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Genetic diversity of *Annona cherimola* Mill in South Central Bolivia

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Genetic Diversity of *Annona cherimola* Mill in South Central Bolivia

By

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submitted in partial fulfillment of the requirements for the degree of

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The thesis: "Genetic Diversity of *Annona cherimola* Mill. in South Central Bolivia" is hereby approved in partial fulfillment of the requirements for the Degree of MASTER OF SCIENCE IN FORESTRY.

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Chapter 1 Introduction

My interest in the subject of genetic diversity came about during my first year of the Masters International program. I began to realize genetic diversity is an important aspect of the management of a forest or a small farm in Bolivia. The level of genetic diversity of natural products upon which we depend was discussed from varying angles throughout the courses. Anxious about what I might do as a thesis I developed a preliminary plan of what I could possibly do in Bolivia, an area, at that time, which I knew nothing about. My plan was to look at a crop or fruit tree, which was important to the Bolivian farmer, and determine how much genetic diversity was present on their farms. Knowing how much diversity in a common crop is an important step towards conservation. Placing value on the varieties present could prevent another vital agricultural crop from losing diversity because of monoculture cropping. Therefore not only would I have a thesis but also I could make an impact. This eased my anxiety and made me focus. Once I arrived in Bolivia I began thinking about what I could do. During the first week of my training my language instructor mentioned a fruit, the *chirimoya,* and asked us if we had heard of it and what was the name in English. The dictionary incorrectly translated it as custard apple. Once I heard of this fruit I began asking around about the fruit and it turn out my host family had a tree in their patio. My Peace Corps trainer Dr. Jose Salinas found the correct name for it: cherimoya, *Annona cherimola.*

I was quickly intrigued by the fruit and began looking for a site through "site fair"(Peace Corps Bolivia, 1999) where there would be cherimoya or at least other fruit trees. Las Carreras fit the description of a site, which could possibly have cherimoya. I chose this site and my APCD placed me there. Las Carreras unfortunately did not have cherimoya but a town just north of it did: Villa Abecia. To this day I still insist to people at my site they must at least try to grow the tree and some farmers have taken me up on it. Invoking competition usually helps since they are rivals of Villa Abecia.

During my investigation I found out how valuable the fruit is. In Bolivia it was expensive but the demand was also as high. Encouraging rural farmers to cultivate the tree would increase their income and perhaps improve their livelihoods. Bolivia currently is suffering an economic crisis. During my time in Bolivia the economy worsened. Once fair prices for high demand crops now have decreased. Fruits such as the cherimoya can only help the rural Bolivian farmer.

The tree grows well along slopes and riverbanks preventing erosion better then perhaps the plum or peach. Although rather strange looking on the outside, it is a favorite among Bolivians, Peruvians, Argentineans and Chileans. When mentioning my interest in the fruit to my mother, a Peruvian, she immediately became nostalgic for her favorite fruit.

Through my investigation I found the cherimoya was not a unique fruit to Bolivia although Bolivia is within the range of its origin. Spain and the United States are major commercial producers of the fruit. Limited research had also been done on the fruit and tree because of its importance in other areas of the world such as Australia, New Zealand, Israel, Italy and other countries with small commercial productions of the cherimoya.

Within a month of arriving at my site I began developing my proposal and I was anxious to get started. Going along with a Peace Corps theme I felt my investigation should at least benefit the farmer as well as my own research goals. Therefore, as way to thank them for their help in answering my questions, I would help local Bolivians solve some of their basic problems.

Typical Bolivian farming communities, the three research sites Villa Abecia, Acchilla, and La Merced, struggle to increase their annual incomes and to decrease their debt. The sites have a mixture of commercial and small landholder farmers. Improvements of the cherimoya tree is vital for each of these farmers in each of these regions. The research sites are in a larger region where Spanish and European settlers have had the largest impacts. Spanish is the language spoken by all sites, Acchilla is the only site with Quechua influence but the Spanish culture has an equal impact on this site.

My objective for this thesis was to analyze the genetic diversity of the cherimoya in south central Bolivia. First I looked at diversity on a region to region level then I compared diversity between the commercial farmers and the small landholder farmers. Chapter two describes the background of Bolivia: history, the environment and agriculture. In Chapter three the background of each of the sites I chose is thoroughly discussed. In Chapter four the description of the fruit, the tree, its production, and current and past research is also discussed. Chapter five describes the methods used in this investigation. In Chapter six is the results of the genetic analysis and a discussion of what I found in the field and laboratory. Finally in chapter seven conclusions are drawn, recommendations are made for each group involved (*e.g.* Peace Corps, sites) and the importance of future research.

Chapter 2 Bolivia

2.1 Background

A land-locked country, Bolivia is surrounded to the southwest by Brazil, to the north by Peru, to the south by Argentina and obstructed by Chile to access to the Pacific Ocean in the west. Bolivia's total land and water area is 1,098,580 square kilometers; about three times the size of Montana (CIA, 2003) (Figure 2.1). Bolivia was named after one of its independence fighters, Simon Bolivar. Simon Bolivar helped Bolivia win its independence in 1825 from Spain. Throughout its history, Bolivia has had many political problems including two hundred coups and counter coups. Political problems still plague Bolivia, however efforts to overcome these problems are evident. Funds are not evenly distributed, natural resources are misused and poverty plagues 70% of the population (CIA, 2003). A significant problem, which also plagues Bolivia, is corruption on many levels. Currently it is ranked number seven in the world, tied with several others, on a list of the most corrupt countries in the world. Bolivia had a score of 2.2 where a rank of ten is the cleanest and one as the most corrupt. A score lower than 5.5 by a country indicates serious problems with corruption (IPA, 2002). In recent years efforts to eliminate corruption are evident from legislation including *Ley de Participacion Popular* or Law of Popular Participation, which was put in law in 1994(PDM-*Plan Desarrollo Municipal* 2000, Peace Corps Bolivia 1997).

Figure 2.1- Map of Bolivia (www.aguabolivia.com)

20th Century History

The single most important event, which had a significant impact on the farmers of today, is the revolution of 1952. Many of the farmers in my research area attribute this event to the way they farm today. Six years before the electoral victory in1951 MNR, a political party which represented many people of all classes, was gaining power and was a major opponent of the ruling party. MNR's win was denied with the use of military force by the outgoing president. In 1952, the economy was worsening; social unrest increased in all sectors of the society and the military was severely demoralized. MNR took back their rightful seat in government and a revolution began. Many changes including a "far reaching" agrarian reform and universal suffrage was established. Peasants who were illiterate or held no land could vote (Library of Congress, 1989).

As of 1950 large landowners owned 92% of the arable land and the average farm was 1,000 hectares or more. This unequal proportion instigated the agrarian reform in January 1953. A commission was established and decreed the law of Agrarian Reform in August in the same year. This law "abolished forced labor and established a program of expropriation and distribution of the rural property of the traditional landlords to the Indian peasants" (Library of Congress, 1989). Large landlords were forced to divide their land and the peasants (*campesinos)* who worked the land now owned the land. Farmers told me that by simply working the land the property could be legally titled to those who work the land. In the past landlords had vast amounts of land with many peasants working the land for them in the manner of serfs (Library of Congress, 1989).

2.2 Climate and Geography

Bolivia

Bolivia has a diverse climate and geography. Altitude varies throughout the country, diversifying the climate. Climatic zones differ with regard to humidity and rainfall; semi- arid and arid cold; hot and temperate. Geography ranges from the rugged steep Andes Mountains with a highland plateau, (called the *Altiplano*) at altitudes of 3000 meters above sea level, hills and valleys at 3000-1500 meters above sea level to lowlands of altitudes of 1500 meters above sea level and lower. Because of the rugged terrain only 2% of the land is arable. However, approximately 1,280 square kilometers are irrigated (CIA, 2003). Natural hazards occur; flooding in the northeast occurs during the rainy season, fruit crops are destroyed by hail in the south and mudslides are common along the major roads and near La Paz in extremely eroded areas (CIA 2003)

Climate and Geography of Chuquisaca and Tarija

Chuquisaca and Tarija are in the southern central end of Bolivia. Chuquisaca is north of Tarija. Chuquisaca consists of valleys, which are surrounded by *altiplano* on the northwest side and lowlands on the east side. Sucre is the capital, which is in the northern end of the department. Although a much smaller department, Tarija is also diverse in geography and climate. Tarija also has *altiplano* in the northwest, lowlands in the south, valleys in the center and what is called *el Chaco* in the northeast bordering Paraguay. The city of Tarija is the capital of the department and is situated in the western central area. The majority of the land is valley and the climate is subtropical in both departments. The combination provides for an ideal growth area for the cherimoya tree, the subject of this thesis.

2.3 Natural Resources

Bolivia has a diverse natural environment and many natural resources. Tin, natural gas, petroleum zinc, tungsten, antimony, silver, iron, lead, gold, timber, and hydropower are some of the natural resources currently exploited. Silver and copper are resources historically mined. A large part of the economy since the fifteen hundreds has been silver mining. Mining for natural resources remains a significant part of the Bolivian economy (CIA, 2003).

Environmental issues for Bolivia are numerous. Demand for timber, poorly managed woodlots and demand for *leña* or firewood results in deforestation. Poor agricultural practices have led to land degradation and soil erosion. Pollution from unregulated factories in rural areas is also an issue.

2.4 Agriculture

Agriculture is a significant part of the Bolivian economy. Forty-three percent of the people farm about 3% of the arable land. Subsistence farming dominates the agricultural economy (Figure 2.2).

Figure 2.2 Carrot Harvesting in Las Carreras

Bolivia cultivates corn, potatoes, wheat, barley, rice, *haba,* sugarcane, coffee, yucca, soybeans, carrots, and cotton. Some products are used as cash crops. Historically potato has been a cash crop for Bolivian farmers (Fuertes Medina,1987). Sugar cane is grown in southern provinces of Tarija. Bolivia is the third highest producers of coca in South America. As the mines in Potosi began closing, many went to the *Chapare* and *Yungas* for coca farming (CIA 2003). Coffee production is expanding and Bolivia currently exports coffee to Europe.

2.5 The People

8,445,134 people live in Bolivia. The census was completed on September 5th , 2001(INE, 2001). The population in Bolivia is on the rise. Migration to the cities has increased the city populations while rural areas suffer from out migration.

Bolivia is known for a mixture of different ethnic groups, each with a significant presence in its society. Three different ethnic groups live in Bolivia. Quechuas predominate with about 30% of the population, Aymara are present in the northwest part of the country and are also 30% of the population, and Spanish descendants are about

15% of the population. The remainder of the population is a mixture of these groups, Guarani and Europeans, such as the Germans and Italians, whom migrated in the nineteen hundreds (CIA, 2003). The official languages are Spanish, Quechua and Aymara. Guarani is spoken by small border populations near Argentina, Paraguay and Brazil. Many of the citizens are bilingual, especially the children. During most of the 20th century Quechua and Aymara in school were discouraged but now it is being taught and the languages are viewed as important languages. Jobs for farm extension workers require bilingual candidates. The literacy rate the CIA gives seems high at 83.1% of the entire population. Until recently rural female children did not go to school and many women 35 years and older are illiterate, especially in the rural areas of Bolivia. (Figure 2.3)

Figure 2.3 Quechua schoolgirls

The main religion is Roman Catholic, however especially in the rural areas, the Evangelical Methodist church has a large influence (Figure 2.4).

Figure 2.4 Old Catholic Church

In some places the number of Roman Catholics and Evangelicals are nearly equal while the entire country is 95% Catholic. Bolivian Catholicism has a mixture of the indigenous religion and one might say they are Catholic but the indigenous culture influences the way many Bolivians worship.

2.6 Culture and Festivities

Bolivians take pride in their festivals and celebrations. The religious festivals are important and are celebrated by everyone. The holidays include La Cruz, May 3rd, *Todo los Santos* or all Saints Day which is the 1st of November, San Pedro which is the 29th of June, and Virgen del Rosario which is the 7th of October. Each town or city has a patron saint and on the saint's designated day the people parade with all of the traditional costumes. Traditional food and drink is always a fixture of these festivals. For example, Cochabamba is famous for its corn drink *chicha*, in Tarija *singani* and wine are the common celebration drinks (PDM –*Plan Desarrollo Municpal* 2000).

They will also celebrate days such as All Saints Day, Christmas, Three Kings Day and Carnival which is forty days before Easter (Figure 2.5).

Figure 2.5 Children among flowers for All Saints Day

The festivities are usually celebrated one day or sometimes they will last a week. Bolivian Independence Day, August 6th and the department's foundation day are important holidays for Bolivians as well. During government related celebrations a parade of all the officials and the school children is an important feature. School children will practice for days for the celebration. A ceremony includes the National Anthem, the department songs, and politicians' speeches. (Figure 2.6 and Figure 2.7)

Figure 2.6 Pre-K-12 Students fidgeting

Figure 2.7 My students taking a break from practicing

Local celebrations also occur all year round. The anniversary of a town or a school is a two-day process with activities associated with national holidays. (Figure 2.8)

Figure 2.8 Little girl-carrying crown for School Queen in a School festival

Traditional Tarijan dances, based on Spanish dances, are typical of all celebrations. Sometimes students practice months ahead of time for such festivals. Dance groups are hired for these special occasions. (Figures 2.9 and 2.10)


Figure 2.9 and 2.10 Traditional Dances from the Tarija Region and Research Areas

2.7 Education

Concurrent with reforms made by the local government education was also restructured. According to teachers, basic education changed due to "*La Reforma*". Children's rights were established as well. A number of trial schools began developing a new curriculum (PDM-*Plan Desarrollo Municipal*, 2000). In the past education in the rural area was vastly different from education in urban areas. City education was stringent with high standards while rural education concentrated on the basics. Also, the education requirements of a city teacher were higher when compared to those of a rural teacher. A rural teacher was required to have a minimum of a sixth grade education while a city teacher was required to have a high school degree. The curriculum was different for those who wanted to be a rural teacher than that of the city teacher. This has changed, now all teachers must have a high school degree and are encouraged to obtain a Bachelors in Education.

The reform changed many things including restructuring the school system to include schools in villages which at one time did not have a school. In addition, high schools were built to ensure a high school education where it was once not possible. (Figure 2.11)

Figure 2.11 Newly built elementary school in the rural community of Las Carreras

Currently several agencies are helping the schools. As I was leaving, Peace Corps Bolivia designed a plan to work with the schools on *La Reforma.* Implementing a system of computers for rural schools was mentioned as important by different agencies. Money from international organizations was focused on health and schools.

2.8 Alternative Education

Alternative education is important in Bolivia. A significant number of Bolivians have only completed a fifth grade education. NGOs and government aid agencies such as DANIDA (Denmark's aid agency), ADRA (Adventist Development and Relief Agency), CIAC (The Center for Research and Rural Aid) and others help the rural farmer and the family gain skills and understanding of various aspects of farming, laws and artisan trades (Figure 2.12).

Figure 2.12 Fruit basket made by Guarani rural women

They usually provide seminars to the communities about different laws explaining people's rights. Other seminars teach different farming techniques, help one obtain their high school degree, learn to weave, and make preserves. The latter are usually nine months long and are during the school year (PDM-*Plan Desarrollo Municipal*, 2000).

2.9 Health

In the last few years the health care system has greatly improved. Bolivia is receiving money from other countries for health care. All children receive free vaccinations. Older children and adults also receive free vaccinations if supplies are adequate. Priority is given to children, girls, women and then boys and men. The medical staffs in each area are present for the sick, but also educate the population about disease and illnesses and how to prevent them. A main clinic with at least two doctors in charge are located in the municipalities capital and then in each rural community a nurse is always present at a local health post. Doctors make rounds to the community as well as help with education and prevention.

2.10 Political System

Popular participation, a new program in Bolivia, has helped revolutionize how governments and politics function. In the past, solely the president or congress controlled and determined every project executed. Personal connections were necessary to receive funding for a program. In other words a small town such as my site, a site far from the capital of the department and twenty-four hours from La Paz, had little chance to do a project benefiting the community. Now programs such as popular participation have modified the function of the government.

The Bolivian Congress passed the Law of Popular Participation (LPP) in April 1994. I t was meant to enforce the *Plan de Todos* which was a plan to encourage development. LPP was part of an overall plan to decentralize the government, capitalize on many state owned enterprises and promote education reform (Figure 2.13)

Figure 2.13 Community leaders conducting meeting

The Law of Popular Participation (LPP) has three goals: enhance economic development for all Bolivians, strengthen democracy, and improve Bolivia's territorial demarcations and management. These goals are based on the belief that every citizen, no matter the background, has the inherent right to participate in defining their own community's needs and to play a role in deciding how funds directed to the area are to be used. (Peace Corps Bolivia 1997)

The first phase of implementing this law was to establish the boundaries of each department, province, and *municipal*. This included settling border disputes. The demarcation of municipalities helped in the management and distribution of resources. From this the government could directly deposit money to be used for projects into the accounts of the municipalities (Peace Corps Bolivia 1997). Now each municipality has its own money and this money can be used in agreement with the community goals. Currently the municipalities are in the process of implementing this.

In Bolivia nine departments comprise the country: Chuquisaca, Tarija, Cochabamba, Beni, Pando, La Paz, Santa Cruz, Oruro and Potosi (CIA, 2003). Each department consists of provinces, the provinces are divided into sections or *secciones.* For example Las Carreras (Figure 2.14), my Peace Corps site, was in the department of Chuquisaca in the province of Sud Cinti and the third section of the province.

Figure 2.14 Las Carreras

Sucre is the capital of Chuquisaca and Villa Abecia is the capital of the province. Within these sections several groups help govern the area. These are the mayor and council members, community leaders, and the *Comité de vigilancia*, which is a representative body of community and group leaders who balance the mayor and council members in decision-making.

Although now the communities can participate in many local decisions, the governing system is still party based. Determining leaders is based on party. This system also affects jobs. The winning party has the right to appoint party members to different government posts, NGO jobs, embassy jobs, schools, and hospitals.

Chapter III Research Sites

Not only does this thesis include genetic analysis of a commercially important fruit tree species but also it documents the essential details of the *chirimoya* tree and how it is affected by the environment and the potential for loss of diversity. In order to understand the diversity and variation of the trees one has to consider the geography, politics, climate, and culture. For example, at one site the cherimoya tree is becoming less important because of disease. Another site is rural and development is hindered by inadequate transportation although fruits are of high quality (Figure 3.1).

Figure 3.1 Conditions typical of Cherimoya farming

Bolivia is a developing country with the gross domestic product per capita of $US 970, (Lopez Levy, 2001) or about eighty dollars a month. In rural areas, farmers are fortunate if they obtain eighty dollars a month. These farmers depend on their cash crops every season. At times they borrow money from high interest micro-lenders. They pay the lenders with the income they receive from selling their agricultural products.

Whether they go into debt or not depends largely on the weather and market prices. Given yield variability, farmers depend on only a few crops which require limited care and resources for high yield and high market prices. In all three sites they grow many of the same crops: carrots, onions and potatoes. These research sites use typical farming practices of Bolivian farmers. Dependence upon a few crops has negative consequences. Price fluctuation for new and different crops results in farmers' hesitancy to try the new products

Options for each of the research sites make it possible for the farmers to diversify the farm system. Fruit trees produce well in these sites. Potentially these fruit trees could be both economically and environmentally important in the farm system of these research sites. The benefits would be seen with less erosion, higher yield, and decreased pests and diseases. Transfer of fruit tree management skills by farm extension workers is limited. An unhealthy or low yielding tree is likely to be harvested for firewood.

3.1 Farmers and Farm System

Farmers in each of the sites had much in common. Most of the farmers had no more then twenty hectares of land. The areas are semi-arid and the most productive land was situated near the rivers in each of the sites. Commercial farmers and small landholders are relatively poor. Commercial farmers are slightly more successful then their small land holder counterparts. Any fluctuation in the market prices of their crash crops could easily plunge them into debt.

