    set speciesPotCNorm to speciesPotCSum / (nCultures * nCultures * nDiseases
* nSpecies)
                                                    set cellName to (disease & " P")
                                                    set cell cellName of speciesRec to speciesPotCNorm
```

```
                end repeat -- disease
            end repeat -- cSpeciesRec
        end tell
        return rslt & speciesPotCNorm & " " & speciesPotCSum & " " & nCultures
    end tell
```

After all the runs, average and standard error statistics were gathered on all the calculated potentials for each species to treat each disease in each culture $P_{s,d,c}$, for each species to treat each disease in any culture $P_{s,d}$, for each species to treat any disease in any culture P_{s}, for each culture to treat any disease $P_{d,c}$, and the overall potential of the research project $P_{d,c}$, which can be used to compare different studies' efficacy. The calculations were done once on just the 69 plants used to treat the parasitic diseases and then a second time on the 146 plants used to treat all eight diseases. The calculated potentials for the plants with bioassay data for the latter case are shown in Table 7.5.

The four-disease calculation took twelve hours and the eight-disease calculation took nine days on a dual-core 2.16 GHz MacBook laptop computer. This is prohibitively long to allow experimentation with the relational efficacy model equation, using approximate randomization statistics to allow for identification uncertainty where this calculation must be run hundreds of times, and dealing with mixtures and synergy where more complex equations and permutations must be evaluated. This slowness is probably due to Applescript's general slowness as an interpreted language and more significantly due to all the interprocess communication that must occur between the script, Excel, and Filemaker Pro, which means programs must be swapped into memory from disk, causing many delays. There was an apparent memory leak in Filemaker Pro that caused it to

balloon from a starting resident memory size of 700 megabytes to more than 3000 megabytes as the script ran. With only 1024 megabytes of physical memory on the computer, this causes constant memory swapping and processes progressing at an unusable glacial pace, so the script and Filemaker Pro had to be periodically restarted during the calculations to recover this memory. The calculation program needs to be rewritten in another language such as Python, Java, or Objective C, and all the data from the Filemaker Pro relational database and the distance matrices from Excel must be read into the program at the outset so that the constant interprocess communication inside the loop that is executed millions of times is not necessary. Without optimizations like checking if a disease is treated in a particular culture at all to avoid calculations on any species in that culture for that disease, the inner loop in the program is executed $N_c^2 N_d^2 N_s^2$ times, where N_c is the number of cultures, N_d is the number of diseases, and N_s is the number of species. For the eight-disease calculation (two cultures and 146 species) this comes to $8^2 2^2 146^2$ or 5,456,896 calculations in the inner loop alone, so it is clear why optimization of this block of code is essential. I will continue to improve on this code, translate it a operating-system-independent and fast language, and release this code as open source so other researchers can use the relational efficacy calculation and improve upon it.

Table 7.5. Calculated plant potential efficacy (P) for all eight diseases with the inhibition percentage at 9.6 μg/mL bioassay data for Chagas, African sleeping sickness, leishmaniasis, and malaria, for analysis with plant-based-disease-taxonomy disease distances.

species ID	Country	chagas P	chagas %I	sleeping P	sleeping %I	leish-mania sis P	leish-mania sis %I	malaria P	malaria %I	asthma P	diabetes P	eczema P	fibroids P
klkl2	Mali	4.127	26.268	15.133	0.000	14.966	32.335	110.354	16.377	29.622	45.419	35.098	44.151
gnu2	Mali	4.178	0.000	84.751	0.000	14.971	31.725	40.736	21.386	29.759	45.419	35.259	44.324
dskm2	Mali	4.126	0.000	84.349	45.453	15.041	24.784	43.527	19.734	31.881	45.354	37.057	46.064
nr2	Mali	4.283	0.000	15.389	0.000	15.088	16.579	110.449	12.095	30.349	45.603	104.833	45.935
tra2	Mali	4.367	12.727	17.237	41.447	15.612	21.405	116.834	35.094	35.206	51.558	40.985	50.835
ghr2	Mali	5.135	11.775	86.626	0.000	15.861	30.440	51.874	13.520	37.206	57.971	45.240	59.299
knss2	Mali	5.196	16.486	88.136	14.689	15.943	31.517	52.613	35.339	40.173	122.315	111.772	59.182
p1	Peru	5.375	33.014	16.014	0.000	16.048	17.439	46.586	6.772	173.773	49.410	180.797	189.382
smya2	Mali	4.339	35.694	16.951	0.000	16.299	19.861	115.355	2.275	34.277	50.210	39.092	49.703
klkr2	Mali	5.244	5.978	89.734	7.949	16.543	10.906	132.197	25.091	41.782	62.686	50.334	59.961
tkn2	Mali	4.775	36.842	86.150	0.000	16.573	31.831	117.358	7.517	36.496	51.782	42.319	120.194
ksf2	Mali	6.059	0.000	16.535	44.917	16.813	31.309	117.874	27.889	36.329	57.772	40.142	54.528
sg2	Mali	6.266	0.000	86.409	11.223	16.940	11.053	48.350	88.331	37.194	57.956	110.037	56.485

species ID	Country	chagas P	chagas %I	sleeping P	sleeping %I	leish-mania sis P	leish-mania sis %I	malaria P	malaria %I	asthma P	diabetes P	eczema P	fibroids P