South central Bolivia is different from the rest of Bolivia. Few people from indigenous populations are present in the area. Some of the farmers are of Andean descent and have left the mountains to look for land to farm in this area. The original settlers were of European descent. The main language is Spanish and very little Quechua is spoken. Preservation of the language of Quechua is a priority in the school system and this is a national effort. Quechua is taught to everyone to preserve the language and it has become vital for young educated Bolivians to know Quechua in order to get jobs. Clearly spoken Spanish is a feature of the area. The only research site where Quechua is spoken is Acchilla. Most of the residents are fluent and bilingual. Quechua in other areas of the country is common in business transactions. However in south central Bolivia, Spanish is the language spoken for such business.

A farm, whether commercial or small landholder, had a mixture of crops and fruit trees on their land. Formal orchards were rarely present. Farmers in Villa Abecia, for example, called their plots of lands *viñas* or vineyards. At one time the farms mainly grew grapes for wine. However, farmers were forced to diversify because of the diseases and pests, which eventually affected the grape. Now farms in Villa Abecia, as well as the other research sites, have a mixture of fruit trees along the edges of the properties, cash crops in larger sections, small plots of experimental products and crops for home consumption in another corner of the land. Commercial farmers have plots of trees, which were planted randomly as the farmer expanded his or her production. Trees were randomly situated rather than in a traditional orchard.

Farmers at the three research sites for this investigation on the cherimoya utilize small-scale mixed farming; they integrate animal husbandry and crop production (Beets, 1990). However, many of the farmers are beginning to specialize in crop production and others solely in animal husbandry (Figure 3.2 and Figure 3.3).

Figure 3.2 Carrot farming

Figure 3.3 Sheep corral

The remaining farmers integrate both of these. Within those who integrate both aspects, some farmers are physically separating the two facets. For example many of the farmers have their land near a river or a water source and have their animals in the hills distant from their farms. Fruit tree production for home consumption or market is common in the area, indicative of small-scale mixed farming. In all three sites farmers grew apricots, citrus, plums, peaches and cherimoya. In Villa Abecia the fruit trees are used as a hedgerow, which defines property lines or near the river where the land tends to slope or erode. The commercial farmers establish small orchards on their land. In La Merced farmers use both orchards and hedgerow planting of fruit trees. Acchilla farmers separate their fruit orchards and their fields of corn or onions. Fruit orchards in Acchilla consist of a mixture of the different fruit trees. For example the lemon tree or the avocado tree may be planted close to a cherimoya tree.

3.2 Politics

The Law of Popular Participation came in effect in 1994, thus changing the manner local government functions. Between 1994 and 1997 local government underwent substantial reorganization. The most significant changes occurred with resolution No. 216961 on March 14th 1997 (PDM-*Plan Desarrollo Municipal*, 2000).

For example Las Carreras was established as a new municipality with its own mayor. Prior to Law of Popular Participation, Las Carreras was a district of the municipality of Villa Abecia. For a few villages, Villa Abecia was a day's trip to address government issues. As this law was executed, the area received money to build an office building and other infrastructure improvements. Las Carreras became a new municipality in February 15th 1994.

A municipality is broken up into districts and each district has several communities (PDM-*Plan Desarollo Municipal*, 2000). When an election takes place each district votes for the party they would like to represent them. The winning party chooses the mayor. The representative usually makes it clear whom they will vote for as mayor. At anytime the winning party may decide to change the mayor. Periodically, the mayor will change for reasons unknown to the public. For example, the party may think the mayor is not fulfilling his promises so they replace him. However, he remains as a council member. In the larger districts, sub mayors are appointed by the mayor and approved by the council members. In the case of Acchilla, they voted for their own sub-mayor and the mayor and council members felt this method was acceptable.

When resolution No. 216961 was implemented a diagnostic was completed by the mayor's office to evaluate the needs of its people. Then a five-year plan was developed to establish goals. The municipality must execute projects present in this five-year plan and straying from the original plan is against the law. Of course the mayor is not expected to complete all projects but the plan is used as a guide for the projects to be executed over the five-year period. Several meetings were designed to have the community members decide what needs are present and to prioritize these needs. The mayor's office and the community would help determine a strategy to implement projects for the next five years.

With this program each municipality received about forty dollars per person to spend on projects which benefit the community. Often the money was insufficient and not all projects could be implemented in every community. Each community lobbies for their project by showing the municipality they are united and willing to work to complete a particular project. The mayor's office, the council members and a community group decide through extensive dialogue which project will be completed in a particular year. Community groups also participate in the project completion. The group ensures the company hired executes the project properly and the community members participate in its completion. This system, as described, has not been implemented perfectly and only in the last two years in Las Carreras and in Villa Abecia has each group been involved and fully participated. Participation at the local level is new to many and to ensure that community members are capacitated to execute this style of governing many NGOs are helping the municipalities. Villa Abecia and Acchilla are in municipalities which receive aid from DANIDA, the Danish development agency. This agency and others like it help effectuate projects, preventing money from being wasted or stolen for personal uses. In all communities complaints of former mayors stealing money is common. This system ensures completion of projects and the local community members can participate in their local government and have a say in what must be done to improve their livelihoods.

Many times the elected mayor or officers either have a high school degree or only have schooling up to the fifth grade. Although they are among the more motivated individuals in the community, running a local government may be a daunting task.

Therefore, as part of the reform, money was set aside to hire a qualified staff. Offices have an accountant, administrator, agronomist, civil engineer and lawyer either fulltime or part time on their staff. The local government usually does not have enough money to pay the entire salary of these people but several programs with aid agencies such as DANIDA and USAID ensure each town can recruit highly qualified staff. People in these positions have college degrees.

Part of the diagnostic includes the qualified staff trained to determine the needs of the community and fully participate in the execution of projects. Staffs can also initiate small projects in individual communities.

3.3 Acchilla, District 8 San Lucas, Nor Cinti, Chuquisaca, Bolivia

Figure 3.4 The Village of Acchilla

Acchilla is a small town of two hundred people in fifty households (Figure 3.4). The population resides in a small area of about a square kilometer. Amenities are limited to potable water but without electricity or latrines. Communication is by either a rural telephone or a radio, both run by a solar panel and battery. Many of the houses are as old as the village, three hundred years. Additions were constructed as the families grew. Construction material consisted of adobe, stones, and *caña weca*, which is similar to sugar cane but hollow inside and is used to make the roofs. (Figure 3.5)

Figure 3.5 Street in Acchilla

The village is about one hundred meters above the river. It is on a plateau of about a kilometer long and half a kilometer wide. Hills begin outside of the village on the eastern side. The western side of the valley rises dramatically. The road into the valley also runs down this side of the valley.

Acchilla is a rural town located five hours from the capital of the municipality; San Lucas. The dirt road to the capital is long and often times dangerous. Because of its distance, many NGOs choose not to enter the area and prefer working with communities with easier access. Two organizations do enter: the Catholic Church from Spain which has a convent located in the town and the Embassy of Denmark which provides doctors, nurses and farm extension workers. Before the road was constructed about three years ago the only way to enter was along the river valley or with horses.

Geography

The geography of the area has a significant impact on the culture and farming practices. Acchilla is nestled in a deep river valley. The mountains on the west side are rock with areas where it is considered arable farmland. The hillsides are used for grazing (Figure 3.6).

Figure 3.6 Sara B. Me, Patty (L-R) by western mountains of Acchilla

Outside Acchilla the remainder of the municipality consists of altiplano type farming system. At this altitude (3000 masl) the area is dry with some grasses and shrubs. Acchilla, however, is semi-arid and the river provides the water for the area (Figure 3.7). The permanent river provides water for drinking and irrigation all year long. The farmers also indicate they receive little rain but can irrigate their farms year round with river water.


Figure 3.7 Vegetation along riverbank in Acchilla

During the rainy season the river rises from runoff in the higher mountains. To enter Acchilla one must cross the river. The farmers tell me the climate is ideal, neither too cold nor too hot. Compared to other towns in the municipality they say that winter is not significantly cold, therefore their fruit trees sustain little damage. However, in the winter of 2001 they received about an inch of snow and there was damage to their cherimoya trees. The snow was a rare occurrence and many of the older farmers do not recall a winter with snow in their lifetimes.

Culture

The population is a mixture of both indigenous and Spanish descent. The culture is Incan. Quechua is the primary language for all residents. The adults are fluent in both Spanish and Quechua. The children and the elderly prefer to speak Quechua but do understand Spanish. The adults speak Quechua among themselves but are capable of communicating to strangers in Spanish. All town meetings are in Quechua but if a guest were not able to understand, the meeting would be conducted in Spanish or translated out of respect for the visitor.

Education

A fifth grade education is the highest level of schooling provided to the community children. Many families send their children to Camargo or San Lucas to further their education. However, these families are economically advantaged. Although the cost of education is minimal for a high school education, the transportation and living expenses create a hurdle for poorer families.

Preservation of the language and culture and educating the children to the best of their ability are difficult tasks for many teachers. Spanish is rarely spoken in family life and children will speak Quechua among themselves. Spanish is taught as an essential second language in the schools (Figure 3.8)

Figure 3.8 Acchilla boys under a *Chirimoya* Tree

Politics

Acchilla is the capital of District Eight in the Municipality of San Lucas, the Province of Nor Cinti, and the Department of Chuquisaca. (Figure 3.9) Several smaller communities make up the district. In Acchilla, a sub mayor had been appointed and had his office in the center of town. Several committees formed by the community work with the mayor and their activities to ensure project completion.

Figure 3.9 Map of the Province of Nor Cinti (Aguabolivia 2003)

Vegetation and Agricultural Products

The vegetation is typical of a semi-arid climate. Plants such as the aloe plant *Aloe sp.,* grasses, cacti and bromeliads are common. Several different trees surround the village and the farm plots. Many acacias or leguminous trees such as *Tarco Jacaranda mimosifolia*, the pepper tree *Schinus molle,* and various pines and eucalyptus trees are common (Figure 3.10). The eucalyptus tree occasionally surrounds the plots and the effects of the trees are obvious; cherimoya trees will not produce fruit.

Figure 3.10 Molle Tree

The natural vegetation density increases at village level near irrigation canals and the river edge. The vegetation becomes sparser as one climbs the east side of the valley. The irrigation of the small plots of fruit orchards helped some of these natural trees and vegetation survive. The soil is rich in organic matter.

The residents of Acchilla consider their land near the river the perfect place for fruit trees. This confidence enables them to try different varieties of fruits for production and explains the variety available in the area. The community contains motivated individuals willing to try new farming techniques.

Fruit trees are the main agricultural product in the vicinity of the town. Other agricultural products are grown higher in the hills. The main fruit trees are the cherimoya trees and the citrus trees. Several varieties of lemons are present: large lemons, small ball like lemons and lemons meant to be eaten like oranges. Limes are common as well but are included with the lemons and have the same Spanish name. Orange is a common citrus with different varieties present in the area: juice oranges, sweet oranges, tangerines, and mandarins. Other citrus include the grapefruit, although it is not as common.

Several varieties of avocados are also available: small black oval, round green, oval green, and black round fruits. The avocado tree grows next to cherimoya trees in Acchilla. This was an interesting aspect to note because in Spain the cherimoya is cultivated in deep alluvial soil with high lime content where the avocado does not grow well. Farré (1999) also mentions that the cherimoya is not demanding of soil type so it could explain why in Acchilla avocado and cherimoya grow well side by side. Acchilla's avocados are situated on the farms in the same manner as the cherimoya as an orchard, on terrace on sloping hills and hedgerows (Farré, 1999). Other fruit present in Acchilla include the guava and several apple varieties and these fruit trees are used for home consumption.

In the houses, Acchilla farmers have patio gardens where various hot peppers and different spices are grown. If they do not have much room in their patios they usually try growing varieties of spices or necessary herbs for cooking, such as are lemon grass, chives and strawberries, in their smaller plots. Another product grown for home consumption is the sugar cane, a treat for the kids.

In the hills the farmers cultivate their main cash crops: corn, onions, potatoes, peas, and *haba*. These crops are tolerant of harsher climatic elements than the fruit trees. The residents usually have small houses near the cash crop land. During harvest and planting, the farmers and their helpers will spend several days in the hills.

Animal husbandry is another farming activity for the Acchilla farmers. Cows bulls, goats and sheep graze the hillsides. Pigs and chickens are located in the houses and corrals or roam the streets (Figure 3.11).

Figure 3.11 Bolivian pig in corral

Some farmers herd llamas, alpacas and vicuñas in the hills. The farmers use cows and goats for milk and cheese, bulls are used to plow the land. Sheep, alpaca, vicuñas and llamas are used for wool and yarn. Pigs, chickens, goats, sheep and cows are used for meat.

Acchilla's Chirimoya Trees

Around the department of Chuquisaca and Bolivia, Acchilla's cherimoya trees are famous for the quantity of trees grown and the quality of fruit they produce. Stories circulate of the quantity of fruit, and much of the fruit is given away to visitors. Both farmers and residents of the surrounding communities repeatedly told stories of large fruits which have grown from the Acchilla trees. In our collection of the fruit the biggest fruit found was nearly two kilograms (Figure 3.12).

Figure 3.12 Acchilla women showing a large *chirimoya*

Natural forests of cherimoya are thought to be present in the area. Trees need little management to be productive and Acchilla's farmland is considered the ideal soil for the cherimoya tree.

The area has about eight hundred trees, although not all produce fruit. Seedlings located under the parent trees are a common sight. The productive trees range from five to over one hundred years old. The farmers observe erratic fruit production. One year they will have too many to sell and other years they will have few cherimoya fruit. It seems part of the reason is dependent on climate. Winter 2001 was unusually cold and had snowed. Some damage was seen on parts of the trees exposed to wind and sun as a result. (Figure 3.13)

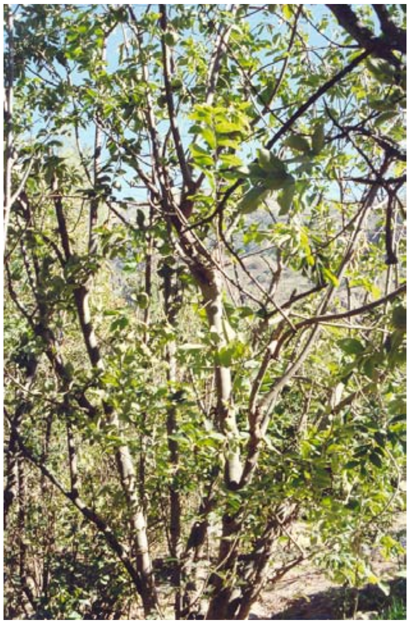

Figure 3.13 Young cherimoya tree in Acchilla

I began surveying and interviewing farmers in Acchilla starting with those farmers close to the river. The farms were situated in various locations along the river, at town level and farther up the mountainside. In certain instances the trees were grown along slopes and some farmers chose terraces for farming. Cherimoya trees grew in areas of mixed shade and sun. Limited exposure to the sun appeared ideal for the trees in Acchilla. On some farms the trees formed a forest. However it was noted the trees grown closer together did not produce as well.

History of the Chirimoya Trees

The residents of Acchilla do not recall when the cherimoya trees came to the area. The trees are permanent fixtures in the oral history of the area, and the presence of the trees is thought to be present as long as the ancestors had settled the area. Ancestors of the Acchilla farmers settled in the area approximately three hundred years ago. Some of the farmers believe their ancestors brought seeds and tree seedlings to the area to be cultivated along with other fruit trees. These ancestors were a mix of Spanish settlers and indigenous people which explains the Spanish looking people with much of the Quechua culture intact.

Farmer Interviews

Through the collection of cherimoya fruit the residents introduced me to the different types of cherimoya. The farmers explained to me the different positive and negative characteristics of each of the cherimoya varieties. The residents also pride themselves on the many good varieties available in the area.

The farmers indicated few problems with pests and diseases. I observed and was informed of an occasional tree with fungus or *gusanos,* fruit with fruit flies. What the farmers did observe, which they felt was a negative aspect, was that the trees may flower in abundance but fall off or do not produce the fruit expected. The farmers also informed me that some trees produce well one year and then suddenly never produce again. They noticed older trees diminished in fruit production, however every once in awhile they would have a tree which would consistently produce every year. It was agreed, however, the prime fruit producing time was between eight and forty years old. The soil in the area had high organic matter so little effort in fertilizing the orchards.

The farmers sell their fruit to merchants in Camargo, Potosi and Sucre. They will also take some fruit to the capital of the municipality, San Lucas. Some years the municipality has cherimoya fruit festivals. The remainder is sold, given to visitors, or sent to family members. During the 2002 harvest season farmers sold their fruit at Bs 5 per kilogram (US$ 0.70 per 2.2 pounds). Some of the farmers have their own trucks to haul out the fruit and other products. The journey out of the valley is difficult; sometimes the farmers are hindered by lower prices offered by the closest buyers.

3.4 Villa Abecia, Sud Cinti, First Section, Chuquisaca, Bolivia

Geography

Villa Abecia is a small town in the department of Chuquisaca in the province of Sud Cinti and in the Municipality of Camataqui of which it is the capital (Figure 3.14) (PDM-*Plan Desarrollo Municipal* 2000). The altitude is 2309 meters above sea level as written on their post as you enter the village. Villa Abecia is in a narrow valley. The west side has red mountains and the east side has white mountains (Figure 3.15)

Figure 3.14 Map of the Province of Sud Cinti. (Aguabolivia 2003)


Figure 3.15 Valley of Villa Abecia

I have been told these mountains have a lot of calcium and gold deposits. The road to Potosi intersects these mountains. A river also comes from the west flowing to the southeast.

Climate

The average annual temperature of Villa Abecia is 16.5ºC. The average minimum is 8ºC and the annual maximum is 38ºC (PDM –*Plan Desarrollo Municipal,* 2000). From my measurements of 2001-2002 it has been as low as 1ºC and as high as 38ºC throughout the year. When it rains it tends to lower the temperature. Since the rainy season is in the summer it helps moderate the temperature. According to the PDM or the five-year plan for the municipality of Villa Abecia the precipitation of rain each year can range between 250-400 millimeters per year (PDM-*Plan Desarrollo Municipal*, 2000). The rainy season is November through April, however the years I was in Bolivia we had rain once on June 30th 2002 and a few rain showers as early as September.

Although the amount of rain each year is an important factor in the agriculture system of Villa Abecia, a well-organized irrigation canal system is present for an added benefit to ensure farming can continue all year long. A high water table keeps the irrigation canal recharged (Figure 3.16).

Figure 3.16 Water pumping in order to construct retaining wall for irrigation canal

For example during the year 2001-2002 I measured two hundred millimeters of rain in my site, Las Carreras, twenty-five kilometers south of Villa Abecia and with a similar climate. It was a dry year and the smaller rivers dried up; however, the irrigation canals were constantly recharged with water. Also in both Villa Abecia and Las Carreras when they were building the dam for the improvement of the canal they dug underneath the riverbed level and had to pump water constantly as it was being built (Figure 3.16). In addition as one ascends the hills away from the rivers the land becomes drier (figure 3.17). All vegetation near the river such as the *molle* tree (*Schinus molle)* is able to stay green throughout the winter(figure 3.18).


Figure 3.17 Riverbed in Valley


Figure 3.18 Hills above river

Politics

Villa Abecia is the capital of the municipality and the capital of the province of Sud Cinti. The sub-governor office and the province's courts are located in Villa Abecia. The sub-governor oversees the activities of the entire province. He or she represented the governor of the department. The courts deal with province crimes and legal issues. In the past, money has been directed into Villa Abecia for many of the development projects because they are the first section and the capital of the province. One can see their community pride in the presentation of their main plaza. In the last twenty years they have made a great effort to improve their town. The plaza has become a park with towering palm trees, an olive tree and pine trees. Many flowers are present, predominantly roses. The plaza is the center of business. Buses from Tarija, La Paz, and Cochabamba stop for lunch and dinner. This helped the community flourish greatly. Currently fruit and refreshment stands are next to the plaza. The cherimoya fruit is sold at these stands and are sold rather quickly to passing travelers. In addition many restaurants are located in the plaza. For the community members it is often a place to socialize after a hard day's work in the offices, schools and on the farms. The Catholic church is also located in the central plaza. Nearby the mayor's office, the police headquarters for the region and other gubernatorial offices are situated.