nkl2	Mali	5.045	5.837	86.761	13.708	17.264	2.381	119.246	93.044	38.567	56.487	45.210	59.950
bsbl2	Mali	5.045	16.848	86.761	5.551	17.264	35.125	119.246	27.963	38.567	56.487	45.210	59.950
trb2	Mali	4.666	0.000	18.636	14.795	17.676	18.869	122.819	32.166	39.357	57.288	44.449	56.317
srt2	Mali	4.927	0.478	19.629	0.000	17.726	29.778	122.819	0.000	37.446	55.213	43.941	55.356
kndl2	Mali	5.715	0.000	18.419	47.922	17.728	32.126	120.111	27.557	107.202	60.539	114.617	64.104
ntmb2	Mali	5.087	0.000	17.825	0.000	18.117	17.003	123.510	11.821	39.145	59.892	47.529	59.680
Lng2	Mali	5.138	11.413	87.443	20.086	18.122	5.262	53.892	28.758	39.282	59.891	47.690	59.853
nma2	Mali	5.242	0.000	87.559	0.000	18.249	14.342	123.627	0.000	39.379	60.025	47.785	59.947
sndj2	Mali	5.360	0.000	18.225	11.357	18.335	8.120	123.738	9.732	40.194	129.813	49.497	129.600
kbt2	Mali	5.225	11.051	17.971	0.000	19.126	38.580	116.746	14.546	37.743	52.592	41.803	51.953
brnk2	Mali	5.382	0.181	18.227	5.178	19.248	27.909	116.841	9.640	38.471	52.776	111.538	53.737
btl2	Mali	5.498	16.848	18.372	0.000	19.343	29.734	116.974	10.188	38.793	122.513	43.772	121.874
dn2	Mali	5.653	25.543	88.107	0.000	19.475	22.852	117.090	5.459	39.027	122.646	44.028	122.141
ssb2	Mali	5.790	14.641	22.230	0.000	19.741	27.648	126.617	25.338	43.879	132.376	51.200	67.176
par2	Mali	6.277	4.306	90.041	21.079	20.457	3.892	60.079	6.302	112.614	66.576	121.372	137.218
dla2	Mali	5.200	10.326	20.119	0.000	21.461	25.668	122.169	16.735	42.241	59.420	114.781	64.034
slfn2	Mali	5.198	27.273	89.598	0.000	21.471	22.748	122.191	11.182	41.748	59.369	45.302	62.517

species ID	Country	chagas P	chagas %I	sleeping P	sleeping %I	leish-mania sis P	leish-mania sis %I	malaria P	malaria %I	asthma P	diabetes P	eczema P	fibroids P
slns2	Mali	5.465	0.000	20.498	0.000	21.675	26.305	122.399	7.094	112.298	129.356	47.742	133.022
zdr2	Mali	5.683	0.000	20.759	65.046	21.897	35.161	192.267	76.547	43.463	199.208	184.980	66.284
c1a	Peru	5.104	9.601	18.399	43.357	85.452	44.394	52.489	8.146	39.858	52.540	111.453	58.852
o1	Peru	4.997	22.297	19.644	0.770	87.334	34.545	53.211	1.722	37.467	55.174	43.968	55.386

7.1. Verification

The only one of these potential efficacies for which we have a good independent measure from the bioassay data is $P_{s,d}$, the potential of each species to treat each disease in any culture. This potential can be compared to the bioassay IC_{50} data by looking at the correlation coefficient between the two and the significance of a linear regression and changing the model to improve this correlation, but rather than the exact values, what we are more concerned with is whether the ranking of the potential efficacies matches the assay-derived ranking of the plants' efficacies, so we can test only the highest ranking plants further in the laboratory.

To this end, linear regressions of the calculated disease-treating potential efficacy vs. the bioassay inhibition percentage and a Spearman ranked correlation coefficient were calculated to see if they were significantly similar, for the four-disease case in Figure 7.1 and Table 7.6 and for the eight-disease case in Figure 7.2 and Table 7.7 respectively. As shown in Figure 7.2 and Table 7.7, there was only significance found for the eight-disease model for leishmaniasis-treating plants.

Though this second analysis with all eight diseases and two cultures has similarly low R^2 and p values for three of the diseases, it does show a 14% R^2 value and a p-value less than 0.05 for leishmaniasis an interesting and significant result in that it implies that 14% of the difference in the actual efficacy of these medicinal plants can be explained by a mathematical model of the ethnobotanical processes of medicinal plant discovery and knowledge transfer. That the first attempt at an equation to model this extremely complex process had some significant correlation with reality is surprising and it shows that there

is enormous potential in trying to model this process further. One thing that is difficult to explain is that though the correlation of the linear regression of the potential efficacy vs. the actual bioassay efficacy was significant for leishmaniasis, the Spearman rank correlation was not significant, a seemingly easier requirement to satisfy that the ranking of the two lists are significantly similar. The statistics merely tell us *that* something is true, but not *why* something is true. Understanding this next step of why only the data for leishmaniasis were significant will take more analysis. It is likely that significance was not reached for Chagas disease and African sleeping sickness because there were so few remedies found for these two diseases as they no longer seemed to be in the area of Paititi nor Kita. There were only 16 species mentioned for sleeping sickness in Mali (19.0% of medicinal Malinké species in the study) and 5 species mentioned for Chagas in Peru (4.6% of medicinal Asháninka species in the study), the lowest percentage of the medicinal flora of any of the eight diseases in both areas. This does not explain the non-significant findings for malaria, however, with its high representation at least in the Malinké medicinal flora (32 species or 14.22% of medicinal Malinké genera in the study, the highest of any disease in Mali), though relational efficacy was able to increase the hit rate for malaria.