The cherimoya farms are located contiguous to the town, which makes cherimoya a concern for the local government. The cherimoya is becoming a priority for the municipality. Research was completed in conjunction with the local government. Since Villa Abecia is fairly small, with approximately five hundred people, the mayor and his staff knew which farmers had cherimoya trees and had someone on staff acting as a mediator in the beginning of my research.

Culture

Culturally the town is quite diverse. Traditionally, few indigenous populations are present. The people are of Spanish descent with a mix of indigenous, Italian, and Germans who arrived at the turn of century and after or during World War II. The first language and the language commonly spoken is Spanish. Quechua is spoken but by older people or those who have migrated from the north for work and opportunity. Rock art and other artifacts indicate the presence of indigenous people in the past (Figure 3.19). It is thought the Incas conquered and absorbed these ancient people into the Inca Empire. Solid evidence of this was present just west of the area. (PDM-*Plan Desarollo Municipal*, 2000)

Figure 3.19 Rock Art

The area was settled as early as 1777 by the Spanish. The entire valley, which includes Las Carreras and Camargo and other communities, was considered important for fruit production. Being a geographic break in the *altiplano*, the climate was ideal for year round agricultural production. It was also an easy way to travel north or south since the valley gradually ascended to the *altiplano*. (PDM- *Plan Desarollo Municipal*, 2000).

Education

The school system is considered at urban level. The teachers are taught at a higher level to teach in an urban area. Special certificates are required by the teachers to teach at this level. Most children can receive a high school education. For those who live farther away there is a boarding house, which charges a low fee for room and board. Although the education is much better than in surrounding towns it still lacks resources. Improvement in education is a goal for the school system. The school system benefited from *La Reforma*. Courses are more intense and requirements for graduation are much higher than high schools in rural areas. Opportunity to take different courses is also present. Although the language spoken in the area is Spanish, Quechua and English are required courses.

Economics

The village of Villa Abecia is about one square mile. The houses are attached to one another. Several small stores are based out of these homes (Figure 3.20, 3.21), which carry a variety of food and supplies. Depending on the day a family will bake bread and sell it.

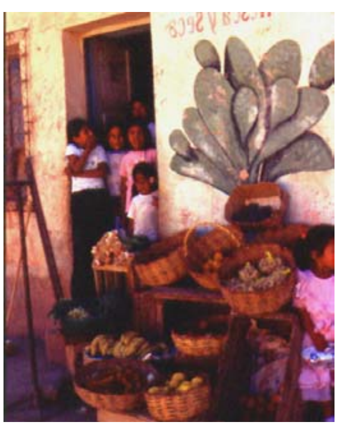

Figure 3.20 Produce store outside of home

Figure 3.21 Family store

They usually indicate they have bread by a white cloth over a chair or a stool outside the door. Usually two to three families a day bake fresh bread. In the main plaza about four families have taken advantage of the their plaza front houses and have dining establishments for the travelers who come through town. A market in the center has about six women who are cooking all day for hungry travelers. Also in this market food, snacks, alcohol (70% ethanol and wine), school supplies, goat cheese, and some fruit and vegetables are sold. In the plaza, several women have fruit stands. The stands are open year round. They sell either fresh fruit in the summer or dried fruit in the winter. One young woman told me she remembered as a young girl running to and from the plaza to her farm to bring fruit to sell. Because some families have small plots of land and a few fruit trees, the crops are not sufficient to send to the city markets, they sell to other travelers. In addition to fruit these small stands sell refreshments made from the fruit. One woman grills chicken pieces, intestine or liver for hungry travelers and townspeople in the plaza. She serves these with corn and boiled potatoes with a side of hot sauce. This happens to be a favorite of the Bolivians. Also one can find a stand selling sandwiches or the Bolivian version of hamburgers.

The main economic function of the community is farming. Families have a small home garden in their patios. Some of the cherimoya farmers actually had their trees in the patio alongside various citrus trees. Farmers live in town while in most cases their land is outside of town along the river. However, two of the commercial farmers own the majority of the land near the village, they are exceptions; their land extends past their patios. The rest of the farms are located along the river.

On the farms a variety of different crops are grown. They have many fruit trees including plums, peaches, figs, oranges, lemons, avocados, apricots, and *chirimoya*. Some farmers are beginning to establish orchards from some of these fruits. (Figure 3.22)

Figure 3.22 Peach or Plum Blossoms

One smallholder cherimoya farmer had a young orchard of plums. When interviewing the farmers many mentioned having land in the higher altitudes where they grew peaches. The peaches prospered in slightly colder areas than at town level. The Villa Abecia farmers had some vegetable cash crops grown at town level: onions, carrots, tomatoes, and potatoes. They also grew corn and alfalfa as fodder for their animals.

They had a variety of animals: sheep, cows, pigs, goats and chickens. The goats were mostly situated in the higher altitudes and are used for cheese making; the cows or bulls are used for labor, milk and meat. Pigs are grown for food around the holidays. The pigs are kept in corrals. Chickens are used for eggs and meat. Chickens are allowed to range free on the patio or farm.

Besides fruit trees, an important fruit they cultivate is the grape. With grapes they are famous for their wine and *singani*. I was told the grapes they grow for *singan*i are the best white grapes and give the best tasting *singani* in all of Bolivia.

The Cherimoya Trees

Villa Abecia is also known for their cherimoya fruit. The fruit trees are exotic to the area. The *chirimoya* has been cultivated in the area for nearly eighty years (Figure 3.23). *Chirimoya* and other fruit are displayed to travelers on their way to Potosi or La Paz.

Figure 3.23 Small land holding farmer proudly showing her *chirimoya* tree

The producers were just one family with a large amount of land. When interviewing some of the older farmers I found that the first trees were bought either in Tarija, Yacuiba or Sucre. Although it was not stated, some of their trees could have come from fruit they bought in Camargo, which would certainly be from Acchilla or the same valley. Many of the smallholders had trees in their patios. The commercial farmers began with trees in their patios and from there expanded to their farms. The commercial farmers each had between twenty and fifty producing trees, while smallholders had between six or seven productive trees (Figure 3.24).

Figure 3.24 Harvesting *chirimoya*

Villa Abecia farmers used these trees along with other fruit trees to control erosion along the banks of the river. One commercial farmer had land along a steeper bank of the river and used the trees in a terrace system.

All farmers had few problems with their trees. Slight pest problems were reported. The pests included aphids on the leaves and fruit flies on the fruit. They used some chemical pesticides and usually sprayed once a season. Some of the farmers were starting to use both chemical fertilizers and manure to increase productivity of the tree. As I was leaving some of the farmers were beginning to see results of fertilizing the trees before flushing of the leaves occurred. They saw healthier trees and flowers as a result.

3.5 La Merced

La Merced is the smallest town of the three but was once a commercial cherimoya-farming center in the area. La Merced has many trees but had many more in the past. Farmers once considered the cherimoya a cash crop. Cherimoya festivals were organized for several years bringing in commercial and non-commercial farmers to display and sell their fruit. Everyone in the area recalled the superior taste of the fruit and its quality. In some ways all of the farmers at one time would be considered commercial farmers, however now nearly all are considered smallholder. Nearly ten years ago an unidentified fungal disease entered the area. Trees began producing low quality fruit with an undesirable appearance (Figure 3.25).

Figure 3.25 Diseased leaves and fruit

Sometimes the fruit is inedible and the seeds are not viable (Figure 3.26).

Figure 3.26 Diseased fruit in La Merced

Some fruit product is still marketed by commercial farmers and they are putting a significant amount of effort into saving their trees. None of the farmers could explain what was attacking their trees. They said many farm extension agents came to analyze their problem many times in the last decade.

Geography

La Merced is located in the province of Arce of the department of Tarija (Figure 3.27). It is south of Tarija on the way to the southern small city of Bermejo, a border city on the Bolivia-Argentina border. It is the main access point into Argentina. La Merced is situated on the eastern side of the river Orosas (Aguabolivia, 2003). This river is permanent and fast moving. The rainy season does help augment the river. Population pressure is not a local concern. Plenty of water is present for all of the farmers. The irrigation canals are used in the farming system. Like the other sites La Merced is located in a narrow valley. On the west side of the town a paved road is located for travel to the north to Tarija or travel to the south to the city of Bermejo. Their road has been paved for about two years. Arriving into the area one travels down into the valley and the altitude drops significantly. The altitude is 1700 masl, which is the average for the area.

Figure 3.27 Map of Province of Arce (www.aguabolivia.org)

Before entering the La Merced valley, the drive to the town is in a dry area (Figure 3.28) and as one drives south of La Merced it becomes tropical and humid.

Figure 3.28 Land before entering La Merced

Climate

Data from La Merced was less available because it was the smallest of all the communities I surveyed. Through the data collection, interviews and simple observation I found many similarities in climate between La Merced and Acchilla. The vegetation was similar to Acchilla, indicating a semi-arid area. The rainy season starts in November and ends in March or early April in the same manner as the other sites. The primary difference was temperature. They told me their summers were hot and humid and the winters were not cold.

Vegetation and Agriculture

The hills had many grasses, shrubs and cacti. The area had many trees, Eucalyptus, *Schinus molle, Acacia spp.* and various different pines. Stands of pines and eucalyptus have been planted along the hillsides in reforestation efforts led by NGOs. The presence of epiphytes on the branches of the bushes and trees, indicate higher heat and humidity in the area. Lichen was also present in many of the older trees.

The farmland is present along the river with fruit trees, corn, alfalfa, and tomatoes. Citrus such as oranges, mandarins, and grapefruits are beginning to replace the cherimoya. They also have avocados next to the cherimoya trees. The farmers produce corn, tomatoes, and onions as their main cash crops.

The farmers own cows, bulls, and pigs. The bulls are used for labor and the cow and the pigs are used for milk and food respectively.

Culture

La Merced is in the southern part of Bolivia. Spanish and other Europeans settled the area. The area has been disputed many times by Bolivia and Argentina. La Merced is about twenty miles from an accessible border of Argentina. Oil has been recently discovered in the area making the region a priority for development and another reason for further disputes. As a result the farmers have better access to new technologies, markets, and *tecnicos* (farm extension workers) from different NGOs. Its access makes it ideal for these organizations to implement projects. The people can make day trips rather than staying overnight making it comfortable to complete projects often required of these agencies.

The farmers are a mixture of families present for generations and those who would be considered recent migrants to the area. In 1952 a revolution occurred which ensured those farmers who worked the land owned the land and many went to southern Bolivia to establish their own farms. Currently migration from region to region is considered a problem. Increasingly many from the *altiplano* of Bolivia are heading south to look for opportunity in farming. The community tends to be individualistic by family and is less likely to share new techniques. Usually a cluster of houses consists of extended families. The first three farms are of a sister, a brother, and a cousin. They share experience and knowledge among each other but not with many others. The lack of information sharing may be because of the fact the farmers are dispersed and they do not see each other as often as other communities do. The farmers of the area in general were receptive to me and were willing to elucidate the history of their plants. They had many problems and they were looking for answers to save their precious trees.

The Cherimoya trees

The entire town has about eight hundred cherimoya trees. Not all of the trees produce fruit. Everyone in Tarija knew about the cherimoya from La Merced. La Merced had orchards of cherimoya. They considered it one of their main cash crops. For a couple of years they set up festivals after the harvest to sell and show the best cherimoya in the area. Stands were lined up on the streets close to the farmland. Travelers coming through had access to the fruit. According to the farmers many people from many different parts of the municipality also brought their cherimoya. The festival, meant to be annual, occurred for only two years. Soon after the cherimoya fruit production started to decline and the fruit became infected with the disease. Although the fruit was of bad quality, two farmers can still sell their fruit to market. They had the majority of the trees and could gather sufficient fruit to sell. Also some of the small holders would sell locally or to travelers on their way to Bermejo.

Chapter 4 The Cherimoya Tree

Annona cherimola **Mill,** the cherimoya tree and its fruit the *chirimoya* is a subtropical tree found in many parts of the Americas. The cherimoya tree historically is an important aspect of the culture of South America. Evidence in the "form of terra cotta vases apparently modeled after cherimoya fruits" was found in Peru in an indigenous archeological site (Smith *et al*.1992). The cherimoya fruit has been growing as an economically important fruit throughout the Americas and gaining recognition in the world. In Bolivia, its importance as an alternative crop is being investigated to increase the income of the Bolivian farmer. These reasons justify the need for further research on many facets of the cherimoya tree and its fruit. The background provided here is essential to understand the main focus of the thesis, the genetic diversity of the cherimoya in southern Bolivia.

4.1 *Annonaceae*

Annona cherimola is in the family of Annonaceae within the order of Magnoliales, a primitive angiosperm. The Annonaceae is considered an important family because of its evolutionary, ecological and economic status in the world (Chatrou, 1999). The family provides the world with 'exquisite but often little known fruits'.

The genera of *Annona* contain one hundred and fifty species. A few species of *Annona* are adapted to drier conditions such as *Annona cherimola,* while the majority of the genera occur in lowland rainforests as in *Annona squamosa,* the sugar apple (Smith *et al*. 1992). The scientific names have only recently been established and reorganized in the family. Fifty new species were described for Annonaceae between 1995-1998 including several in the genus Annona (Chatrou, 1999). The cherimoya is often misnamed as either *Annona reticulata* or *Annona squamosa.* In Bolivia, the scientific community uses an older version of the Latin name *Annona cherimolia*. Cherimoya's common name varies in spelling from region to region; *cherimoya* in the United States, *chirimoyo* in Peru and *chirmorriñon* in Venezuela and *chirimoya* in Bolivia. *Annona* means annual harvest and *cherimola* or cherimoya comes from the Quechua name *chirimuya* which means cold seeds. (Pinto de Queiroz *et al*. 2002)

Annona cherimola produces the most esteemed fruit of the *Annonas* (Morton, 1987). Its fruit has been described as 'deliciousness itself' by Mark Twain and "Queen of the subtropical fruits" (Popenoe *et al.*, 1989). by others and the finest fruit in the world by Tijero (1992). The fruits of Annona are underutilized fruits growing mainly in underdeveloped countries of South America and Africa. Popenoe *et al.* (1989) names the cherimoya as one of the "Lost Crops of the Incas: little-known plants of the Andes with promise for worldwide cultivation".

4.2 Origin and Distribution

The cherimoya tree originates in South America in the subtropical valleys of the Andes. Controversy surrounds where the cherimoya originates and depending on the sources, the reported origin can range significantly (Smith *et al.* 1992). The general agreement is the origin exists in the Andean valleys of 1500 masl to 2600 masl (Fuertes Mendina, 1999). One of the assumed origins is in Ecuador in the Loja province in the south bordering northern Peru. Morton (1987) says Ecuador and Bolivia, but not Peru, is the origin. Others say Columbia, Ecuador, and Peru and not Bolivia are countries of origin (Popenoe *et al*. 1989). Once the Spanish conquered South America the seeds were transported throughout the subtropical world (Smith *et al.* 1992). The tree is commercially produced in Mexico, United States (California), Spain, Italy, Israel, Egypt, Taiwan, Australia, Brazil, Portugal and Central America. Markets around the world often have cherimoya fruit piled as high as apples and oranges. World demand for the fruit is high, Japan and the US are willing to pay $US 20 to $US 40 per kilogram (Popenoe *et al*, 1989). Since the publication of these references, the distribution and demand for the fruit has increased. Currently, one can purchase a cherimoya fruit for $US 2.99 in supermarkets around the US. (Wegmans, 2002)

4.3 Climate

A specific climate is required for cherimoya cultivation. Cherimoya trees do not tolerate excessive heat or cold but are resilient in dry highlands (Smith *et al.* 1992). The optimum climate for the cherimoya tree is an annual temperature range of 10°C and 30°C with an average temperature between 19°C and 25°C. The tree prospers in semi-arid areas with an annual precipitation between 650 mm to 1250 mm. The relative humidity favored by the tree is seventy-five to eighty-five percent. (Bydekerke *et al*., 1999) Other sources indicate varying climate conditions for the tree. The sources only differ by a few degrees in temperature or meters in altitude. Bydekerke *et al.* (1999) established a research protocol to determine ideal conditions in Ecuador.

4.4 Geography

Originally found between 1500 meters above sea level to 2600 meters above sea level the cherimoya tree has been domesticated at coastal altitudes in Peru and in California (Morton 1987, Popenoe *et al.* 1989). Tijero (1990) indicates the trees are found and grow along slopes in river valleys in the Andean Mountains. Many farmers use the tree along the rockier slopes and riverbanks of their farmland.

4.5 Soil requirements

Soil requirements are a sandy loam or sandy clay loam, slightly acidic at a pH of 5-6.5 and one to five percent organic material (Bydekerke *et al.*, 1999). Well-drained soil is another important factor. This was cited in Ecuador where it is considered to be ideal for the best cherimoya production. Other sources suggest an ideal pH is 6.5 to 7.6 (CRFG, 1996).

4.6 *Annona cherimola*: Morphology and Physiology

A small to medium tree, the cherimoya can grow to heights of seven to thirty feet or taller (Figure 4.1). Categorized as a briefly semi-deciduous tree it loses its leaves in early spring then quickly begins developing new leaves. In the early spring the tree will flush longer then most trees.

Figure 4.1 Cherimoya tree

The roots are initially tap roots and then spread laterally at a relatively slow pace. The root system is thought to be a weak system compared to other fruit trees (Pinto de Queiroz *et al* 2002, Morton 1987, Popenoe *et al*. 1989).

The leaves are single and alternate. It has two to four ranked leaves. They are classified as ovate-lanceolate to elliptical in shape. The leaves average two to eight inches long. In Bolivia the regions investigated showed a variation of leaf lengths. Some trees had many shorter ones and others were larger. The colors of the newly flushed leaves are a yellow green. Adult leaves are a darker green on the adaxial side and tomentos gray on the abaxial side of the leaf (Pinto de Queiroz *et al* 2002, Morton 1987, Popenoe *et al*., 1989).

4.7 Flowers

Flowering of the cherimoya tree begins between two and six years. The flowers are single and small and found on bare parts of the branches. Sometimes they are alone other times they are clustered in one area. Thick fleshy petals are stiff in touch and yellow-brown in color. In the center of the flower is a cone shaped syncarpium of stigmas where it receives the pollen. Classified as hermaphroditic, the flower enters two distinct phases of male and female stages. Although hundreds of flowers bloom in spring, time of flower maturity varies from flower to flower. The first phase is the female phase for approximately thirty-six hours then it enters the male stage. The female organ is no longer receptive when it enters the male stage so it is thought it is nearly impossible for self-fertilization to occur within the same flower or the same tree. In the female stage it emits a fragrant and sweet smelling odor until it enters the male stage. This is for the attraction of pollinators (Morton 1987, Popenoe *et al*. 1989, Pinto de Queiroz *et al* 2002).

4.8 Fruit

The fruit is somewhat heart shaped. It can be between four and eight inches long and four inches in width. It can weigh between three hundred grams and three kilograms with an average between three hundred and five hundred grams. The skin can be either thin or thick with a variety of textures; smooth, fingerprint indentations, protuberances and a combination. The skin is a yellowish green color and on the inside is a fleshy white with brown-black seeds; each has a sack in which it is enclosed (Figure 4.2)

Figure 4.2 Chirimoya guarding her cherimoya fruit

Numerous cultivars have been identified in the cherimoya industry. They have names for each of them in both California and Spain. Defined botanical forms have been determined for the cherimoya. *Forma impressa* is cherimoya fruit with fingerprint like indentations. *Forma laevis* is a smooth form which is sometimes considered cherimoya *lisa*. Wart like tubercles or a protuberances come from the 'fingerprints'; which in Spanish it is referred to *tetillas* (small breasts): *Forma mamillada.* The last form is *Forma umbonada* which is characterized by thicker skin and many seeds (Pinto de Queiroz *et al* 2002,). Morton (1987) discusses many of the different varieties characterized by Peruvian and Californian cherimoya farmers. Some of the names given to these varieties are "*Lisa*- Almost smooth *Impresa* with fingerprints, *Umbonado* with round protrusions; *Papilonado or Tetilado* with fleshy nipple-like protrusions; *Turberculada* with conical protrusions". Names used in Peru include *Chavez, Ñamas, Sander, Rio Negro, Concha Lisa Bronceada, Concha picuda terciopelo* and *Basta.* Names in California include *Bays, Whaley, Deliciosa, Booth, Mcpherson, Carter, Ryerson, White, Chaffey, Ott Horton, Golden Russet, Loma, Mira Vista,* and *Sallmon* (Morton, 1987). These names are not only given for skin texture but for average weight and time of harvest. For example some of the earlier varieties can be harvested as early as March in Bolivia and others will have fruit as late as August or September. Different varieties have different average seed numbers and within a variety larger fruits typically have more seeds. (Morton, 1987, Popenoe*, et al*., 1989, Pinto de Queiroz *et al. 2002)*

4.9 Production

Although South America may be the origin of the cherimoya it has one of the lowest commercial production rates in the world. Spain, Chile, and California are the main commercial producers of cherimoya. California has approximately one hundred and twenty hectares of cherimoya orchards producing 900 tons per year. Chile has seven times more cherimoya orchards and Spain has thirty times more orchards than California. Fruit is sold to supermarket chains and specialty fruit stores. Demand for the fruit is increasing. (Grossberger, 1999)

Cherimoya fruit is not just sold fresh but used for different fruit products. Ice cream, sherbet and cookie filling are some uses of the cherimoya in South America. However, consuming the fruit fresh or in a fruit salad remains preferred.

4.10 Propagation

Propagation is an important aspect of the tree in terms of production and cultivation. George and Nissen (1987) cite recommendations by Leslie (1922), Campbell and Phillips (1983) not to propagate from seed because *Annona* species generally have an uneven and irregular germination rate, primarily due to genetic diversity and the cherimoya seeds need to be treated. It is thought the seed coat protects the embryo resulting in the need for scarification. Another thought is the seeds are dormant for periods of time. De Smet and Van Damme (1999) analyzed the problem by applying different treatments. Pinto de Queiroz *et al* (2002) suggests each study has different conditions and different seeds would explain the variation. It is agreed that the seeds need an ideal medium to germinate and dormancy occurs until the environmental conditions are ideal (Pinto de Queiroz *et al.,* 2002).

Seed germination results in trees which give low quality fruit. Seeds taken from trees which produced high quality fruit for some reason had progeny which did not produce the fruit of the same quality. This was observed by farmers in Bolivia. Farmers in commercial areas use grafting as a way to accelerate flowering, increase fruit quality and increase yield (Tijero 1992, Popenoe *et al.*1989, Morton 1987). In California and in commercial orchards grafting has become an almost exclusive practice. For economic reasons commercial farmers must have high quantity and high quality fruit. (Grossberger, 1999)

Propagation by seed needs an ideal balance of conditions. George and Nissen (1987) cite Saneweski (1985) and state an optimum germination temperature is between 28ºC and 32ºC and germination occurs within three weeks. At lower temperatures germination can be delayed for two months (George and Nissen 1987). Once germinated the seedlings grow relatively quickly. Year one through five the tree concentrates on growth. Flowering begins in year four, sometimes as early as year two or as late as year six.

In the commercial orchards different techniques are used for ensuring high quality trees and fruit. Trees in California are irrigated with mini-sprinklers or drippers. Fertilizers are applied through the irrigation system. Potassium and potassium sulfate is commonly used. Pruning is another technique used to improve fruit quality and quantity. Currently California growers are researching the best pruning styles (Grossberger, 1999).

4.11 Pollination

In South America, research studies by George *et al.* (1992) establish the nitidulid (*Carpophilus* and *Uroporus*) beetles as possible pollinators. Rotting pineapple attracted more of the nitidulid to the cherimoya trees. Increased populations of the beetle increased fruit set. Pollination of the cherimoya trees is one of the horticultural obstacles to fruit production. In areas where the tree is not native, there is no natural pollinator. Honeybees and other bees do visit the cherimoya flowers but are ineffective (Morton, 1987).

In an area where the nitidulid beetle is not native hand pollination is the alternative for ensuring pollination. When a pollinator is not present, the flower only has less than 1.5% chance of being pollinated by the wind or self-pollination (George *et al.* 1987). An economically effective hand pollination became established in 1941 in California. Other areas of the world began using this technique soon after. A painter's brush is used to collect pollen from open flowers in the male stage. The pollen is put into a "Condor cup" then the pollen is brushed or blown on the female flowers within twelve hours before the pollen looses viability. The pollen can be stored in a refrigerator. It is labor intensive and requires exact timing because of the different flowering stages. On one tree hundreds of flowers can be present but each is in its own stage. The first stage is the female stage where the petals are tightly closed. As it moves into the male stage the petals open widely. Early in the flowering season only male flowers or only female flowers are present, making this technique difficult (Grossberger 1999).

4.13 Pests

In South America the Cherimoya fruit and tree is susceptible to different pests and diseases. The fruit fly (*Ceratitis capitata, Anastrepha sp.)* lays larva on the skin of the fruit and the larva burrows into the fruit. As a result the fruit falls earlier and entrance of fungus and bacteria is easily facilitated. The fruit's flavor and color changes making the fruit unmarketable. Other pests include *Pinnaspis aspidistrae* and *Phyllocnistis sp.* (Tijero, 1992). In areas where the farmer has pest problems with the other fruit trees, the cherimoya is equally susceptible.

4.14 Diseases

At each stage of the development of the tree a disease is described for the cherimoya. During seed propagation the tree is vulnerable to a fungus called *Rhizoctonia solani*. *R. solani* attacks the seed, roots and neck. *Odium Sp* attacks the leaves, flowers, fruits, and stems leaving a white powder. *Botrytis cinerea* is a fungus, which attacks the flowers in humid climates.

The fruit itself has its own diseases increasing the difficulty of marketing the fruit. The disease agents *Botrytis cinerea, Rhizopus stolonifer, Penicillium,* and *Phomopsis sp.*

are secondary attackers when the fruit is injured or handled carelessly during transport to market (Tijero, 1992).

4.15 Other Issues

The cherimoya tree has been described as delicate and finicky about the conditions of climate and when the climate is not ideal the susceptibility of the tree to pests and diseases increases (Tijero, 1992). Sweet (1990) states "freezing weather in the winter and low humidity combined with high summer temperatures are the most detrimental factors limiting commercial production." Popenoe *et al.* (1989) indicate the tree is frost sensitive and less hardy than the avocado or oranges. Damage is present on adult trees after a few nights of frost and freezing temperature in Bolivia. Fruit production after a winter of harsher temperatures can hinder the fruit production the following season. At temperatures above 82°F cherimoyas produce less fruit (Popenoe *et al*., 1989). However the tree does grow well in heat (Sweet, 1990).

Soil can facilitate diseases and other problems, Tijero (1990) informs his readers to avoid planting the tree in soil with little drainage where the tree is susceptible to root rot.

4. 16 Genetics of Cherimoya

Early Genetics

Interest in genetics, migration, evolution, and ecological history dominated the study of this genus during the 1960's and 1970's. Annonaceae is an old angiosperm in the order of Magnoliales. This fact has had many of the earlier geneticists interested in looking at the genetics of the family. Walker (1972) discusses the research of what was the original chromosome number of Angiosperms. *Annona* genus has seven and fourteen chromosome numbers. Walker (1972) suggests *Annona* tribe branched off early in the evolution of the family. He also suggests five polyploid changes and seven aneuploid have occurred within the family.

1980s

The 1980s brought acknowledgement of the potential of the cherimoya. Morton (1987) and Popenoe *et al.*(1989) both wrote about the cherimoya explaining its potential. Morton (1987) discusses some of the challenges and what perhaps can be used for genetic work. Popenoe *et al.,* (1989) discusses the research needs of *Annona cherimola*. The first on his list is germplasm.

> *The danger of losing unique and potentially valuable types is high. A fundamental step, therefore, is to make an inventory of cherimoya germplasm and to collect genetic material from the natural populations as well as from gardens and orchards, especially through the Andes.*

Popenoe (1989) further discusses the need to look for improved varieties, finding seedless varieties, determining the pollinator perhaps to move away from hand pollination, defined cultural practices and other horticultural improvements.

In 1987 Ellstrand and Lee (1987b) clarified confusion different cultivars by developing isozyme markers. Ellstrand and Lee (1987a) also looked at the loci between cultivars. The researchers found variation in isozyme patterns among cultivars so a particular cultivar can be distinguished using this technique.

1990's

Genetic research was dominated by Perfectti *et al.* (1996, 1998,1999) and colleagues (Pascual and Perfectti,1993) . He continued work in cultivar identification of the Spanish cherimoya and further research in isozyme loci. Randomly Amplified Polymorphic DNA markers were used to identify *Annona* cultivars. Successful use of fifteen primers to identify differences between *Annona cherimola, Annona reticulata, Annona muricata*, *Annona squamosa* and their hybrids. Ronning *et al*.(1995) felt since RAPDs were found to be useful for studying conifers, usage in old angiosperms should be successful as well.

Current

Genetic research is occurring in Spain at the agriculture station "La Mayora" near Granada. Currently researchers are developing simple sequence repeat (SSRs) markers for better identifying different varieties in the germplasm bank present at the institute. In another part of the institute work on micropropagation and transformation is being conducted. A protocol has been established for tissue culture using adventitious organogenesis (Encina 1999). This has led to work on the transformation of the cherimoya to down regulate the ACC gene to slow ripening of the fruit. This has been successful using the agrobacterium mediated transformation. (Encina 1999 and personal communication 2003)

Chapter 5 Materials and Methods

While the main objective of my research is looking at genetic diversity, it required six preliminary steps. The first stage of preliminary fieldwork consisted of gathering qualitative data informally and to determine the research sites. The second part was visiting the communities and obtaining initial information about each research site. In the month of June 2002, I traveled to all sites to obtain fruit samples from different farmers from each site. I interviewed the farmers and systematically selected fruit to be harvested for seeds. The seeds were then taken to the US and planted in our greenhouse. Once the seedlings were grown the young leaves were used to extract DNA. The DNA was analyzed using the polymerase chain reaction or PCR based randomly amplified polymorphic DNA or RAPD technique. The data finally was analyzed then conclusions drawn about the genetic diversity at regional, and commercial versus small landholder farms

5.1 In the Field

Preliminary Field work

In the fieldwork I used participant observation, an anthropological technique. According to Bernard (2002)"Participant observation fieldwork is the foundation of cultural anthropology" (Bernard, 2002). Since I was a Peace Corps Volunteer part of my training included skills in development, obtaining information, and diagnosis of community needs. This skill helped me become a better volunteer and researcher. This method also involves significant amount of qualitative collection of data. Data include notes of what one sees and hears in an environment, photographs of the area, the people and their every day actions (Bernard 2002) (Figure 5.1 and 5.2)

Figure 5.1 Young girl in Las Carreras

Figure 5.2 Girls in the Plaza

Once at my site, I observed everyday events people participated in and I took pictures of both the natural setting and the people. Participant observation also involves learning a new language and experiencing the lives of the people (Bernard 2002). As a volunteer I improved my Spanish and adjusted to the community. I accomplished this by having lunch or dinner with various families. (Figure 5.3)

Figure 5.3 Eating a special lunch with Students

I also went to the farms in the community. Through observation and community involvement, I was able to establish rapport with the people in the community and I was able to gather much information I needed to do this thesis.

Even though Las Carreras was not one of my research sites, my role as a participant observer helped me gather the information needed before I could thoroughly develop a way to procure information about the three research sites. Since Las Carreras is located geographically in the middle of the area I was studying, I was able to collect sufficient background to prepare me to efficiently acquire information from the research sites. The people and cultures were quite similar at each site. Since all these research sites consider themselves southern Bolivian, history and development are essentially analogous. The community consisted of people who were familiar with at least one of the three sites. Many of the residents' families have been in the area for generations, and knew where cherimoyas were grown both currently and historically. When I explained my interest in the cherimoya, the names of the three research sites came up often because they each were known for quality cherimoya fruit. Many of the people in this community had contacts or could direct me to someone to guide me to these sites.

Fieldwork

For the second part of my fieldwork for this thesis, I used informal interviews or what Bernard (2002) would call semi-structured interviewing. He defines this as open-ended interviews. These types of interviews follow a general script and cover a list of topics (Bernard 2002). For each of the regions I developed a set of questions I could ask but were sufficiently flexible so I could modify and apply them according to the site and situation. Figure 5.4 is the questions I used as a guide. I chose the route of a semi-structured interview because it was formal enough so the farmers knew why they were being interviewed but not so formal that the farmers were intimidated. In some cases there was distrust from past experiences with farm extension workers. However, this technique helped keep the farmers relaxed. I gained rapport and professionalism, which Bernard mentions as a benefit of this technique.

Interview Questions

1. **How old are the trees?**
2. **Are they grafted?**
3. **What chemicals do you use to combat problems?**
4. **What kinds of problems are there with the trees or the fruits?**
5. **Where do the trees come from (seed source)?**
6. **Are the trees consistent in producing fruit?**
7. **How often are the trees watered?**
8. **Are there any techniques used commonly to improve production?**

Figure 5.4-Questions asked

The research sites I chose are situated around Tarija. The first site was Villa Abecia, which is Northwest of Tarija, the second site was La Merced, south of Tarija, and the last site was Acchilla, which is directly north of Tarija. Each site was deliberately chosen. The research sites were relatively distant from one another to develop the best possible picture of regional diversity. For example, another town within the same area of Villa Abecia would not be considered a unique site because of its proximity to Villa Abecia. Each of the sites chosen had different soil types, mountain formations and altitudes.

In each site the environmental conditions, number of trees, number of farmers, the problems which arose in production, the growth strategy and markets for the fruit were noted. Informal interviewing was applied with a focus on issues surrounding their trees. Additional information, to confirm what was seen, was collected from the mayor's office of each province. In one of the situations I was able to have a group meeting with some of the farmers. During the visit I had an informal group discussion and asked questions about their *chirimoya* trees (Eckman, 1996). When necessary I asked for clarification of what each individual had to say. The community leader acted as an intermediary by clarifying different issues, while others nodded their agreement. This discussion led to a tour of the nearby farms, which further clarified different aspects of what I was researching. During this time, I was able to focus the questions that I needed to ask. Specific information was later gathered by interviewing the farmers as we collected the fruit in June 2002. The original lists of questions were modified according to the site. For example, whether the trees had been grafted was not asked after the first site visit. Some answers were basically the same for all farmers in one area.

In all cases, I had someone in the community or a Bolivian *técnico* to help clarify some of the questions asked. This technique has become an important aspect in scientific research in other countries where there is a language barrier or simply a cultural barrier. Boa *et al.* (2001) describes *el técnico* as a semi-professional who either has a high school degree or a basic university graduate with no post graduate degree. I chose a high school graduate who knew the area, its people and culture. She also grew up in the area helping her parents farm their land. She became a cultural broker (Boa *et al*, 2001). She was also able to help at the other sites. While I could ask a question in Spanish, sometimes the farmer would misunderstand what I intended from the question. Therefore a Bolivian mediator could explain what I actually meant in local terms.

Each site was visited two or three times, Villa Abecia receiving the most visits since it was relatively close and Acchilla having the least visits since the distance was long and the accessibility was treacherous. However in this case contact by phone was used often. The farmers were organized, which helped me collect the necessary information. Usually the first visit was to determine the number of farmers, explain what

I was doing and ask for their assistance. I also began noting the environment, geography and climate. In addition other observations included the location of rivers or irrigation canals, the water source, how the soil looked at each site, the general geography and ecology. The culture of the area was also noted with the interactions I had with the farmers and the villagers in general. I compared the three areas and made some conclusions.

My first attempt to collect genetic samples was in November 2001. Leaves were collected and they were preserved in silica gel. I put twenty grams in Ziploc bags. The silica gel was distributed rudimentarily because of the lack of scales and other instruments in a small village. Fresh leaves were collected and torn into small pieces. Chase and Hills (1991) felt tearing the leaves rather than cutting the leaves with scissors actually did less damage to the DNA. The leaves were put in the bags with the silica gel. After twelve to forty-eight hours the leaves were checked for brittleness. The first indication of insufficient drying occurred at this time. The leaves did not appear as brittle as Chase and Hills (1991) indicated they should be. When the leaves appeared dry and after the twelve hours required drying time I removed excess gel and left a few grams to ensure no moisture entered the sample (Chase and Hills, 1991).

In this pilot trial, I collected from three farms, from approximately five trees at each farm. I tried to obtain a variety of trees. For example, we collected from older trees and from younger trees trying to make the distribution as even and representative of the farm as possible. We prevented the clustering of samples by walking around the farm in a systematic way. We started at one end and made a circle around the farm. We also randomly picked from a group of trees in the center of the farm.

I brought the samples to the US and mailed them to Michigan Tech. The DNA was extracted with the DNeasy® protocol by Qiagen Inc. (2000) from the samples and analyzed for concentration and quality of DNA. Electrophoresis gel was used to determine quality of the DNA. The concentration was determined by the Nanodrop (Nanodrop 2002), an instrument equipped with a computer program, which measures the absorbance and extrapolates the concentration of the DNA.

The pilot trial of collecting leaves was not successful; little DNA could be extracted from the samples. We went to the next option, collecting fruit and harvesting the seeds. The collection in June 2002 followed the protocol established in the pilot trial. From each site we collected from forty to sixty trees, and two to three fruits from each tree. We tried to get one big fruit and one small fruit from each tree.

I separated the farmers from commercial and non-commercial farmers. There are three commercial farmers and six to eight smallholder farmers at each site. I defined the farmers in the preliminary visits, and from initial interviews. I differentiated between the farmers by the following criteria: commercial farmers have nine-three hundred trees, fruit is sold to a major city, and the farmers consider the fruit trees a substantial part of their income. These farmers were conscious of increasing fruit production by using different chemical pesticides or fertilizers. Smallholder farmers had between one and eight trees either on their land or in the patios of their houses. Smallholders used their fruit for home consumption, which meant they ate their own fruit, sold or gave the fruit to neighbors or sent their fruit to relatives in other cities.

A plot of land where there is continuous farming defined a ‘farm’. In some cases farmers owned several plots of land, but each plot were considered a farm. During the collection process we gave each tree a tag with a specific code so we could return later. Fruit from the trees were carefully coded in the same manner on their skin and on the newspaper in which they were encased. Observations of the fruits were written down for further use if necessary. On all the farms the code was unique. I usually asked for two fruits from each tree, although I received between one and five fruits. After the fruit was collected they were wrapped in newspaper and allowed to ripen in a warm room where the sun entered. The seeds were taken out of the ripe fruit carefully by spoon or by hand. The germination rate of seeds in contact with saliva is much lower according to the local impression, so the cherimoya fruit was not eaten. Later, if there were left over fruit we made milk shakes from it.

Some of the seeds showed signs of developing fungal contamination. With the advice of Dr. Jose Salinas, the seeds were washed in warm water to get rid of any fungus, mold or other plant material, which could end up being rejected by the USDA-APHIS. The seeds were dried in open air for one or two days and packed in zip lock bags (Salinas, personal communication 2000-2002).