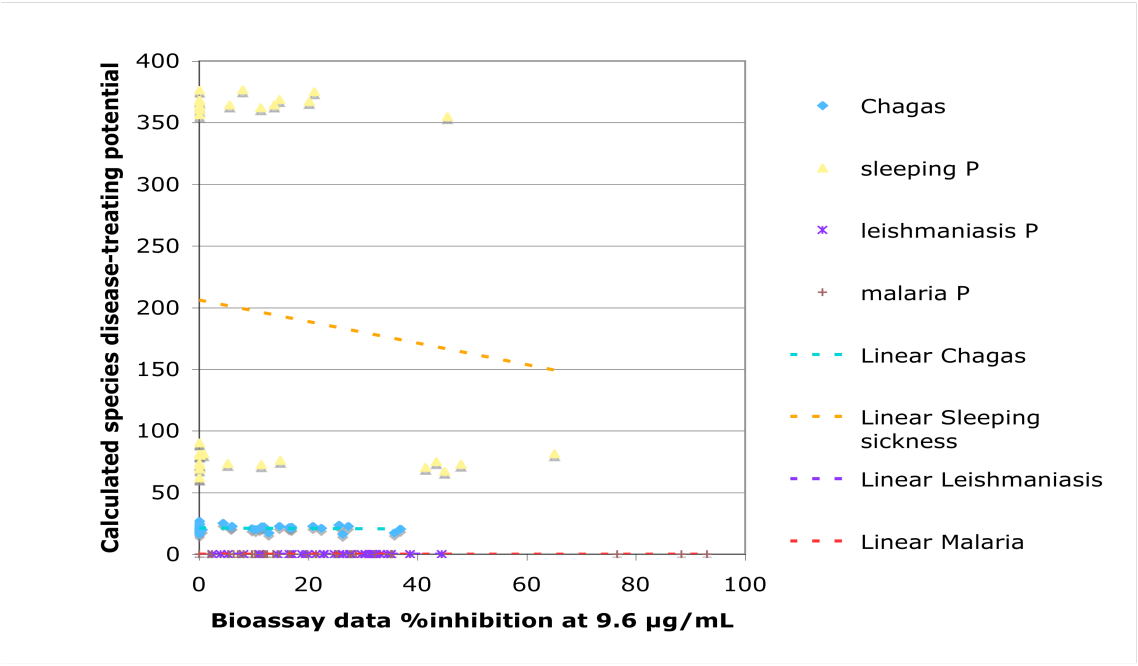

Figure 7.1. Calculated plant potential efficacy for Chagas, African sleeping sickness, leishmaniasis, and malaria vs. the inhibition percentage at 9.6 μg/mL bioassay data for the same species treating the same diseases, with linear regressions for each disease (Chagas $R^2 = 0.0077$, sleeping sickness $R^2 = 0.012$, leishmaniasis $R^2 = 0.0452$, malaria $R^2 = 0.0612$, $p > 0.05$ for all regressions) for four-parasitic-disease analysis with amino-acid-based disease distances.

Table 7.6. Linear regression and Spearman correlation for matched rank statistics between predicted potential and percent inhibition from bioassay data, with significance p, for four-parasitic-disease analysis with amino-acid-based disease distances.

Potential vs. inhibition %	**Linear R^2**	**Linear regression p-value**	**Spearman Rho**	**Rho p-value**
Chagas	0.00769	0.6219	0.0942	0.5961
sleeping sickness	0.011961	0.5381	0.0168	0.9247
leishmaniasis	0.045241	0.2272	0.1352	0.4460
malaria	0.06119	0.1584	-0.0121	0.9459

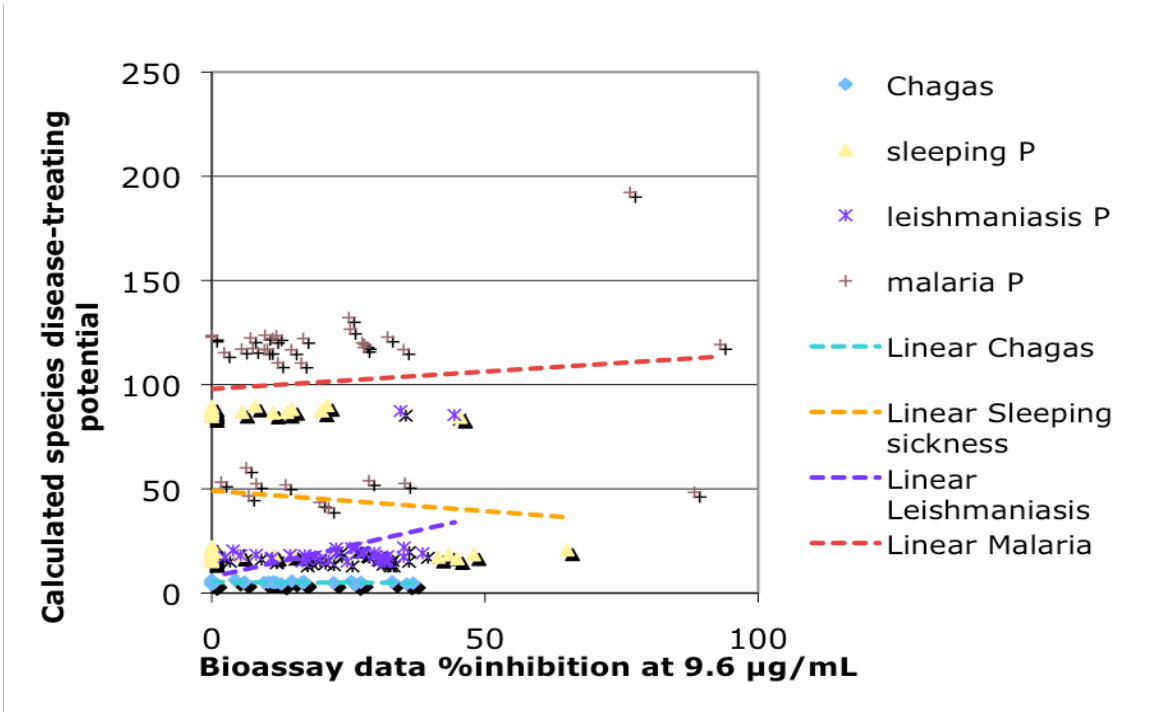

Figure 7.2. Calculated plant potential efficacy for Chagas, African sleeping sickness, leishmaniasis, and malaria vs. the inhibition percentage at 9.6 *µ*g/mL bioassay data for the same species treating the same diseases, with linear regressions for each disease (Chagas $R^2 = 0.038333$, sleeping sickness $R^2 = 0.010704$, leishmaniasis $R^2 = 0.139919$, malaria $R^2 = 0.01116$, $p > 0.05$ for all regressions except leishmaniasis with $p<.05$) for eight-disease analysis with plant-based-disease-taxonomy disease distances.

Table 7.7. Linear regression and Spearman correlation for matched rank statistics between predicted potential and percent inhibition from bioassay data, with significance p, for eight-disease analysis with plant-based-disease-taxonomy disease distances.

Potential vs. inhibition %	Linear R^2	Linear regression p-value	Spearman Rho	Rho p-value
Chagas	0.038333	0.2671	-0.1654	0.3499
sleeping sickness	0.010704	0.5604	0.1057	0.5520
leishmaniasis	0.139919	0.0293	0.1135	0.5226
malaria	0.01116	0.5521	0.0159	0.9289

A big point of the relational efficacy system is to increase the hit rate of finding effective medicinal plants over the current 8% of random plant searches and 30% of ethnomedical searches so this can be analyzed as well. The difficulty lies in where to set the threshold for a hit. Normally with these bioassays, something over 90% inhibition or near that of the positive control would be considered a hit. But in the case of leishmaniasis, none of the tested plants have an inhibition percentage near 92.5% of the positive control Miltefosin, with a range of only 0 to 44.4%. So instead let us consider any plant in the 90th percentile of the inhibition percentage a hit, and see how many species in the 90th percentile of potential efficacy fit this criteria for hits to determine our hit rate. This calculation was done with Microsoft Excel 2008 to show that the 90th percentile of inhibition percentage for leishmaniasis is 34.89% and for potential efficacy it is 21.61 for the species with bioassays, with four species in the 90th potential percentile

(slns2, zdr2, c1a, and o1), of which two (zdr2 and c1a) have an inhibition percentage over 34.89%, making the hit rate 50%. This 50% hit rate is well above the 12% hit rate we would get if we randomly picked a species from the ones that have been bioassayed, the 8% hit rate Lewis et al. (1995) states for random plant screenings, and even the 30% hit rate Lewis found for picking anti-HIV plants from anti-infective ethnobotanical screenings.

Even though other measures for the malaria potentials were not significant, and this is not as impressive as the numbers for leishmaniasis, if we apply the same reasoning to the malaria potentials, there is one plant (zdr2) out of the four in the 90th percentile of potentials with a malaria inhibition percentage over the 90th percentile (35.24%), leading to a 25% hit rate, which is still three times better than Lewis's 8% random screening rates, and twice as good as random screening within our bioassayed plants (4 of all 34 bioassayed plants in the 90th inhibition percentage, or 11.76%). For Chagas disease and African sleeping sickness, the hit rates are both zero. So, all in all, the relational efficacy system accomplishes what I set out for it to do for selected diseases: improve the hit rate of finding effective medicinal plants. I hope the model can be improved upon enough to do the same for the other diseases that have been studied.

7.2. Prediction

In order to test the power of the relational system to predict the disease-treating efficacy of species that are not recorded as being used by any of the cultures in the database, and perhaps have not been reported before anywhere as medicinal, I picked an appropriate genus for this predictive test based on a genus with a well-elucidated phylogeny that has measurable relations (via dating, branch lengths, or molecular data)

and that was already part of my study, preferably in more than one culture, to give it a point of reference. This limited it to a few genera. I selected the tribe Bignonieae in Bignoniaceae which has a complete and solid phylogeny based on three genes with data available (Lohmann 2006) from which to derive a patristic distance matrix for the species concerned.

I planned to run the entire system through with these unstudied species to derive their potential efficacy. Any of these species that had reports in NAPRALERT for any of the 8 diseases then would have their efficacy (IC_{50} values) compared with the predicted efficacy of these species to see if the two measures had good correlation.

This matrix would then used with the existing plant treatment, disease phylogeny, and cultural phylogeny data to calculate potential efficacy for each of the Bignonieae taxa and these potential efficacies would be compared with what data on the IC_{50} values for these taxa could be found on NAPRALERT. It would be good to be able to integrate this Bignoniaceae data into the larger angiosperm phylogeny data to get distances between all of the species, but given that these phylogenies are based on different gene sequences, this is difficult to do effectively. Unfortunately the distance matrix for this tribe could not be obtained in time for this publication from the author, another reason open databases like TreeBASE need to be used more effectively.

7.3. Identification uncertainty and plant compound synergy

I had planned to run the relational efficacy calculation hundreds of times with the identification of non-completely identified species selected randomly from the possible species identifications, in essence a approximate randomization statistics approach to find potential efficacies for even these incompletely identified species, but due to the slowness

of the calculations in the current incarnation of the program (nine days for 146 plants), I was unable to run this repeated calculation. The same slowness issue derailed the calculation of potentials for plant mixtures to determine if there are synergistic effects of certain plant clades, where a certain clade never appears by itself in a remedy, but always appears with other clades to activate the compounds in the main plant or reduce toxic side effects. This synergy calculation

$$P_{(s_1 s_2 \cdots s_n),d,c} = \frac{1}{N_{s'_1 s'_2 \cdots s'_n} N_{d'} N_{c'}} \sum_{(s'_1 s'_2 \cdots s'_n),d',c'} \frac{\prod_{x=1,y=1}^{N_s,N_{s'}} R_{s_x s'_y} R_{d,d'}}{R_{c,c'}} \qquad \text{Eq. 18}$$

with the many permutations of the products in the numerator, make it prohibitive with the speed of the current incarnation of the program. I plan to try both of these calculations once I can sufficiently speed up the relational efficacy program, perhaps by running it as a grid computing application where calculations are divided up over many individual computers, something that is very easy to do with this algorithm since the calculation of the potential of each species is completely independent of other species.

7.4. Conclusion

The preliminary analysis pointed in the direction of significant overlap of the two cultures' pharmacopoeias: the Asháninka and Malinké had significantly more similarity in their medicinal flora than in their overall flora, implying that they are both concentrating on particular taxa for medicinal uses. Given that this prerequisite hypothesis seems to be true, in order to test the relational efficacy hypothesis, the potential efficacy values were calculated from the large database of collected medicinal plants and these potentials were then compared to the efficacy values derived from

parasite bioassays to determine if there was a correlation between the two measures. This revealed for the one disease leishmaniasis a significant correlation between the two measures in a linear regression for the eight-disease case, but not for the four-disease case, perhaps indicating that a larger dataset will lead to more significant results with relational efficacy. The hit rate for finding antileishmaniasis and antimalarial plants has been increased four times and two times respectively over random plant searches in this model. Although the potential efficacy did not correlate to the bioassay data in all cases, this may have been due to the small sample size, the relatedness measures used, or the particular modeling formulas. I would therefore encourage the formation of an open-source public ethnobotany database for research that does not have intellectual property attached to it, so that researchers can submit their data to a common database, ethnobotany can enter the era of bioinformatics, and we can mine the wealth of data on medicinal, edible, wearable, and otherwise useful plants to find essential species for our future. I hope this is just a start, and these quantitative ethnobotany techniques can be improved upon over time by me and other researchers until they become useful and common tools with practical and predictive value.