5.2 Greenhouse

Once the seeds arrived at Michigan Technological University space was set up in the greenhouse for them. Before planting the seeds in the greenhouse I developed a protocol with seeds in Bolivia for growing the seedlings. A large planting pot was obtained and filled with two-thirds organic matter (highly decomposed manure) and one-third sand for drainage. The dry cherimoya seeds were directly planted in a large planting pot. The pot was watered every two days until germination. The first seedling germinated in three weeks. Up to twenty out of the two hundred planted germinated within two months. However, winter was arriving and the temperature was falling. I protected the seedlings with a cotton dishcloth every night throughout the winter. Eight seedlings survived the winter. The seeds were planted in March and by May germination seemed to finish. As we entered the month of September the temperature started rising and about twenty additional seedlings germinated.

In the greenhouse we tried to replicate this natural setting. Two to six seeds from each sample bag were planted in a small plastic pot with rich potting soil. Several different protocols were attempted to see which one would work better. They were watered every day when they were first planted to thoroughly soak not only the soil, but allow enough moisture for seeds to absorb the water. The greenhouse was constantly at 70ºF/21ºC. The first set of seedlings received twenty-four hours of light while the second sample seedlings have been getting twelve hours of daylight. The first seedling from the second group germinated at three weeks and thirty additional germinated in the fourth week. Within two months 60% of the plants had germinated. Of the Acchilla seeds all but two pots with five seeds had germinated. Seeds from trees which did not germinate were replanted and if there was no seeds left to plant from the particular seed set the second set of seeds from the same tree was planted. During this replant seeds from fruit, which had previously not been planted, were propagated.

The germination rates were higher then the documented 10% germination rates for untreated seeds. (De Smet and Van Damme, 1999) Therefore a separate random germination test was done to establish a rate. In order to obtain a random sample from each region, I first randomly selected from each ziplock bag two or three seeds from each fruit with seeds available. I mixed the seeds up and randomly selected one hundred seeds. A total of three hundred seeds were obtained. I mixed the seeds and two hundred seeds were chosen. In each of the eight pots I planted twenty-five seeds. The pots were watered, according to previous protocol, each day. When one seedling germinated I recorded the date and time then took the seedling out of the pot to ensure it was not counted again.

5.3 Laboratory

After the seedlings were at few inches in height, the fresh leaves were taken to the lab and their DNA was extracted. For the DNA extraction the Qiagen DNeasy® protocol was followed using the buffers provided (Qiagen, 2000). The DNA was then run on an electrophoresis gel to determine its quality. The final concentration was determined by using a Nanodrop instrument (Nanodrop, 2002). With these materials, I was able to begin the Randomly Amplified Polymorphic DNA (RAPD) analysis for polymerase chain reaction (PCR) based DNA amplifications using primers with arbitrary nucleotide base sequences (Biotools, 2003), in the thermocycler (Perkin Elmer 1995). "PCR is a technique for the in vitro amplification of specific DNA sequences by the simultaneous primer extension of complementary strands of DNA" (McPherson *et al*, 1991). These reactions were necessary to assess polymorphic bands on an electrophoresis gel. In order to do this, the protocol was set up through some trial runs in the Thermocycler (Perkin Elmer 1995).

Components	ul/rxn	Concentrations
10x REDTaq buffer	**2.5ul/rxn**	**1X**
$MgCl_2$	**1.9ul/rxn**	**3mM**
dNTPs	**.25ul/rxn**	**10uM**
Taq polymerase	**1ul/rxn**	**.05 units**
Primer	**3.5ul/rxn**	**28uM**
DNA template	**1ul/rxn**	**~25ng**
ddH_2O	**14.85ul/rxn**	**up to 25ul**

Table 5.1 - PCR Mix

The PCR was conducted according to the information provided by Sigma REDTaq™ Polymerase (Sigma-Aldrich, 2003). In order to establish the unique protocol for *Annona cherimola*, I also followed a similar protocol by Promega (Promega, 2001) in some of the trials. Graduate students in the Plant Biotechnology Research Center in the School of Forest Resources and Environmental Science provided other information. The final levels for all these components are listed in Table 5.1

The PCR reaction was carried out with a thermocycler (Perkin Elmer 1995). As in its name implied, the reactions were cyclic. The procedure is also variable and must be optimized depending on the species or process. For RAPDs and *Annona cherimola* I programmed the machine to do the following:

- Preheat step to 94°C for five minutes;
- One minute at 94°C to denature the DNA,
- Temperature lowered to 37°C for annealing for one minute
- A rise in temperature to 72°C for extension.
- Repetition for forty-five cycles
- Final step: ten minutes at 72°C.

- Finish at 25°C.

Thirty random primers were screened for polymorphism or PCR amplificability (Perkin Elmer 1995). The twenty best were chosen for analysis of genetic variation. Nine demonstrated clear polymorphic bands. Table 5.2 shows the primers with their sequences, which were chosen for the analysis

Primer code	Sequence 5' to 3'
1. OP-26-05	**GGAACCAATC**
2. OP-26-16	**GATCACGTAC**
3. OP-26-09	**TCGGTCATAG**
4. OP-26-14	**GATCAAGTCC**
5. OP-26-07	**TCGATACAGG**
6. OP-26-08	**TGGTAAAGGG**
7. OP-26-15	**GATCCAGTAC**
8. OPM-6	**CTGGGCAACT**
9. OPM-9	**GTCTTGCGGA**
10. OPM-11	**GTCCACTGTG**
11. OPM-12	**CGACGTTTGG**
12. OPM-13	**GGTGGTCAAG**
13. OPM-16	**GTAACCAGCC**
14. OPBF-04	**GACAGGTTGG**
15. OPAB-12	**CCTGTACCGA**
16. OPAB-08	**GTTACGGACC**
17. OPAB-17	**TCGCATCCAG**
18. OPAB-16	**CCCGGATGGT**
19. OPBF-06	**TCCACGGGCA**

Table 5.2 Primer Sequences

Various levels were tested for an ideal concentration level. Several different concentrations were attempted. Concentration levels tested ranged from 0.5 ng to 200 ng per 25-µl reaction. A level of ~25ng per reaction was best. Concentration levels of *Taq polymerase, dNTPs,* and buffers were varied to obtain an ideal balance. Ideal results are shown in Figure 5.5.

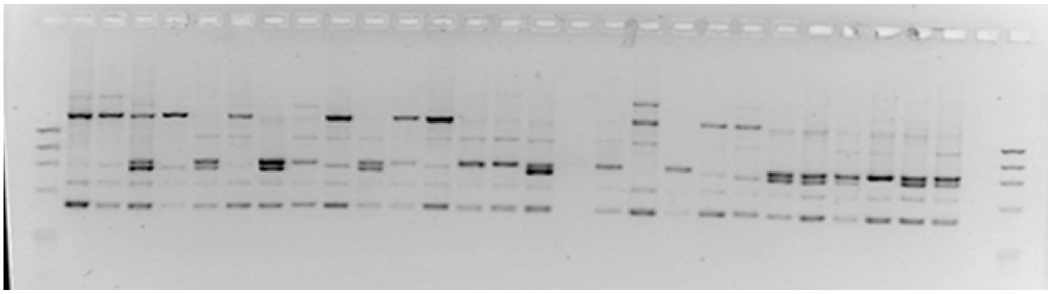

Figure 5.5- Polymerase chain reaction

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Statistical setup

Once the data was collected by the process of RAPDs, the PCR products or the bands seen on the electrophoresis gel were analyzed. One to four polymorphic bands were chosen from each primer and scored as one for present and zero for absence. Zero values were transformed to two for statistical analysis. Twenty-one polymorphic bands were found and used for statistical analysis. The first test using the program TFPGA based on Lynch and Milligan's (1994) Taylor expansion estimate. Heterozygosity (diversity) were determined for each population of the regions studies and for populations between commercial and non commercial farmers. An exact test for population differentiation was also done if there is significance between regions and populations. Finally genetic distance was determined between regions and between populations of small landholders and commercial farmers.

Chapter VI Results and Discussion

This study attempts to address two main questions. First, how genetically similar or diverse are the two groups: commercial farmers and the small landholder farmers. Second, how diverse or similar are the three sites: Acchilla, Villa Abecia and La Merced. Preliminary data was obtained by twenty-one RAPD markers identified from nine primers out of the twenty primers screened. All selected markers are 100% polymorphic according to the statistical program based on Lynch and Milligan suggestions (1994). Ronning *et al* (1995) established their protocol in the same manner as (Figure 6.1).

Primer	**# of loci**	**Location of Base pairs**
I-OPM-6	3	1,400; 872;500
N-OP-26-13	1	890
H-OPM-11	3	1,150;875;300
O-OP-26-14	4	1,200;950;850;750
Q-OP-26-15	3	2,000; 1,395; 872
S OPM-16	1	900;
B OPM-13	2	1,100; 200
G OPAB 8	3	1,600 1,078;600
F OPM 9	1	800

Figure 6.1 Loci used for analysis

6.1 Regional Level

Genetic distance was not related to geographic distance. Villa Abecia and Acchilla were genetically close to one another even though physical distance was a five-hour travel time. La Merced showed the most genetic distance from the other two sites (Table 6.1 and Figure 6.2).

Nei's original (1972) distance			
Population	VA	ACA	LM
VA	**		
ACA	0.0	**	
LM	0.0154	0.0152	**

Table 6.1 Genetic distance

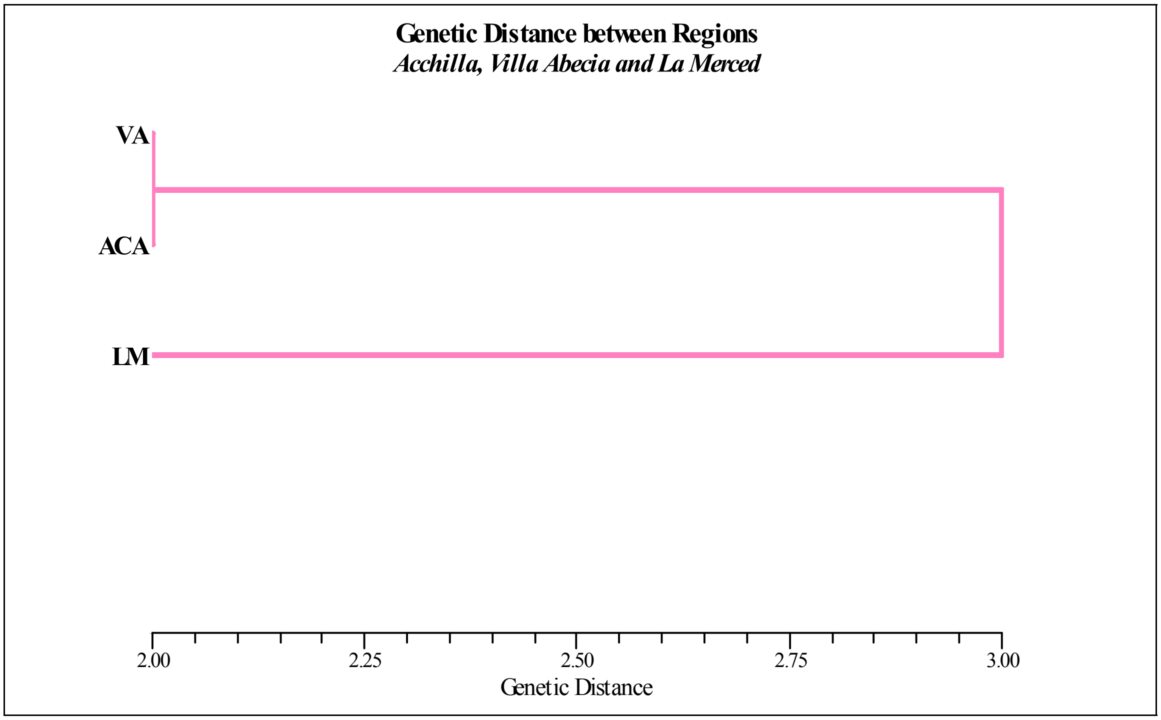

Figure 6.2 Genetic distance between regions

6.2 Commercial and Small Landholders

Differences between the two types of farmers were minimal and not statistically significant. The only significant difference was seen between small landholder of La Merced to small landholder of Villa Abecia and Acchilla. The P levels were 0.0494 and 0.0550 for these sites. According to the genetic distance estimation (Figure 6.3 and Table 6.2), Villa Abecia commercial farmers are genetically closest to Acchilla commercial farmers. In Table 6.2 populations of the three sites are divided between commercial and small landholder. The first digit in the code (*e.g.* 1-1) represents the communities: 1, Villa Abecia; 2, Acchilla; and 3, La Merced. The second digit signifies each site's subpopulation as 1 or 2 for commercial or small landholder

Nei's original (1972) distance

Subpop	1-1	1-2	2-1	2-2	3-1	3-2
1-1	*****					
1-2	0.0166	*****				
2-1	0.0000	0.0166	*****			
2-2	0.0165	0.0000	0.0165	*****		
3-1	0.0326	0.0225	0.0326	0.0223	*****	
3-2	0.0297	0.0363	0.0297	0.0358	0.0405	***

Table 6.2 Genetic distance among Commercial and Small landholder by region

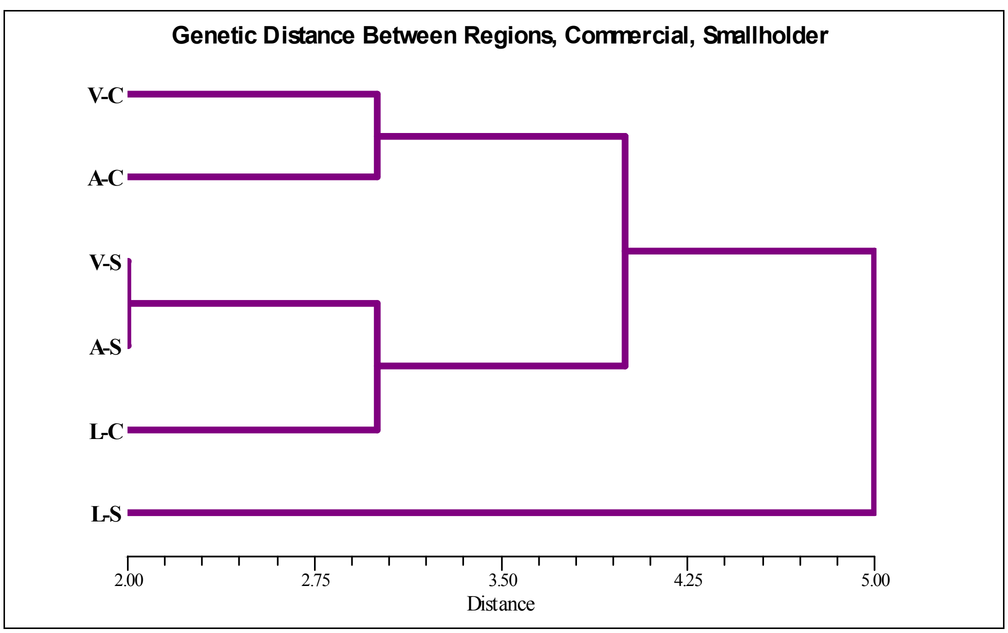

Figure 6.3 Genetic distance between Commercial and Small holders by Region

One explanation from the genetic information and farmer interviews could be the commercial farmers of Villa Abecia purchased fruit which originated from Acchilla. Both sites sell their fruit in Potosi and Camargo region, the two cities closest to both sites where fruit could be sold or sent. Villa Abecia small landholders were virtually the same as Acchilla small landholder suggesting exchange of seeds between small landholders. Within the entire study area, farmers may have familial ties. Further study of migration pattern of people may provide evidence why the tree is genetically similar. When interviewing farmers in both areas I did not delve deeply in this issue. Farmers indicated migrating family members look for land and work in communities such as Villa Abecia. Also, children of the commercial farmers of Acchilla received their education in small cities with easy access to Villa Abecia. It was not difficult to find a young farm extension worker in Villa Abecia who grew up on one of the commercial farms of Acchilla.

The most genetically distant population was the La Merced small land holder farmer. This site is the most geographically distant of all three. It has little contact with the other two sites. Farmers traveling to other areas of the country would have no reason to visit either of these sites. It is also possible that the La Merced seedlings initially came from Argentina or Chile. One of the farmers mentioned a grandfather who obtained seedlings from Yacuiba, a border town. If this farmer obtained the four seedlings, he could have obtained them from populations which are genetically different from the *chirimoyas* originating in the Acchilla area. The other possibility could be that one of the small landholders obtained seeds from *Annona Squamosa.* The fruit and tree of *Annona Squamosa* is sometimes confused with *Annona cherimola*. Ronning *et al.* (1995) state that closely related species readily cross-pollinate giving the hybrid *atemoya.* I suspect this because La Merced appears to be the border of their natural range of both species. The hybrid *atemoya* most resembles the *chirimoya*. Its fruiting range is in the lowlands of the Americas. The La Merced valley quickly changes over to lowlands soon after traveling past La Merced. The fruit of the atemoya can be easily mistaken for one of the different varieties of *chirimoya.*

6.2 Genetic Diversity and Heterozygosity

Heterozygosity is "a measure of the amount of genetic variation at a given locus within a population" (Kendrew, 1994). In other words the higher the heterozygosity the higher diversity there is in the population. The program TFPGA calculated diversity within the population for each polymorphic band. This number was averaged for all loci and the entire population. Diversity within the commercial farmers and small land holders were calculated so that we could determine which population has more diversity. Although all areas have high heterzygosity a trend appeared when one looks at the individual numbers. As expected commercial farmers had slightly lower diversity in all sites while small landholders had higher diversity. However, this is not a statistically significant difference. The estimated genetic diversity for Villa Abecia is 0.3844 or 38.44% heterozygosity, Acchilla has 0.3851 or 38.51% and La Merced 0.3787 or 37.87% heterozygosity. Heterozygosity between the commercial and small landholder were the following; Acchilla: Commercial 35.7 % versus Small landholder 39.89%; Villa Abecia Commercial 35.7 % versus Small landholder 39.79%; La Merced Commercial 35.56 %

versus Small landholder 38.43%; Encina *et al* (1999) states the cherimoya is known for its high heterozygosity Ronning *et al* (1995) found a heterozygosity for both commercial cherimoya varieties of being approximately 30%. Ronning *et al.* (1995) also compared the heterzygosity to the other *Annona* species which he was analyzing and they were much lower then the cherimoya. Appendices 1-6 show the data generated from the statistical analysis.

6.3 Geography and Climate of the Cherimoya Tree in Bolivia

Common features were present in each of the sites such as what the cherimoya tree preferred in terms of geography and climate. The assumptions that can be made based on these sites are that the cherimoya tree favors a semi-arid area with low humidity. Location of the sites was in narrow valleys and situated along permanent rivers. The farming system kept the area an ideal for the tree because in all three cases a well maintained irrigation system was present. The farmers provided the water necessary for all trees.

According to Bydekerke *et al.* (1999) all three sites fit in the good and moderate suitability classes. Villa Abecia and Acchilla have an ideal temperature range for the cherimoya tree. The winters in the three research sites were cool enough to give the cherimoya its required cold to induce the brief deciduous stage (Morton, 1987). All sites also have long growing periods for the cherimoya tree and other crops. In most cases farmers were able to have a cash crop for each season. Villa Abecia and Acchilla had one aspect, which places them in the unsuitability class; they receive less then six hundred millimeters of rainfall per year; permanent rivers nearby compensate for the lack of water. The sites also had lower humidity than what was expected for an ideal cherimoya tree site (Bydekerke *et al*. 1999). Studying all three sites suggests the tree in Bolivia actually did much better in lower relative humidity as long as it received the necessary water, or the equivalent of receiving 650 mm to 1250 mm of rainfall (Bydekerke *et al*. 1999). An indicated fungal disease problem was observed at La Merced site. High temperatures and humidity could be a factor affecting the problem. The tree is sensitive to high temperatures and high humidity. Evolution of the tree indicates an avoidance of the lowlands where humidity and high temperatures dominate.