Chapter 8. Conclusion

This thesis has touched on a wide range of topics related to ethnobotany, and it presents the development of four new techniques that should be helpful to this field and related sciences:

- quantitative synthesis of large databases to select new plant-medicine candidates through relational efficacy;
- dealing with intellectual property and confidentiality;
- using species accumulation curves to improve field collection efficiency; and
- returning results to the non-literate with talking books.

These are all clearly first versions of these techniques; they have built on previous work by others and it is hoped they will be employed by and improved upon by other researchers. The data I have collected has called into question the long-held assumption that more effective plant medicines are found through asking about symptoms rather than naming diseases, at least in the case of the parasitic diseases that are fairly common. This area needs more study to determine in which cases naming diseases might be better than describing symptoms. Statistics also show that one important assumption of the relational efficacy system is true: people in the vastly different cultures of the Malinké and Asháninka have converged on related plants to treat the same diseases, as their respective medicinal floras are significantly more similar than their respective general floras. Yet the inconsistency in this significance as a function of what grouping or taxon rank is analyzed shows that this relation needs to be looked at in a new way.

This research has already shown several plants to be effective for treating problematic diseases of the developing world, and through returning this information in the form of

printed and talking books to the indigenous Asháninka and Malinké groups with whom I work and to researchers in the countries as research papers, I hope we can at least reduce the severity and mortality of these diseases. I do not want this research to stop here, but rather I would like it to be adopted and adapted by other researchers to quickly and effectively document indigenous plant and medicinal knowledge, work with these groups to find even more effective medicinal plants, and spread the use of these plant treatments where they are most needed. This continued development is key in the current era of increased rates of diabetes from the obesity epidemic and abandonment of traditional diets, worsening parasitic infections partly due to global climate change, and loss of efficacy of many pharmaceuticals due to evolving drug resistance.

This should also serve as a jumping-off point from which I and, I hope, other researchers can improve the relational efficacy technique by adjusting the model, collecting more data to be integrated, and adding other variables to the model such as plant part used, method of preparation, toxin removal, and harvesting environment. Perhaps adding in these variables will improve the model's significance and predictive power enough to make it a useful bioinformatics tool for ethnobotanists.

To this end I encourage all systematists and ethnobotanists to make what data they can available on the Internet for all to work with. Databases like NAPRALERT (http://napralert.org), ebDB (http://olorien.org/ebDB/login.php) (Skoczen and Bussmann 2006), and Duke's USDA Phytochemical and Ethnobotanical Database (http://www.ars-grin.gov/duke/) work well in their domain, but there are slow to grow because the managers of the database have to enter data themselves from newly published articles, some have a limited geographic coverage, it is difficult to obtain data from these

databases in a usable table format rather than as merely text, and NAPRALERT is costly for subscriptions. Of these, ebDB is the most advanced since it allows user submission of data, but this can currently only be done one plant at a time, not in batches, and data cannot be downloaded in a table form, so it is not fully accessible yet. If these problems can be overcome and the ethnobotany journals required submission of non-intellectual property restricted data to the databases prior to publication, ethnobotany could enter the bioinformatics age as molecular biology has with GeneBank (http://www.ncbi.nlm.nih.gov/Genbank/), systematics has with TreeBase (http://www.treebase.org/treebase/) and the Encyclopedia of Life (http://www.eol.org/), and floristics has with the Global Biodiversity Information Facility (http://www.gbif.org/). There is a huge untapped amount of data that could be mined and analyzed for key edible, medicinal, textile, and construction plants from the field's knowledge if it were accessible and available in a useful format, in what could be dubbed the Human Ethnobiosphere Project. The issue of how the original knowledge holders of this information should be compensated when new medicines are derived from a large collection of data needs to be addressed. One possibility is keeping a record of every download of a particular piece of information and having contracts for use that require a percent of royalties, some of which is given to each of the original knowledge holders whose information was used to develop the new medicine, an approach pioneered by Shaman Pharmaceuticals and Paul Cox (Balick and Cox 1996). Clearly this is something that needs to be discussed further, but this key information needs to be gathered, preserved, returned to its original holders, made widely available, and analyzed for new useful plants in this age of rising epidemics, food shortages, increased food costs, and

disappearing species and indigenous groups. A clearinghouse for this data will help ethnobotanists keep their promise of preserving and finding new medicinal plants, food crops, textiles, and construction and industrial plants that they made years ago when the field was nascent.

Appendix A. Returning Results and Talking Books

Ethnobotanists and anthropologists usually want to give back training tools or documentation of their results to the communities with which they work to help preserve the communities' knowledge, as a sort of social contract, and an ethical guideline of groups such as the International Society of Ethnobiology, but this can often be quite difficult with largely non-literate groups. There have been attempts to use pictures and diagrams to convey this information back to the community members, and they work well, but only up to a point. With just pictures, it is difficult to represent complex and abstract ideas like names, emotions, and relationships. Using a combination of off-the-shelf products, I have constructed what I call "Talking Books"– water-resistant, solar-rechargeable picture books that explain the concepts of each picture with short audio clips in the users' native language and voices that are played when a button next to each picture is pressed. These books are effective tools for retaining and returning traditional knowledge to remote, non-literate communities lacking electricity, and stimulating renewed interest in their own traditional knowledge.

A.1.1. Background

While studying the ethnobotany of the Asháninka community of Paititi in the Yuruá river area of Ucayali, Peru, and the Malinke of Kita, Western Mali, I recorded a large number of plant uses from many informants; both groups requested that I return to them some documentation of this research such as a simple list of plants and uses with photos to use as a reference within their community. This was easy with literate members of the

community (about 15 of the 29 people living in Paititi, and less than half of the 15 Malinke informants) to whom I gave books with pictures, names, uses, and preparations of their useful plants, but this was useless to the rest of the community who could not read this book, only spoke their unwritten native language of Asháninka or Bambara (and not the written Spanish or French), and often had no members of their family who could read. There are small schools in both communities that teach local children reading, arithmetic, and agriculture, but many of the adults had been in the villages before the school was set up, and so had never learned these skills. Also, many of their children are too young to have learned reading yet or have little interest in reading long lists of plant uses.