6.4 Soil

In terms of soil the best site was Acchilla. The soil was fertile with a significant organic layer. Farms in Villa Abecia and La Merced had less than ideal soil type for the cherimoya. Addressing this issue, the farmers in Villa Abecia supplied the trees with manure or chemical fertilizers. This was common practice among these farmers for most of their crops. This helped significantly with their production. Little evidence of fertilizing the trees was found in La Merced. However, one farmer noted that one of his trees which sat on a septic tank performed much better in growth and fruit production than his other trees. As I was finishing my service in Bolivia, some of the smallholder farmers used manure to fertilize their trees and were seeing significant amount of improvement: the trees had more flowers as a result.

6.5 Propagation and Germination

Most sources agree the cherimoya has characteristically irregular germination (De Smet and Van Damme 1999, Pinto de Queiroz *et al*., 2002, George and Nissen, 1987). However, these sources disagree on germination rates, scarification and storage. Some suggest one must plant the seed as soon as possible or it will loose its viability, others recommend saving the seeds for three to five months (Pinto de Queiroz *et al*., 2002). Although germination is irregular seeds germinated sequentially rather than simultaneously. Sources discuss this as a problem with the cherimoya tree. For the small landholder knowing an ideal procedure of germination of the cherimoya tree is vital. Recommendations for procedures to ensure germination are in Figure 6.4.

1. Extract seeds from ripe cherimoya fruit
2. Seeds should be cleaned and dried for protection from common fungal or pest infection
3. Plant in well-drained soil with high organic matter: A mixture of sheep/cow manure with sandy loam soil of 1:1 is ideal. Too little organic matter will hinder germination
4. Keep well watered: Water everyday or every other day
5. Plant during times of temperatures of between 60°F and 80°F. If cold nights are unavoidable cover with cloth to protect from freezing
6. Place pot or section of garden with cherimoya seeds in an area semi-sun/semi-shade for overall protection of heat/cold.
7. Replant to Farm or large pot at three months
8. Protect tree as necessary from animals and climatic adversity

Figure 6.4 Germination protocol

6.6 The Disease

When interviewing the farmers as I began at the highest point of the town and worked my way down the hill into the main area and farther down outside, I saw a natural progression of the fungal disease. Those at the top of the hill remember being infected about ten years ago. At town level they said they began seeing the disease about seven years ago and further south of the town the disease began five years ago.

6.7 Symptoms

According to the farmers the first signs of the disease began after the second year of the annual *chirimoya* fair. During the flowering period the flowers appeared sick and with brown blotches. Also during this period of time it rained more frequently than average. The bark on the cherimoya trees also showed signs of being infected. When the fruiting began some fruit were infected immediately with brown/black splotches until the entire fruit was mummified. A mummified fruit is hard as a rock and dry with little evidence it had once been a green fruit. One farmer had trees growing near the courtyard of their house and she mentioned sweeping the fallen mummified fruit every day. Some fruit made it to the edible stage but would show undesirable and perhaps unmarketable marks on the skin of the fruit. Flavor of the fruit did not change in these cases. However the sections of the fruit which had the undesirable blotches were not edible. The leaves were infected as well with brown black blotches. The fungal spores appeared to travel by air to nearby trees. It is still not clear what causes the disease, however the disease makes the fruit susceptible to secondary fungal disease and pests. The fruit was susceptible to molds and fruit flies making harvesting the seeds undesirable. Farmers informed me of extension workers looking into the disease but not providing any concrete solutions. Some farmers have begun to cut down the cherimoya trees and use it for firewood rather than salvaging the tree for fruit.

6.8 Factors Associated with the Disease

High temperatures and humidity was a major factor observed by the farmers as a hindrance. The farmers noticed hotter summers in the last ten-year period than the previous years. Rainfall was high and, to the farmers, excessive. However, gravitational irrigation systems were well established. Trees were either weakened as a result or were weak from exposure to high temperatures. Calcium is mentioned in articles as an essential macronutrient for the tree in order to build defenses. Lowered defenses from a combination of these stresses may result in the susceptibility to the disease.

6.9 Native or Exotics

When interviewing the farmers on origins of the tree seedling came from, La Merced and Villa Abecia farmers could tell me a grandfather or a large landholder brought seedlings from nearby cities. Others indicate using the seeds from fruit they bought from the local market or city market and while others had one tree where seeds from the particular tree were used for establishing their current orchard. In La Merced most of the trees came from one farm in the area. Villa Abecia seem to have had the most farmers bringing in the tree seedlings from other areas of the country. Acchilla farmers did not bring in any new seedlings. Farmers pride themselves as stewards of the land of the *chirimoya.* The population of cherimoya trees has been in the area for generations. Natural regeneration occurs on the farms and in wild populations of the cherimoya along the river valley.

Acchilla also fits the description of areas where cherimoya grows wild in Ecuador. The sites would be considered in the moderate class and with some characteristics would be considered in the ideal range (Bydekerke, 1999).

6.10 Recommendations and Discussion for Field and Laboratory Procedures

Collection and Preservation of Samples

Collection of the cherimoya samples is difficult due to the distance of orchards from any major city. Two of the three sites were a one-day trip to the city on unpaved roads. Also, in Bolivia liquid nitrogen is hard to obtain. For DNA extraction leaves must be preserved in liquid nitrogen to stop the metabolic actions so the DNA does not deteriorate. Alternatively, the silica gel technique, as described by Chase and Hills (1991), has been developed to preserve the leaves and was attempted. However, this technique was not as useful as hoped. The DNA appeared to have deteriorated because the leaves have defenses against drying. The leaves of most plants have waxes, which prevent the loss of water, and the wax thickness will vary depending on the environmental conditions (Kramer and Kozlowski, 1979). Since the cherimoya tends to grow in areas where a periodic drought exists and is considered semi-arid, naturally the leaves will have the wax needed to prevent loss. The cherimoya had sufficient waxes to prevent the silica gel from taking its water. An example of the cherimoya tree's tolerance to drought came from a farmer who told me he had stopped using land with cherimoya trees, eucalyptus and several other fruit trees. When he returned to his land years later many trees were dying or were dead because they had been accustomed to receiving irrigation. However he did find his cherimoya tree was still alive while his eucalyptus had died.

Further research is needed to determine a better method of collecting leaves from the cherimoya trees for sample preservation and collection because of several difficulties besides the problems listed above and by Chase and Hills (1991). First the collection of fruit on a farm was limited to the trees producing in that year. Most of the farmers could not predict how much fruit would be produced. The amount of production had much to do with the climate in that particular year. The winter before I began collecting in June of 2001 was a rather harsh year in terms of cold. In Acchilla it had snowed. Signs of cold stress were observed in the trees. Dead branches were the result of the snow and the exposure to the wind or were the taller parts of the tree. In La Merced it was not possible to select samples in a systematic way; we were confined to the trees with fruit which were healthy or semi healthy. Some trees had fruit but had been consumed by the disease so the seeds were probably not viable. Some fruit was dried up and stiff with irregular seed appearance. Collection was from as many trees as possible from each farm.

6.11 Recommendations of Laboratory work

PCR work and gel electrophoresis pictures protocols were not consistent in the products. In order to do quality work some time consuming processes are necessary. My recommendation is to rerun the primers which did not give results because PCR or electrophoresis failed. Only nine out of twenty gave consistent results which could be analyzed for preliminary results. Six of the eleven primers which did not have sufficient data for analysis can and should be rerun in order to give a broader and more reliable picture.

Statistical Analysis

Genetic distance, heterozygosity, and diversity was determined by the computer programs for such analysis. However, more in-depth statistical analysis should be done to accurately determine the diversity level of the farms. This is important for management plans of the cherimoya in Bolivia. It can be assumed a good diversity is present because of different phenotypes of fruit.

Chapter VII Conclusions

Several important recommendations and observations have evolved from this research. The Cherimoya tree and fruit is an important agricultural crop for many Bolivian farmers. Its value in terms of environment, prevention of erosion on the farmland and the economic benefits, make conservation and improvement efforts a priority. Overall these populations are diverse, however more can be done to continue to fully reap the benefits of the tree situated in its original ecological range. My preliminary research in the genetic variation in south central Bolivia defines a need for a management plan. Although diversity is relatively high, signs of decline are present. As small landholders become sophisticated in breeding and selection with the cherimoya the danger of monoculture for crops such as the chirimoya is always present. Economic markets demand unbruised smooth cherimoyas. Farmers know what the consumers want. When doing research in all of the sites farmers were very excited to show me their best trees and disregard trees, which produced fruit of lower quality. Knowing this I insisted on gathering samples from those as well. The following paragraphs will be recommendations developed through my research.

7.1 Recommendations for each of the Sites

Technical assistance and information about the tree and fruit is an important request from each of these areas. Acchilla and La Merced are small towns either far from their municipalities or one of the smaller villages. Leaders from these sites need to lobby their town halls for technical assistance. Villa Abecia, the capital of their municipality, is fortunate enough to have a mayor and town council interested in developing the fruit as one of their strengths in fruit production. The mayor of Villa Abecia should request Peace Corps, PASACH and other organizations to receive technical assistance in fruit production, tree health and commercial marketing.

Developing sites for future orchards in the surrounding communities will also assist in increasing production. Throughout my research I observed several sites, which could serve as ideal areas for developing the cherimoya, benefiting the entire area by increasing income and augmenting the local economy. One site is my Peace Corps site Las Carreras. Villa Abecia and Las Carreras could work together to establish cooperatives, further developing the area for fruit production.

Finally when local farmers sell their fruit in other cities the farmers should purchase fruit from other areas of Bolivia to increase the diversity among their cherimoya trees. Also many times the leaders of the area would travel to cities such as La Paz, which have fruit from Peru, Chile and Ecuador. Integrating seedlings from these areas could perhaps produce varieties, which are more resistant to the pests and diseases in the area.

7.2 Recommendation for Peace Corps Bolivia

Since I began my efforts to research the cherimoya and help farmers become aware of the benefits of their trees, other Peace Corps volunteers have also become aware of the cherimoya. As I was leaving, an agricultural volunteer was developing a project for a cherimoya orchard in her site. The cherimoya trees of these three areas are diverse but could benefit from seed exchange from other sites around Bolivia. Peace Corps volunteers could develop an IST for a cherimoya seed exchange, as well as sites where the people could cultivate the cherimoya. Farmers would benefit from an introduction of a fruit tree to diversify their farmland and prevent erosion. Their children can benefit from the nutritional value of the fruit.

Peace Corps could also facilitate a conference among cherimoya farmers, developing plans on simple tree improvement and pruning and other horticultural techniques to produce more and better quality fruit yield. From the sites, where I interviewed farmers, many of the problems can be addressed by simple solutions for the farmer. Other future efforts could include developing better ways to market the fruit and perhaps export the fruit to other areas of the world. This simple project follows the policy of Peace Corps Bolivia. This simple seed exchange and training conference could encourage prevention of erosion, increase farmer income and increase diversity to prepare for a future disease or pest attack, which could destroy a crop upon which a farmer could be partly dependent.

7.3 Recommendations for Future Research for the Cherimoya

The cherimoya tree is an important fruit tree in terms of economics for poverty stricken South American countries. The cherimoya, with a South American origin, indicates the highest diversity is present in the area making the trees a conservation priority. Conservation of the tree needs to be brought to the attention of the governments of these countries so we may not lose the diversity. Europe and the United States have the facilities to conserve the tree. The orchard owners in United States and Spain would also benefit from conservation because of their economic interest in the cultivation of the fruit. A larger genetic base allows for more experimental or new varieties when developing the cherimoya tree. Farmers and researchers of these producing countries should make efforts to emphasize the importance and promote awareness of diversity present in these countries. Information exchange is vitally important as well as conservation efforts.

Limited research efforts are currently underway in Spain, the number one commercial producer of cherimoya. Germplasm banks are present in Spain and California. Spanish researchers have developed a micropropagation protocol and are currently developing SSR marker for genetic analysis of the germplasm. Emphasis on determining genetic variation of the species is an important step towards conservation.

7.4 Improvements of the Cherimoya

Further research in improving the cherimoya is also important. Even though heterozygosity (diversity) is high among these populations they are still susceptible to diseases, pests and climatic extremes. The cherimoya is sensitive to high temperatures and freezing temperatures. Research in improving its resistance to many of these problems such as cold and disease resistance should be a priority. Production in all areas is limited to a narrow temperature range. With evidence of global temperature change losing a species such as the cherimoya may one day be a possibility thus losing an evolutionary and ecologically important species.(Chatrou, 1999) Currently limited research suggests the *Annona cherimola* is capable of being transformed using *Agrobacterium tumefacins* and a micropropagation protocol has been established. These factors open many possibilities for genetic improvement. An improved *Annona* *cherimola* tree which has its own defenses against fungal diseases, pests and climatic adversity will not only benefit the cherimoya's survival but the farmers who cultivate it around world. Farmers from countries such as Bolivia will not be hesitant to grow the cherimoya tree on their land, knowing little spraying and protection is needed thus solving many environmental factors affecting the farmers. These factors also hinder the farmers' productivity. Improved cherimoya trees could prevent erosion in a susceptible subtropical region, reduce the pesticide and herbicide which not only saves the farmer money but results in a cleaner environment (water and air) and finally another fruit product to increase their annual income.

Cherimoya, belonging to an evolutionarily older genus and family than most species studied at the molecular and genetic level, could make a case for traditional genetic studies. Perhaps making it a model species for fruit trees and other trees in functional genomics and biotechnology will benefit other species with similar problems. Once research is done on this species because of its position in the evolutionary line, the information found about it could easily transfer to other trees. For example increasing its cold tolerance could be a step towards looking at increasing the cold tolerance of a highly valuable tropical wood or pulp tree. Cold tolerance and disease resistance genes have been transformed and expressed in different plants. However, for cold tolerance, the model genome used is *Arabidopsis*, a weed. Certainly it is valuable model genus especially for basic research in the field of cold tolerance, however transferring this knowledge to trees may be difficult. Work has been done on the *Populous* (cottonwood) to be used as the model tree species, however, it is not a tropical species.

Finally the genetics, the problems farmers are presented with, and the potential of cherimoya should not stop here. This research is preliminary and should be a stepping-stone for in-depth study in all aspects of fruit tree genetics, tree improvement and improving the livelihoods of South Americans farmers.

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Appendices

Exact Test

28/4/2003 12:27:21 PM
Analysis of C:\TFPGA\KRISTI.DAT
Data set contains genotypes of individuals sampled from
subpopulations within populations.
Organism Type: Diploid
Marker Type: Dominant
H-W Equilibrium Assumed.
Allele frequencies estimated based on Lynch and Milligan's (1994) Taylor expansion estimate.

Exact tests for population differentiation (Raymond and Rousset 1995)
# of dememorization steps: 1000
# of batches: 10
# of permutations per batch: 2000

Pairwise analysis of all subpopulations
Groups compared: 1-1 vs. 1-2
locus 1 : p = 0.7401 S.E. :0.0042
locus 2 : p = 0.3943 S.E. :0.0037
locus 3 : p = 0.0981 S.E. :0.0050
locus 4 : p = 0.7546 S.E. :0.0046
locus 5 : p = 0.7360 S.E. :0.0047
locus 6 : p = 0.4924 S.E. :0.0040
locus 7 : p = 0.2023 S.E. :0.0044
locus 8 : p = 0.5123 S.E. :0.0052
locus 9 : p = 0.1141 S.E. :0.0059
locus 10 : p = 0.2125 S.E. :0.0081
locus 11 : p = 1.0000 S.E. :0.0000
locus 12 : p = 0.3193 S.E. :0.0110
locus 13 : p = 1.0000 S.E. :0.0000
locus 14 : p = 0.7369 S.E. :0.0036
locus 15 : p = 1.0000 S.E. :0.0000
locus 16 : p = 0.1440 S.E. :0.0036
locus 17 : p = 1.0000 S.E. :0.0000
locus 18 : p = 0.1933 S.E. :0.0051
locus 19 : p = 0.7564 S.E. :0.0031
locus 20 : p = 0.3520 S.E. :0.0095
locus 21 : p = 1.0000 S.E. :0.0000

Results over loci
X-sq   : 34.3790
df   : 42
Overall: p = 0.7924

Groups compared: 1-1 vs. 2-1
locus 1 : p = 1.0000 S.E. :0.0000
locus 2 : p = 1.0000 S.E. :0.0000
locus 3 : p = 1.0000 S.E. :0.0000
locus 4 : p = 1.0000 S.E. :0.0000
locus 5 : p = 1.0000 S.E. :0.0000
locus 6 : p = 1.0000 S.E. :0.0000
locus 7 : p = 1.0000 S.E. :0.0000
locus 8 : p = 1.0000 S.E. :0.0000
locus 9 : p = 1.0000 S.E. :0.0000
locus 10 : p = 1.0000 S.E. :0.0000
locus 11 : p = 1.0000 S.E. :0.0000
locus 12 : p = 1.0000 S.E. :0.0000
locus 13 : p = 1.0000 S.E. :0.0000
locus 14 : p = 1.0000 S.E. :0.0000
locus 15 : p = 1.0000 S.E. :0.0000
locus 16 : p = 1.0000 S.E. :0.0000
locus 17 : p = 1.0000 S.E. :0.0000
locus 18 : p = 1.0000 S.E. :0.0000
locus 19 : p = 1.0000 S.E. :0.0000
locus 20 : p = 1.0000 S.E. :0.0000
locus 21 : p = 1.0000 S.E. :0.0000

Results over loci
X-sq   : 0.0000
df   : 42
Overall: p = 1.0000

Groups compared: 1-1 vs. 2-2
locus 1 : p = 0.7384 S.E. :0.0029
locus 2 : p = 0.3928 S.E. :0.0065
locus 3 : p = 0.0902 S.E. :0.0057
locus 4 : p = 0.7639 S.E. :0.0038
locus 5 : p = 0.7387 S.E. :0.0038
locus 6 : p = 0.5053 S.E. :0.0072
locus 7 : p = 0.2102 S.E. :0.0071
locus 8 : p = 0.5177 S.E. :0.0049
locus 9 : p = 0.0959 S.E. :0.0025
locus 10 : p = 0.2155 S.E. :0.0085
locus 11 : p = 1.0000 S.E. :0.0000
locus 12 : p = 0.2993 S.E. :0.0088
locus 13 : p = 1.0000 S.E. :0.0000
locus 14 : p = 0.7405 S.E. :0.0029
locus 15 : p = 1.0000 S.E. :0.0000
locus 16 : p = 0.1508 S.E. :0.0036
locus 17 : p = 1.0000 S.E. :0.0000
locus 18 : p = 0.2091 S.E. :0.0099
locus 19 : p = 1.0000 S.E. :0.0000
locus 20 : p = 0.3621 S.E. :0.0046
locus 21 : p = 1.0000 S.E. :0.0000

Results over loci
X-sq   : 33.9519
df   : 42
Overall: p = 0.8074

Groups compared: 1-1 vs. 3-1
locus 1 : p = 1.0000 S.E. :0.0000
locus 2 : p = 0.3554 S.E. :0.0076
locus 3 : p = 0.7268 S.E. :0.0039
locus 4 : p = 0.4833 S.E. :0.0046
locus 5 : p = 0.1381 S.E. :0.0053
locus 6 : p = 0.2819 S.E. :0.0082
locus 7 : p = 0.0455 S.E. :0.0035
locus 8 : p = 0.2986 S.E. :0.0071
locus 9 : p = 1.0000 S.E. :0.0000
locus 10 : p = 0.7165 S.E. :0.0043
locus 11 : p = 0.1319 S.E. :0.0037
locus 12 : p = 0.1612 S.E. :0.0093
locus 13 : p = 0.3147 S.E. :0.0053
locus 14 : p = 0.7326 S.E. :0.0036
locus 15 : p = 1.0000 S.E. :0.0000
locus 16 : p = 0.0049 S.E. :0.0009
locus 17 : p = 1.0000 S.E. :0.0000
locus 18 : p = 1.0000 S.E. :0.0000
locus 19 : p = 1.0000 S.E. :0.0000
locus 20 : p = 0.0229 S.E. :0.0029
locus 21 : p = 1.0000 S.E. :0.0000