Returning results or other benefits to the communities we work with can take many forms (Shanley & Laird 2002). There have been uses in the past of laminated sheets of plant photos and names as a way to give back somewhat weather-proof plant documentation to the communities (Foster 2000, 2004) which work well to provide a long-lasting reference for indigenous groups performing inventories of their own as long as they can read the plant names. Alexiades et al. (2004) have produced a book for the Ese Eja of Peru and Bolivia that has transcriptions of many Ese Eja songs and mythology in both Spanish and Ese Eja, but this type of documentation can be difficult to produce if the written form of newly studied languages is variable or lacking. In their two books *Fruit Trees of the Forest in the Lives of Amazonians* (Shanley et al. 1998) , describing forest ecology and economics, and *Recipes without Words: Medicinal Plants of Amazonia*, (Shanley et al. 1996a) giving medicinal plant remedies, these authors have used largely non-verbal images to try to convey these complex concepts to non-literate

communities. Their non-verbal illustrations give these books widespread appeal, as they can be understood by people around the world (Shanley & Gaia 2002; Shanley et al. 1996b). However, the abstract concepts of emotions, relations, and local names can be quite difficult to convey without the use of some language, so a book that can literally speak these names and concepts to its "readers" is needed.

A.1.2. Construction

I considered several ways to represent plant uses by employing audio or images such as laminated plant images with cassettes (some people in the communities had tape players) to explain the plants uses, but there was no way to link each image easily to a section on the tape and have it be a tool that someone could easily pick up and learn about a few plants quickly. The optimal solution would be to have a picture book that explains the name and uses of each plant on a page as you open each page, can be recharged using solar power for communities without electricity and are far from a supply of batteries, is resistant to the elements so it will actually preserve the traditional knowledge for a significant amount of time, and is cheap enough that each non-literate family in the community can be provided with a copy of the book.

There were greeting cards made in the 1990's that had a cheap microchip and a little microphone and speaker in them that allowed one to record a short audio greeting for the receiver of the card. Using many of these back to back was one possible way to build the talking books, but these are bulky (5 mm thick for each card), expensive (around $8), and difficult to find now, presumably because they did not work very well and were not very popular.

I was able to locate several talking photo albums on the World Wide Web that could hold 10, 24, or 36 4"x6" photos in plastic sleeves, and for each photo one could record up to 12 seconds of audio through the built in microphone on the album, and cost under $30 (http://www.sharperimage.com/us/en/catalog/productview.jhtml?pid=58939600, http://www.creativebuys.com/taphal.html, and http://store.breakingnewsproducts.com/ig-pi84066.html). The audio clips could then be played back by pressing a button next to each photo. The photo albums use 2 AAA batteries, and hold their recordings in a solid state chip even when the batteries run out. This is not the best configuration, as each photo is smaller than the optimal size for presentation of plant photos, the batteries were not easily rechargeable or replaceable, and having to press a button to hear the recording is not as easy simply hearing the recording as soon as you open the page, but this book contained most of the features I sought in an off-the-shelf, relatively cheap product.

To recharge the batteries, I found several small solar panels with built-in charge controller circuits (to keep the battery from overcharging) such as the Insten AAA Battery Solar Charger (http://store.yahoo.com/exchangecellular/aaabasoch.html) and the Solar Panel & Battery Charger (http://www.modernoutpost.com/gear/details/mv_solarpanel.html). The latter is slightly larger, more powerful, and has external wires that can be connected to the batteries in the talking book, whereas the former is smaller, less powerful, and needs to be opened up to connect the battery wires, but has a built-in bright LED light that can double as a flashlight. These solar panels can be glued firmly to the back of the talking book and have wires running to the battery case inside the book (Figure A.8.4), but it is much more durable to open up the electronics compartment of the talking book, drill a hole in the

back cover, pass the charging wires from the solar panel through the hole into the electronics compartment of the book, and solder the wires directly to the battery compartment power leads. The positive wire of the solar panel must be connected to the positive lead of the talking photo album's battery compartment, and the negative to the negative. When in doubt, one must remember that the positive end of most batteries has a bump or dimple on it, which connects to the positive lead of the battery compartment, whereas the negative end of the battery is usually flat (see Figure A.8.4). Rechargeable batteries, preferably of high capacity (i.e. greater than 500 milliamp hours [mAh]), must be placed in the battery compartment of the talking photo album. The standard non-rechargeable AAA batteries that the photo album comes with did not recharge properly with the solar panel.

Since there is a fair amount of space in the electronics compartment of the talking photo album, the most durable construction involves cutting a rectangle in the back of the photo album just large enough to accommodate only the solar panel of the solar battery charger. Then the battery charger can be deconstructed and its electronics and solar panel can be directly glued inside the photo album, while the solar panel can still be exposed to the sun (see Figure A.8.4).

With the solar panel solidly in place, the book can be left out in the sun for 2-6 hours to recharge if the batteries are running low. The charging time will vary with all the factors that effect the brightness of the light hitting the solar panel: latitude, time of day, angle to the sun, and cloud cover. A good sign that the batteries are low is when the sound playback sounds slowed down or distorted. An advantage of the 36-photo talking photo albums mentioned above is that they retain their audio recordings even when their

batteries run out, so there is no requirement to keep the batteries constantly charged. The talking book can be recharged in the sun once there is a need for it. I have not had experience with all the models of talking photo albums, so I cannot say whether they all have this same feature. Although it would be nice to have a ruggedized talking book made for the outdoors, it is unlikely that something like this was manufactured. In order to protect the book against rain and humidity, especially when it is left out to charge in the sun unwatched, the book can be placed in a large clear watertight plastic container (see Figure A.8.5 *)*. These plastic containers are readily available in kitchen stores in many cities around the world. In such a container, the book can be left outside to recharge for long periods, even in the rain. It should not, however, be left in an area where it can become completely submerged, as the plastic containers are not completely watertight.

One could go further and just use the electronics from the talking photo albums, but rearrange the pictures and pages into larger formats and change the triggering buttons to play each page's recording when the page is simply opened. If the playback of the audio clips is changed to be activated on opening each page, there needs to be only one recording per page, and only on one side of each page. This would make it easier for children to use the book, as they would not have to manually press the small button next to each photo to hear the plant's uses.

A.1.3. Images and Audio

The illustrations or photos of each plant, disease, or other concept shown in each image in the talking book should be simple and show features that are salient to the community members using the book. For example, a picture of the anther attachment of a flower is useful to a botanist, but is most likely not used by informants to recognize a

plant in the field. I try to use a collection of images for each plant to show the features people are most familiar with: the entire plant as it would appear in the field or garden where it is located, a bark slash if applicable, a drawing of the plant, and a close-up of the leaves, the fruit, and the flower if available (see Figure A.8.6). Complex preparation and administration techniques can be shown for medicinal plants as well. For diseases, externally visible symptoms of the disease are shown if they exist. It is best if these images are as large and clear as possible on the page, and that they are in color to be familiar to the book's users.

The audio clips must often squeeze a lot of information into only 12 seconds: the plant's common name or names, how it is identified, where it is found, what diseases it treats, and how it is prepared and administered. This information can be split across several images and audio clips if it is too much to fit into 12 seconds. For example, audio for *Catahua* (*Hura crepitans* L.) might say in Asháninka, "This plant is *catahua*, which is found as a large tree with purple flowers growing in the low jungle. It is used to treat wounds by drawing the resin from the trunk of the tree, cooking it until stiff, and applying it to the wound for seven days."

Obviously, it is best to record the audio clips on the talking books in the native language of the people that was using it the most, but a researcher not fluent in this language can use a translator to record the audio clips in the field. This is the approach I initially took, but it does pose problems: there are only 10-12 seconds of audio for each photo and it is difficult to push the translator to squeeze long plant preparation techniques or narratives into this short time. Therefore, an easier option is to record informants explaining their narrative or preparation at a leisurely pace in the field and then, when

one has access to a computer, to edit down the audio to the essentials, speed it up, and perhaps split it into several 10-12 second snippets using audio editing software. The edited audio clips can then be recorded into the talking book with the computer's speakers. A way to cleanly and quickly transfer the audio to the books would be a nice option, but this is beyond the capabilities of these cheap books.

Corrections can be made and additional information gained by reviewing informant interviews with the informants and recording the audio clips directly with the informants once the talking book images are prepared. So as not to lose the benefits of this review when recording the audio clips from the computer, all the edited clips should be listened to by the informants in the field if possible, and some audio can be re-recorded if corrections are necessary using a portable computer or directly from the informant. The record functionality (pressing a separate single record button and the play button for a particular picture simultaneously on the models with which I have worked) should eventually be disabled before leaving the field site so it is not accidentally triggered and the hard-earned recordings erased.

A.1.4. Results

I have just left the first versions of these talking books in the field, so I have yet to see how they survive the rigors of life in the field, how much the community will use them, and what benefits the community will derive from them, but the initial reaction is quite favorable towards the books. Everyone in the community was interested in trying out the books and helping me record the plant preparation recipes, even those who were literate and could use the perhaps slightly less interesting text and picture books. Most encouraging was the fact that many children in the community, with their natural

penchant for new toys and gadgets, were intrigued with the book, pressing the buttons for the novelty of hearing their parents and elders voices coming out of a machine (Figure A.8.7). I am most concerned about the children's interest in medicinal plant knowledge, so it is encouraging to see that the talking books may help with the preservation of this knowledge. Returning to these communities will show me how the community is really responding to and using the books, and if they need to be modified. Perhaps a separate book, designed specifically for children with more interesting pictures and simpler explanations would engage them even more. All the photos in the book might need to be laminated and the electronics sealed watertight to help the book survive high humidity, copious dust, and heavy use. Suggestions and inquiries are encouraged on this topic.

These books could be used to return documentation and preserve knowledge of construction techniques, food preparation, plant identification for surveys, hunting techniques, mythology, genealogy, traditional ecological knowledge, and ethnohistories, in addition to the medicinal plant knowledge for which I have used them. The solar-powered weather-resistant talking books presented have initially shown promise in community education, traditional knowledge documentation and preservation, and stimulating the interest of future generations. This is just the first design of such a device and improvements will be made as its long-term role in the community becomes clear. It shows that that simple, cheap applications of technology can be quite useful and beneficial.

Figure A.8.1. The cover of a talking book, made from a talking photo album and a solar panel to recharge its batteries.

Figure A.8.2. The 12-second messages associated with each photo in the talking book can be played by simply pressing the button next to each photo.

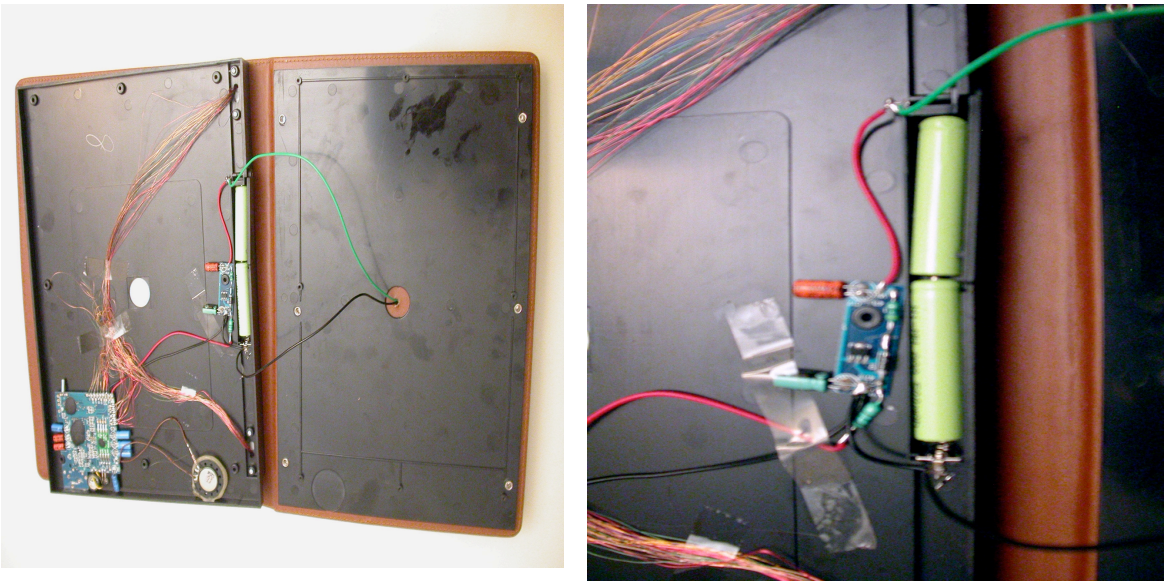

Figure A.8.3. Open electronics compartment of talking book showing wires passing through back cover from the solar panel affixed to the book and the connections to the talking book's battery compartment, with positive wire (light wire on upper right) connected to positive battery lead, and negative wire (dark wire on lower right) connected to negative battery lead.