Results over loci
X-sq   : 48.7538
df   : 42
Overall: p = 0.2197

Groups compared: 1-1 vs. 3-2
locus 1 : p = 0.0687 S.E. :0.0048

locus 2 : p = 0.3273 S.E. :0.0034
locus 3 : p = 0.4570 S.E. :0.0054
locus 4 : p = 1.0000 S.E. :0.0000
locus 5 : p = 1.0000 S.E. :0.0000
locus 6 : p = 1.0000 S.E. :0.0000
locus 7 : p = 0.3285 S.E. :0.0042
locus 8 : p = 0.2509 S.E. :0.0042
locus 9 : p = 0.0596 S.E. :0.0033
locus 10 : p = 0.7082 S.E. :0.0037
locus 11 : p = 0.2124 S.E. :0.0067
locus 12 : p = 0.2479 S.E. :0.0062
locus 13 : p = 0.4654 S.E. :0.0049
locus 14 : p = 0.2688 S.E. :0.0057
locus 15 : p = 0.4641 S.E. :0.0032
locus 16 : p = 0.0057 S.E. :0.0006
locus 17 : p = 1.0000 S.E. :0.0000
locus 18 : p = 1.0000 S.E. :0.0000
locus 19 : p = 0.4583 S.E. :0.0062
locus 20 : p = 1.0000 S.E. :0.0000
locus 21 : p = 0.4436 S.E. :0.0052

Results over loci
X-sq : 45.5817
df : 42
Overall: p = 0.3253

Groups compared: 1-2 vs. 2-1
locus 1 : p = 0.7393 S.E. :0.0050
locus 2 : p = 0.3952 S.E. :0.0063
locus 3 : p = 0.0939 S.E. :0.0034
locus 4 : p = 0.7634 S.E. :0.0037
locus 5 : p = 0.7413 S.E. :0.0051
locus 6 : p = 0.4907 S.E. :0.0052
locus 7 : p = 0.1909 S.E. :0.0070
locus 8 : p = 0.5213 S.E. :0.0046
locus 9 : p = 0.1083 S.E. :0.0059
locus 10 : p = 0.2062 S.E. :0.0044
locus 11 : p = 1.0000 S.E. :0.0000
locus 12 : p = 0.3127 S.E. :0.0065
locus 13 : p = 1.0000 S.E. :0.0000
locus 14 : p = 0.7415 S.E. :0.0031
locus 15 : p = 1.0000 S.E. :0.0000
locus 16 : p = 0.1497 S.E. :0.0043
locus 17 : p = 1.0000 S.E. :0.0000
locus 18 : p = 0.1971 S.E. :0.0070
locus 19 : p = 0.7520 S.E. :0.0049
locus 20 : p = 0.3468 S.E. :0.0072
locus 21 : p = 1.0000 S.E. :0.0000

Results over loci
X-sq : 34.6324
df : 42
Overall: p = 0.7833

Groups compared: 1-2 vs. 2-2
locus 1 : p = 1.0000 S.E. :0.0000
locus 2 : p = 1.0000 S.E. :0.0000
locus 3 : p = 1.0000 S.E. :0.0000
locus 4 : p = 1.0000 S.E. :0.0000
locus 5 : p = 1.0000 S.E. :0.0000
locus 6 : p = 1.0000 S.E. :0.0000
locus 7 : p = 1.0000 S.E. :0.0000
locus 8 : p = 1.0000 S.E. :0.0000
locus 9 : p = 1.0000 S.E. :0.0000
locus 10 : p = 1.0000 S.E. :0.0000
locus 11 : p = 1.0000 S.E. :0.0000
locus 12 : p = 1.0000 S.E. :0.0000
locus 13 : p = 1.0000 S.E. :0.0000
locus 14 : p = 1.0000 S.E. :0.0000
locus 15 : p = 1.0000 S.E. :0.0000
locus 16 : p = 1.0000 S.E. :0.0000
locus 17 : p = 1.0000 S.E. :0.0000
locus 18 : p = 1.0000 S.E. :0.0000
locus 19 : p = 1.0000 S.E. :0.0000
locus 20 : p = 1.0000 S.E. :0.0000
locus 21 : p = 1.0000 S.E. :0.0000

Results over loci
X-sq : 0.0000
df : 42
Overall: p = 1.0000

Groups compared: 1-2 vs. 3-1
locus 1 : p = 0.7591 S.E. :0.0049
locus 2 : p = 1.0000 S.E. :0.0000
locus 3 : p = 0.3074 S.E. :0.0062
locus 4 : p = 0.2539 S.E. :0.0054
locus 5 : p = 0.3104 S.E. :0.0065
locus 6 : p = 0.7359 S.E. :0.0043
locus 7 : p = 0.5615 S.E. :0.0046
locus 8 : p = 0.5598 S.E. :0.0049
locus 9 : p = 0.0704 S.E. :0.0062
locus 10 : p = 0.0769 S.E. :0.0049
locus 11 : p = 0.1260 S.E. :0.0063
locus 12 : p = 0.7388 S.E. :0.0049
locus 13 : p = 0.2454 S.E. :0.0058
locus 14 : p = 0.3608 S.E. :0.0081
locus 15 : p = 0.7643 S.E. :0.0045
locus 16 : p = 0.0960 S.E. :0.0060
locus 17 : p = 1.0000 S.E. :0.0000
locus 18 : p = 0.0432 S.E. :0.0032
locus 19 : p = 0.5557 S.E. :0.0062
locus 20 : p = 0.1162 S.E. :0.0050
locus 21 : p = 1.0000 S.E. :0.0000

Results over loci
X-sq : 47.9459
df : 42
Overall: p = 0.2441

Groups compared: 1-2 vs. 3-2
locus 1 : p = 0.1113 S.E. :0.0057
locus 2 : p = 1.0000 S.E. :0.0000
locus 3 : p = 0.0078 S.E. :0.0017
locus 4 : p = 0.7442 S.E. :0.0039
locus 5 : p = 1.0000 S.E. :0.0000
locus 6 : p = 0.4940 S.E. :0.0064
locus 7 : p = 0.0061 S.E. :0.0011
locus 8 : p = 0.5240 S.E. :0.0062
locus 9 : p = 0.7475 S.E. :0.0042
locus 10 : p = 0.5242 S.E. :0.0077
locus 11 : p = 0.1588 S.E. :0.0051
locus 12 : p = 1.0000 S.E. :0.0000
locus 13 : p = 0.4979 S.E. :0.0083
locus 14 : p = 0.0549 S.E. :0.0044
locus 15 : p = 0.3196 S.E. :0.0094
locus 16 : p = 0.1313 S.E. :0.0044
locus 17 : p = 1.0000 S.E. :0.0000
locus 18 : p = 0.0954 S.E. :0.0055
locus 19 : p = 0.2058 S.E. :0.0060
locus 20 : p = 0.3368 S.E. :0.0084
locus 21 : p = 0.4893 S.E. :0.0062

Results over loci
X-sq : 58.1851
df : 42

Overall: p = 0.0494

Groups compared: 2-1 vs. 2-2
locus 1 : p = 0.7381 S.E. :0.0047
locus 2 : p = 0.3823 S.E. :0.0055
locus 3 : p = 0.0895 S.E. :0.0051
locus 4 : p = 0.7650 S.E. :0.0033
locus 5 : p = 0.7397 S.E. :0.0050
locus 6 : p = 0.5054 S.E. :0.0072
locus 7 : p = 0.1942 S.E. :0.0042
locus 8 : p = 0.5152 S.E. :0.0100
locus 9 : p = 0.0983 S.E. :0.0061
locus 10 : p = 0.2136 S.E. :0.0058
locus 11 : p = 1.0000 S.E. :0.0000
locus 12 : p = 0.3124 S.E. :0.0057
locus 13 : p = 1.0000 S.E. :0.0000
locus 14 : p = 0.7323 S.E. :0.0054
locus 15 : p = 1.0000 S.E. :0.0000
locus 16 : p = 0.1444 S.E. :0.0045
locus 17 : p = 1.0000 S.E. :0.0000
locus 18 : p = 0.1967 S.E. :0.0055
locus 19 : p = 1.0000 S.E. :0.0000
locus 20 : p = 0.3563 S.E. :0.0092
locus 21 : p = 1.0000 S.E. :0.0000

Results over loci
X-sq : 34.3325
df : 42
Overall: p = 0.7941

Groups compared: 2-1 vs. 3-1
locus 1 : p = 1.0000 S.E. :0.0000
locus 2 : p = 0.3589 S.E. :0.0034
locus 3 : p = 0.7303 S.E. :0.0022
locus 4 : p = 0.4833 S.E. :0.0070
locus 5 : p = 0.1370 S.E. :0.0034
locus 6 : p = 0.2726 S.E. :0.0058
locus 7 : p = 0.0443 S.E. :0.0037
locus 8 : p = 0.2831 S.E. :0.0081
locus 9 : p = 1.0000 S.E. :0.0000
locus 10 : p = 0.7192 S.E. :0.0048
locus 11 : p = 0.1365 S.E. :0.0059
locus 12 : p = 0.1588 S.E. :0.0053
locus 13 : p = 0.3099 S.E. :0.0040
locus 14 : p = 0.7442 S.E. :0.0047
locus 15 : p = 1.0000 S.E. :0.0000
locus 16 : p = 0.0042 S.E. :0.0008
locus 17 : p = 1.0000 S.E. :0.0000
locus 18 : p = 1.0000 S.E. :0.0000
locus 19 : p = 1.0000 S.E. :0.0000
locus 20 : p = 0.0362 S.E. :0.0025
locus 21 : p = 1.0000 S.E. :0.0000

Results over loci
X-sq : 48.3062
df : 42
Overall: p = 0.2330

Groups compared: 2-1 vs. 3-2
locus 1 : p = 0.0642 S.E. :0.0038
locus 2 : p = 0.3261 S.E. :0.0060
locus 3 : p = 0.4614 S.E. :0.0057
locus 4 : p = 1.0000 S.E. :0.0000
locus 5 : p = 1.0000 S.E. :0.0000
locus 6 : p = 1.0000 S.E. :0.0000
locus 7 : p = 0.3324 S.E. :0.0056
locus 8 : p = 0.2730 S.E. :0.0069
locus 9 : p = 0.0615 S.E. :0.0043
locus 10 : p = 0.7037 S.E. :0.0038
locus 11 : p = 0.2177 S.E. :0.0064
locus 12 : p = 0.2461 S.E. :0.0043
locus 13 : p = 0.4604 S.E. :0.0065
locus 14 : p = 0.2751 S.E. :0.0062
locus 15 : p = 0.4631 S.E. :0.0063
locus 16 : p = 0.0064 S.E. :0.0008
locus 17 : p = 1.0000 S.E. :0.0000
locus 18 : p = 1.0000 S.E. :0.0000
locus 19 : p = 0.4609 S.E. :0.0027
locus 20 : p = 1.0000 S.E. :0.0000
locus 21 : p = 0.4472 S.E. :0.0065

Results over loci
X-sq : 45.1659
df : 42
Overall: p = 0.3410

Groups compared: 2-2 vs. 3-1
locus 1 : p = 0.7550 S.E. :0.0037
locus 2 : p = 1.0000 S.E. :0.0000
locus 3 : p = 0.3273 S.E. :0.0053
locus 4 : p = 0.2307 S.E. :0.0078
locus 5 : p = 0.3189 S.E. :0.0051
locus 6 : p = 0.7325 S.E. :0.0046
locus 7 : p = 0.5575 S.E. :0.0069
locus 8 : p = 0.5634 S.E. :0.0076
locus 9 : p = 0.0841 S.E. :0.0059
locus 10 : p = 0.0875 S.E. :0.0055
locus 11 : p = 0.1183 S.E. :0.0077
locus 12 : p = 0.7433 S.E. :0.0041
locus 13 : p = 0.2520 S.E. :0.0119
locus 14 : p = 0.3526 S.E. :0.0072
locus 15 : p = 0.7565 S.E. :0.0042
locus 16 : p = 0.0918 S.E. :0.0040
locus 17 : p = 1.0000 S.E. :0.0000
locus 18 : p = 0.0418 S.E. :0.0043
locus 19 : p = 0.7642 S.E. :0.0048
locus 20 : p = 0.1129 S.E. :0.0062
locus 21 : p = 1.0000 S.E. :0.0000

Results over loci
X-sq : 47.0668
df : 42
Overall: p = 0.2727

Groups compared: 2-2 vs. 3-2
locus 1 : p = 0.1053 S.E. :0.0058
locus 2 : p = 1.0000 S.E. :0.0000
locus 3 : p = 0.0081 S.E. :0.0017
locus 4 : p = 0.7459 S.E. :0.0028
locus 5 : p = 1.0000 S.E. :0.0000
locus 6 : p = 0.4796 S.E. :0.0052
locus 7 : p = 0.0055 S.E. :0.0013
locus 8 : p = 0.5192 S.E. :0.0092
locus 9 : p = 0.7378 S.E. :0.0049
locus 10 : p = 0.5329 S.E. :0.0039
locus 11 : p = 0.1648 S.E. :0.0062
locus 12 : p = 1.0000 S.E. :0.0000
locus 13 : p = 0.5095 S.E. :0.0059
locus 14 : p = 0.0534 S.E. :0.0050
locus 15 : p = 0.3167 S.E. :0.0054
locus 16 : p = 0.1346 S.E. :0.0051
locus 17 : p = 1.0000 S.E. :0.0000
locus 18 : p = 0.0915 S.E. :0.0034
locus 19 : p = 0.3273 S.E. :0.0050
locus 20 : p = 0.3287 S.E. :0.0069

locus 21 : p = 0.4865 S.E. :0.0076

Results over loci
X-sq : 57.5902
df : 42
Overall: p = 0.0550

Groups compared: 3-1 vs. 3-2
locus 1 : p = 0.0402 S.E. :0.0033
locus 2 : p = 1.0000 S.E. :0.0000
locus 3 : p = 0.1614 S.E. :0.0038
locus 4 : p = 0.5121 S.E. :0.0074
locus 5 : p = 0.2494 S.E. :0.0020
locus 6 : p = 0.2545 S.E. :0.0075
locus 7 : p = 0.0018 S.E. :0.0007
locus 8 : p = 1.0000 S.E. :0.0000
locus 9 : p = 0.0681 S.E. :0.0042
locus 10 : p = 0.4613 S.E. :0.0039
locus 11 : p = 1.0000 S.E. :0.0000
locus 12 : p = 1.0000 S.E. :0.0000
locus 13 : p = 1.0000 S.E. :0.0000
locus 14 : p = 0.4915 S.E. :0.0072
locus 15 : p = 0.4997 S.E. :0.0052
locus 16 : p = 1.0000 S.E. :0.0000
locus 17 : p = 1.0000 S.E. :0.0000
locus 18 : p = 1.0000 S.E. :0.0000
locus 19 : p = 0.7250 S.E. :0.0039
locus 20 : p = 0.0258 S.E. :0.0019
locus 21 : p = 0.4446 S.E. :0.0054

Results over loci
X-sq : 48.9354
df : 42
Overall: p = 0.2144

Matrix of combined probabilities for each pairwise comparison

	1-1	1-2	2-1	2-2	3-1	3-2
1-1	*****					
1-2	0.7924	*****				
2-1	1.0000	0.7833	*****			
2-2	0.8074	1.0000	0.7941	*****		
3-1	0.2197	0.2441	0.2330	0.2727	*****	
3-2	0.3253	0.0494	0.3410	0.0550	0.2144	*****

Appendix II Genetic Distance between Commercial and Small Landholders

28/4/2003 12:25:11 PM
Analysis of C:\TFPGA\KRISTI.DAT
Data set contains genotypes of individuals sampled from
subpopulations within populations.
Organism Type: Diploid
Marker Type: Dominant
H-W Equilibrium Assumed.
Allele frequencies estimated based on Lynch and
Milligan's (1994) Taylor expansion estimate.

GENETIC DISTANCES

NEI'S (1972/1978) IDENTITIES/DISTANCES

Nei's original (1972) identity

Subpop	1-1	1-2	2-1	2-2	3-1	3-2
1-1	*****					
1-2	0.9836	*****				
2-1	1.0000	0.9836	*****			
2-2	0.9837	1.0000	0.9837	*****		
3-1	0.9680	0.9777	0.9680	0.9779	*****	
3-2	0.9707	0.9643	0.9707	0.9648	0.9603	*****

Nei's original (1972) distance

Subpop	1-1	1-2	2-1	2-2	3-1	3-2
1-1	*****					
1-2	0.0166	*****				
2-1	0.0000	0.0166	*****			
2-2	0.0165	0.0000	0.0165	*****		
3-1	0.0326	0.0225	0.0326	0.0223	*****	
3-2	0.0297	0.0363	0.0297	0.0358	0.0405	*****

Nei's unbiased (1978) identity

Subpop	1-1	1-2	2-1	2-2	3-1	3-2
1-1	*****					
1-2	0.9983	*****				
2-1	1.0185	0.9983	*****			
2-2	0.9984	1.0114	0.9984	*****		
3-1	0.9843	0.9907	0.9843	0.9909	*****	
3-2	0.9905	0.9806	0.9905	0.9811	0.9783	*****

Nei's unbiased (1978) distance

Subpop	1-1	1-2	2-1	2-2	3-1	3-2
1-1	*****					
1-2	0.0017	*****				
2-1	-.0183	0.0017	*****			
2-2	0.0016	-.0113	0.0016	*****		
3-1	0.0159	0.0094	0.0159	0.0091	*****	
3-2	0.0095	0.0196	0.0095	0.0191	0.0220	***

Appendix III Genetic Distance between population

28/4/2003 13:27:30 PM
Analysis of C:\TFPGA\KRISTI.DAT
Data set contains genotypes of individuals sampled from
subpopulations within populations.
Organism Type: Diploid
Marker Type: Dominant
H-W Equilibrium Assumed.
Allele frequencies estimated based on Lynch and
Milligan's (1994) Taylor expansion estimate.

GENETIC DISTANCES

NEI'S (1972/1978) IDENTITIES/DISTANCES

Nei's original (1972) identity

Population	1	2	3
1	*****		
2	1.0000	*****	
3	0.9847	0.9849	*****

Nei's original (1972) distance

Population	1	2	3
1	*****		
2	0.0000	*****	
3	0.0154	0.0152	*****

Nei's unbiased (1978) identity

Population	1	2	3
1	*****		
2	1.0071	*****	
3	0.9927	0.9930	*****

Nei's unbiased (1978) distance

Population	1	2	3
1	*****		
2	-.0071	*****	
3	0.0073	0.0071	*****

Appendix IV
Heterozygosity of populations

Analysis of
C:\TFPGA\KRISTI.DAT
Data set contains genotypes of individuals sampled from subpopulations within populations.
Organism Type: Diploid
Marker Type: Dominant
H-W Equilibrium Assumed.
Allele frequencies estimated based on Lynch and Milligan's (1994) Taylor expansion estimate.