Figure A.8.4 . Solar panel recessed inside electronics compartment, a more durable construction.

Figure A.8.5 . Talking book in a sealable clear plastic container, charging in the sun.

Figure A.8.6. An example of the images that appear in the talking book for each plant, in this case *Catahua* (*Hura crepitans* L.), with its overall form, leaves, flowers, and an illustration of the key features (illustration courtesy of Samantha Tsistinas).

Figure A.8.7. Recording plant remedy recipes with Frederico Sanchez, one of the most knowledgeable Asháninka from Paititi, Peru, for a talking book that will speak the recipes back to the young people of this village in their native language as they leaf through the pages, looking at images of the plants described.

Appendix B. Sample Herbarium Confidentiality Agreement

As an example, a model confidentiality contract is shown that can be used as a template for similar agreements

Contract Governing Confidentiality of Herbarium Submissions

Purpose of Agreement

The purpose of this agreement is to protect and keep confidential the provenance and medicinal uses of the plant specimens deposited in herbarium. Such information is protected by the Convention on Biological Diversity under Article 16, "Access to and Transfer of Technology"[1], which was enacted in order to prevent the degradation of biodiverse areas as well as the cultural heritage and integrity of peoples who occupy these biodiverse areas and have intimate knowledge of their natural resources.

This agreement is not intended to limit bona fide research in the fields of taxonomy, systematics, floristics, ecology, conservation, or biogeography, but to bar unethical, unauthorized, or unlawful dissemination of medicinal plant knowledge.

Parties to this Agreement

1. The _____ herbarium (hereafter referred to as "the herbarium") and all its subsidiaries, agents, and employees, both current and those who succeed such positions, duties, and organizations, including, but not limited to the Herbarium, the Information Resources Department, the Science Department and anyone who controls or has access to

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[1] At present, the United States government has not yet signed or ratified this convention, but the principles and practices embodied in this convention are adhered to internationally by the community of scientists and professional societies working with issues of intellectual property rights.

the Herbarium specimens, nor is it limited to the individual currently holding the title of Herbarium Director, but is applicable to whomever assumes the duties of the office of Herbarium Director.

2. The depositing researcher, _____ (hereafter referred to as "the depositor"), currently residing at _____. As part of his/her research, _____ has collected and deposited tangible botanical specimens into the custody of the herbarium. In addition to this agreement, all of these specimens are covered by an Informed Consent Agreement (annexed hereto) approved by the Institutional Review Board with local collaborators in the field who gave him/her the medicinal plant information about these herbarium specimens.

Agreement

It is agreed between and among the above parties that the herbarium will not release information on the depositor (_____), the date of specimen collection, or the uses of these plants to anyone outside the herbarium or to visiting researchers, either directly or by releasing information that allows such information to be inferred, without the prior written consent of the depositor or until a pre-set time limit expires, the depositor's will allows it, research agreements and prior informed consent (PIC) forms expire, or the owners of the intellectual property disband as a group or go extinct. These are hereafter referred to as "trigger events." Herbarium staff will check on a yearly basis the state of these trigger events to determine if the restrictions on these specimens need to be revised. Visiting researchers for this purpose are defined as individuals who are allowed access to the herbarium and its specimens but who are not part of the herbarium staff as defined above.

Plant collection and use information will not be entered in the herbarium computer database ("the database",) either when the specimens are deposited in the herbarium, or at later points in time when the depositor's specimens are part of a family or genus that is being entered into a database. To ensure this outcome, all specimens deposited by the depositor will be stamped with the words "Do not database." This restriction will be honored by the database staff.

Specimens from the geographic area _____ of which the herbarium currently has very few collections will be kept in a separate locked cabinet until the time that the herbarium has over 2000 specimens from _____, allowing the depositor's _____ collections to be sufficiently diluted so as not to be easily located within the herbarium. Once the herbarium has over 2000 specimens from _____, the depositor's specimens from _____ may be merged into the regular herbarium, but should still not be entered into the database.

In return for permission to have access to the herbarium, all visiting researchers must sign an agreement with the herbarium agreeing to not remove any plants, plant parts, or information on the depositor's specimens in accordance with this contract.

Taking administrative burdens into account, the depositor will work with the herbarium to establish mutually agreeable procedures to implement the points of this contract. To this end, the depositor must do their best to uphold their research agreements and PICs while not excessively restricting non-ethnobotanical, non-commercial research (e.g. taxonomic, ecological, floristic, biogeographic) on these specimens. The depositor will also attempt to deposit specimens in a primary herbarium that has a large number (20

times the number of deposits) of collections from the same geographic area to obviate the need to keep specimens from rarely collected geographic areas in a separate locked cabinet.

The links that connect these specimens with other herbaria deposits with the same collections in this herbarium (i.e. barcodes) will be blocked until the collections in these his herbaria from the same geographic area are greater than 20 times the number of ethnobotanical specimens, or one of the trigger events removes the confidentiality on these specimens. Once these blocks are removed, all use, locality, and collector information from the other herbaria will be copied to the specimens in this herbarium.

Signed

_____ _____

Depositor Date

_____ _____

Authorized to sign on behalf of the herbarium Date

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