DESCRIPTIVE STATISTICS

****
****
**
RESULTS FOR EACH POPULATION.

Villa Abecia

Locus 1 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 14 0.1732
12.6007 0.2864
2 30 0.8268
12.6007 0.2864

Locus 2 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 37 0.5951
21.2048 0.4819
2 7 0.4049
21.2048 0.4819

Locus 3 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 13 0.1596
11.8049 0.2683
2 31 0.8404
11.8049 0.2683

Locus 4 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 25 0.3404
19.7586 0.4491
2 19 0.6596
19.7586 0.4491

Locus 5 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 29 0.4129
21.3324 0.4848
2 15 0.5871
21.3324 0.4848

Locus 6 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 30 0.4325
21.5987 0.4909
2 14 0.5675
21.5987 0.4909

Locus 7 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 19 0.2446
16.2594 0.3695
2 25 0.7554
16.2594 0.3695

Locus 8 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 15 0.1870
13.3766 0.3040
2 29 0.8130
13.3766 0.3040

Locus 9 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 17 0.2152
14.8646 0.3378
2 27 0.7848
14.8646 0.3378

Locus 10 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 21 0.2751
17.5498 0.3989
2 23 0.7249
17.5498 0.3989

Locus 11 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 17 0.2152
14.8646 0.3378
2 27 0.7848
14.8646 0.3378

Locus 12 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 15 0.1870
13.3766 0.3040
2 29 0.8130
13.3766 0.3040

Locus 13 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 29 0.4129
21.3324 0.4848
2 15 0.5871
21.3324 0.4848

Locus 14 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 29 0.4129
21.3324 0.4848
2 15 0.5871
21.3324 0.4848

Locus 15 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 30 0.4325
21.5987 0.4909
2 14 0.5675
21.5987 0.4909

Locus 16 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 5 0.0582
4.8226 0.1096
2 39 0.9418
4.8226 0.1096

Locus 17 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 26 0.3578
20.2196 0.4595
2 18 0.6422
20.2196 0.4595

Locus 18 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 19 0.2446
16.2594 0.3695
2 25 0.7554
16.2594 0.3695

Locus 19 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 19 0.2446
16.2594 0.3695
2 25 0.7554
16.2594 0.3695

Locus 20 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 25 0.3404
19.7586 0.4491
2 19 0.6596
19.7586 0.4491

Locus 21 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 12 0.1463
10.9901 0.2498
2 32 0.8537
10.9901 0.2498

Results over all loci

Ave. sample size: 44.0000
Ave. heterozygosity: 0.3800
Ave. heterozygosity (unbiased): 0.3844

Ave. heterozygosity (direct
count): 0.3800
% polymorphic loci (no
criterion): 100.0000
% polymorphic loci (99%
criterion): 100.0000
% polymorphic loci (95%
criterion): 100.0000

Acchilla

Locus 1 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 14 0.1732
12.6007 0.2864
2 30 0.8268
12.6007 0.2864

Locus 2 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 37 0.5951
21.2048 0.4819
2 7 0.4049
21.2048 0.4819

Locus 3 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 13 0.1596
11.8049 0.2683
2 31 0.8404
11.8049 0.2683

Locus 4 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 25 0.3404
19.7586 0.4491
2 19 0.6596
19.7586 0.4491

Locus 5 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 29 0.4129
21.3324 0.4848
2 15 0.5871
21.3324 0.4848

Locus 6 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 30 0.4325
21.5987 0.4909
2 14 0.5675
21.5987 0.4909

Locus 7 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 19 0.2446
16.2594 0.3695
2 25 0.7554
16.2594 0.3695

Locus 8 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 15 0.1870
13.3766 0.3040
2 29 0.8130
13.3766 0.3040

Locus 9 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 17 0.2152
14.8646 0.3378
2 27 0.7848
14.8646 0.3378

Locus 10 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 21 0.2751
17.5498 0.3989
2 23 0.7249
17.5498 0.3989

Locus 11 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 17 0.2152
14.8646 0.3378
2 27 0.7848
14.8646 0.3378

Locus 12 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 15 0.1870
13.3766 0.3040
2 29 0.8130
13.3766 0.3040

Locus 13 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 29 0.4129
21.3324 0.4848
2 15 0.5871
21.3324 0.4848

Locus 14 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 29 0.4129
21.3324 0.4848
2 15 0.5871
21.3324 0.4848

Locus 15 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 30 0.4325
21.5987 0.4909
2 14 0.5675
21.5987 0.4909

Locus 16 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 5 0.0582
4.8226 0.1096
2 39 0.9418
4.8226 0.1096

Locus 17 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 26 0.3578
20.2196 0.4595
2 18 0.6422
20.2196 0.4595

Locus 18 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 19 0.2446
16.2594 0.3695
2 25 0.7554
16.2594 0.3695

Locus 19 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 20 0.2597
16.9185 0.3845
2 24 0.7403
16.9185 0.3845

Locus 20 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 25 0.3404
19.7586 0.4491
2 19 0.6596
19.7586 0.4491

Locus 21 # obs. at locus= 44
allele: # obs: allele freq:
hets: het freq:
1 12 0.1463
10.9901 0.2498
2 32 0.8537
10.9901 0.2498

Results over all loci

Ave. sample size: 44.0000
Ave. heterozygosity: 0.3808
Ave. heterozygosity (unbiased):
0.3851
Ave. heterozygosity (direct
count): 0.3808
% polymorphic loci (no
criterion): 100.0000
% polymorphic loci (99%
criterion): 100.0000
% polymorphic loci (95%
criterion): 100.0000

La Merced

Locus 1 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 14 0.2391
12.0071 0.3639

2 19 0.7609
12.0071 0.3639

Locus 2 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 26 0.5329
16.4287 0.4978
2 7 0.4671
16.4287 0.4978

Locus 3 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 16 0.2797
13.2966 0.4029
2 17 0.7203
13.2966 0.4029

Locus 4 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 14 0.2391
12.0071 0.3639
2 19 0.7609
12.0071 0.3639

Locus 5 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 25 0.5017
16.4998 0.5000
2 8 0.4983
16.4998 0.5000

Locus 6 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 23 0.4447
16.2980 0.4939
2 10 0.5553
16.2980 0.4939

Locus 7 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 13 0.2196
11.3100 0.3427
2 20 0.7804
11.3100 0.3427

Locus 8 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 16 0.2797
13.2966 0.4029
2 17 0.7203
13.2966 0.4029

Locus 9 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 12 0.2005
10.5816 0.3207
2 21 0.7995
10.5816 0.3207

Locus 10 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 11 0.1820
9.8239 0.2977
2 22 0.8180
9.8239 0.2977

Locus 11 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 5 0.0782
4.7601 0.1442
2 28 0.9218
4.7601 0.1442

Locus 12 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 7 0.1115
6.5369 0.1981
2 26 0.8885
6.5369 0.1981

Locus 13 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 16 0.2797
13.2966 0.4029
2 17 0.7203
13.2966 0.4029

Locus 14 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 15 0.2591
12.6702 0.3839
2 18 0.7409
12.6702 0.3839

Locus 15 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 19 0.3453
14.9204 0.4521
2 14 0.6547
14.9204 0.4521

Locus 16 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 14 0.2391
12.0071 0.3639
2 19 0.7609
12.0071 0.3639

Locus 17 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 20 0.3687
15.3617 0.4655
2 13 0.6313
15.3617 0.4655

Locus 18 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 7 0.1115
6.5369 0.1981
2 26 0.8885
6.5369 0.1981

Locus 19 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 19 0.3453
14.9204 0.4521
2 14 0.6547
14.9204 0.4521

Locus 20 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 22 0.4182
16.0588 0.4866
2 11 0.5818
16.0588 0.4866

Locus 21 # obs. at locus= 33
allele: # obs: allele freq:
hets: het freq:
1 11 0.1820
9.8239 0.2977
2 22 0.8180
9.8239 0.2977

Results over all loci

Ave. sample size: 33.0000
Ave. heterozygosity: 0.3729
Ave. heterozygosity (unbiased): 0.3787
Ave. heterozygosity (direct count): 0.3729
% polymorphic loci (no criterion): 100.0000
% polymorphic loci (99% criterion): 100.0000
% polymorphic loci (95% criterion): 100.0000

Appendix V Heterozygosity of Commercial and Non Commercial Farmers

28/4/2003 12:46:21 PM
Analysis of C:\TFPGA\KRISTI.DAT
Data set contains genotypes of individuals sampled from subpopulations within populations.
Organism Type: Diploid
Marker Type: Dominant
H-W Equilibrium Assumed.
Allele frequencies estimated based on Lynch and Milligan's (1994) Taylor expansion estimate.

DESCRIPTIVE STATISTICS

****
**
RESULTS FOR EACH SUBPOPULATION.

Villa Abecia Commercial

Locus 1 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 4 0.1410
3.6346 0.2423
2 11 0.8590
3.6346 0.2423

Locus 2 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 14 0.7077
6.2058 0.4137
2 1 0.2923
6.2058 0.4137

Locus 3 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 7 0.2643
5.8339 0.3889
2 8 0.7357
5.8339 0.3889

Locus 4 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 8 0.3103
6.4204 0.4280
2 7 0.6897
6.4204 0.4280

Locus 5 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 9 0.3595
6.9081 0.4605
2 6 0.6405
6.9081 0.4605

Locus 6 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 9 0.3595
6.9081 0.4605
2 6 0.6405
6.9081 0.4605

Locus 7 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 4 0.1410
3.6346 0.2423
2 11 0.8590
3.6346 0.2423

Locus 8 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 4 0.1410
3.6346 0.2423
2 11 0.8590
3.6346 0.2423

Locus 9 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 3 0.1037
2.7885 0.1859
2 12 0.8963
2.7885 0.1859

Locus 10 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 5 0.1801
4.4297 0.2953
2 10 0.8199
4.4297 0.2953

Locus 11 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 6 0.2211
5.1660 0.3444
2 9 0.7789
5.1660 0.3444

Locus 12 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 7 0.2643
5.8339 0.3889
2 8 0.7357
5.8339 0.3889

Locus 13 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 10 0.4129
7.2722 0.4848
2 5 0.5871
7.2722 0.4848

Locus 14 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 9 0.3595
6.9081 0.4605
2 6 0.6405
6.9081 0.4605

Locus 15 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 10 0.4129
7.2722 0.4848
2 5 0.5871
7.2722 0.4848

Locus 16 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 0 0.0000
0.0000 0.0000
2 15 1.0000
0.0000 0.0000

Locus 17 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 9 0.3595
6.9081 0.4605
2 6 0.6405
6.9081 0.4605

Locus 18 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 4 0.1410
3.6346 0.2423
2 11 0.8590
3.6346 0.2423

Locus 19 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 7 0.2643
5.8339 0.3889
2 8 0.7357
5.8339 0.3889

Locus 20 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 7 0.2643
5.8339 0.3889
2 8 0.7357
5.8339 0.3889

Locus 21 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
1 4 0.1410
3.6346 0.2423
2 11 0.8590
3.6346 0.2423

Results over all loci

Ave. sample size: 15.0000

Ave. heterozygosity: 0.3451
Ave. heterozygosity (unbiased): 0.3570
Ave. heterozygosity (direct count): 0.3451
% polymorphic loci (no criterion): 95.2381
% polymorphic loci (99% criterion): 95.2381
% polymorphic loci (95% criterion): 95.2381

Villa Abecia Small Holder

Locus 1 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 10 0.1887 8.8805 0.3062
2 19 0.8113 8.8805 0.3062

Locus 2 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 23 0.5375 14.4184 0.4972
2 6 0.4625 14.4184 0.4972

Locus 3 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 6 0.1084 5.6072 0.1934
2 23 0.8916 5.6072 0.1934

Locus 4 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 17 0.3528 13.2429 0.4567
2 12 0.6472 13.2429 0.4567

Locus 5 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 20 0.4375 14.2736 0.4922
2 9 0.5625 14.2736 0.4922

Locus 6 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 21 0.4688 14.4434 0.4980
2 8 0.5312 14.4434 0.4980

Locus 7 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 15 0.3020 12.2254 0.4216
2 14 0.6980 12.2254 0.4216

Locus 8 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 11 0.2101 9.6249 0.3319
2 18 0.7899 9.6249 0.3319

Locus 9 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 14 0.2779 11.6390 0.4013
2 15 0.7221 11.6390 0.4013

Locus 10 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 16 0.3269 12.7620 0.4401
2 13 0.6731 12.7620 0.4401

Locus 11 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 11 0.2101 9.6249 0.3319
2 18 0.7899 9.6249 0.3319

Locus 12 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 8 0.1476 7.2988 0.2517
2 21 0.8524 7.2988 0.2517

Locus 13 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 19 0.4079 14.0084 0.4830
2 10 0.5921 14.0084 0.4830

Locus 14 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 20 0.4375 14.2736 0.4922
2 9 0.5625 14.2736 0.4922

Locus 15 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 20 0.4375 14.2736 0.4922
2 9 0.5625 14.2736 0.4922

Locus 16 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 5 0.0895 4.7247 0.1629
2 24 0.9105 4.7247 0.1629

Locus 17 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 17 0.3528 13.2429 0.4567
2 12 0.6472 13.2429 0.4567

Locus 18 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 15 0.3020 12.2254 0.4216
2 14 0.6980 12.2254 0.4216

Locus 19 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 12 0.2320 10.3349 0.3564
2 17 0.7680 10.3349 0.3564

Locus 20 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 18 0.3797 13.6612 0.4711
2 11 0.6203 13.6612 0.4711

Locus 21 # obs. at locus= 29
allele: # obs: allele freq: # hets: het freq:
1 8 0.1476 7.2988 0.2517
2 21 0.8524 7.2988 0.2517

Results over all loci

Ave. sample size: 29.0000
Ave. heterozygosity: 0.3909
Ave. heterozygosity (unbiased): 0.3978
Ave. heterozygosity (direct count): 0.3909
% polymorphic loci (no criterion): 100.0000
% polymorphic loci (99% criterion): 100.0000
% polymorphic loci (95% criterion): 100.0000

Acchilla Commercial

Locus 1 # obs. at locus= 15
allele: # obs: allele freq: # hets: het freq:

 1 4 0.1410
3.6346 0.2423
 2 11 0.8590
3.6346 0.2423

Locus 2 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 14 0.7077
6.2058 0.4137
 2 1 0.2923
6.2058 0.4137

Locus 3 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 7 0.2643
5.8339 0.3889
 2 8 0.7357
5.8339 0.3889

Locus 4 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 8 0.3103
6.4204 0.4280
 2 7 0.6897
6.4204 0.4280

Locus 5 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 9 0.3595
6.9081 0.4605
 2 6 0.6405
6.9081 0.4605

Locus 6 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 9 0.3595
6.9081 0.4605
 2 6 0.6405
6.9081 0.4605

Locus 7 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 4 0.1410
3.6346 0.2423
 2 11 0.8590
3.6346 0.2423

Locus 8 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 4 0.1410
3.6346 0.2423
 2 11 0.8590
3.6346 0.2423

Locus 9 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 3 0.1037
2.7885 0.1859
 2 12 0.8963
2.7885 0.1859

Locus 10 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 5 0.1801
4.4297 0.2953
 2 10 0.8199
4.4297 0.2953

Locus 11 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 6 0.2211
5.1660 0.3444
 2 9 0.7789
5.1660 0.3444

Locus 12 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 7 0.2643
5.8339 0.3889
 2 8 0.7357
5.8339 0.3889

Locus 13 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 10 0.4129
7.2722 0.4848
 2 5 0.5871
7.2722 0.4848

Locus 14 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 9 0.3595
6.9081 0.4605
 2 6 0.6405
6.9081 0.4605

Locus 15 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 10 0.4129
7.2722 0.4848
 2 5 0.5871
7.2722 0.4848

Locus 16 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 0 0.0000
0.0000 0.0000
 2 15 1.0000
0.0000 0.0000

Locus 17 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 9 0.3595
6.9081 0.4605
 2 6 0.6405
6.9081 0.4605

Locus 18 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 4 0.1410
3.6346 0.2423
 2 11 0.8590
3.6346 0.2423

Locus 19 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 7 0.2643
5.8339 0.3889
 2 8 0.7357
5.8339 0.3889

Locus 20 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 7 0.2643
5.8339 0.3889
 2 8 0.7357
5.8339 0.3889

Locus 21 # obs. at locus= 15
allele: # obs: allele freq:
hets: het freq:
 1 4 0.1410
3.6346 0.2423
 2 11 0.8590
3.6346 0.2423

Results over all loci

Ave. sample size: 15.0000
Ave. heterozygosity: 0.3451
Ave. heterozygosity (unbiased): 0.3570
Ave. heterozygosity (direct count): 0.3451
% polymorphic loci (no criterion): 95.2381
% polymorphic loci (99% criterion): 95.2381
% polymorphic loci (95% criterion): 95.2381

Acchilla Small Holders

Locus 1 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
 1 10 0.1887
8.8805 0.3062
 2 19 0.8113
8.8805 0.3062

Locus 2 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
 1 23 0.5375
14.4184 0.4972
 2 6 0.4625
14.4184 0.4972

Locus 3 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
 1 6 0.1084
5.6072 0.1934
 2 23 0.8916
5.6072 0.1934

Locus 4 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 17 0.3528
13.2429 0.4567
2 12 0.6472
13.2429 0.4567

Locus 5 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 20 0.4375
14.2736 0.4922
2 9 0.5625
14.2736 0.4922

Locus 6 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 21 0.4688
14.4434 0.4980
2 8 0.5312
14.4434 0.4980

Locus 7 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 15 0.3020
12.2254 0.4216
2 14 0.6980
12.2254 0.4216

Locus 8 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 11 0.2101
9.6249 0.3319
2 18 0.7899
9.6249 0.3319

Locus 9 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 14 0.2779
11.6390 0.4013
2 15 0.7221
11.6390 0.4013

Locus 10 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 16 0.3269
12.7620 0.4401
2 13 0.6731
12.7620 0.4401

Locus 11 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 11 0.2101
9.6249 0.3319
2 18 0.7899
9.6249 0.3319

Locus 12 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 8 0.1476
7.2988 0.2517
2 21 0.8524
7.2988 0.2517

Locus 13 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 19 0.4079
14.0084 0.4830
2 10 0.5921
14.0084 0.4830

Locus 14 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 20 0.4375
14.2736 0.4922
2 9 0.5625
14.2736 0.4922

Locus 15 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 20 0.4375
14.2736 0.4922
2 9 0.5625
14.2736 0.4922

Locus 16 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 5 0.0895
4.7247 0.1629
2 24 0.9105
4.7247 0.1629

Locus 17 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 17 0.3528
13.2429 0.4567
2 12 0.6472
13.2429 0.4567

Locus 18 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 15 0.3020
12.2254 0.4216
2 14 0.6980
12.2254 0.4216

Locus 19 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 13 0.2546
11.0074 0.3796
2 16 0.7454
11.0074 0.3796

Locus 20 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 18 0.3797
13.6612 0.4711
2 11 0.6203
13.6612 0.4711

Locus 21 # obs. at locus= 29
allele: # obs: allele freq:
hets: het freq:
1 8 0.1476
7.2988 0.2517
2 21 0.8524
7.2988 0.2517

Results over all loci

Ave. sample size: 29.0000
Ave. heterozygosity: 0.3920
Ave. heterozygosity (unbiased): 0.3989
Ave. heterozygosity (direct count): 0.3920
% polymorphic loci (no criterion): 100.0000
% polymorphic loci (99% criterion): 100.0000
% polymorphic loci (95% criterion): 100.0000

La Merced Commercial

Locus 1 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 5 0.1396
4.5638 0.2402
2 14 0.8604
4.5638 0.2402

Locus 2 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 15 0.5296
9.4668 0.4983
2 4 0.4704
9.4668 0.4983

Locus 3 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 7 0.2022
6.1304 0.3227
2 12 0.7978
6.1304 0.3227

Locus 4 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 7 0.2022
6.1304 0.3227
2 12 0.7978
6.1304 0.3227

Locus 5 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 16 0.5882
9.2045 0.4844
2 3 0.4118
9.2045 0.4844

Locus 6 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:

1 15 0.5296
9.4668 0.4983
2 4 0.4704
9.4668 0.4983

Locus 7 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 12 0.3861
9.0070 0.4741
2 7 0.6139
9.0070 0.4741

Locus 8 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 9 0.2702
7.4933 0.3944
2 10 0.7298
7.4933 0.3944

Locus 9 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 4 0.1099
3.7177 0.1957
2 15 0.8901
3.7177 0.1957

Locus 10 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 5 0.1396
4.5638 0.2402
2 14 0.8604
4.5638 0.2402

Locus 11 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 3 0.0812
2.8352 0.1492
2 16 0.9188
2.8352 0.1492

Locus 12 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 4 0.1099
3.7177 0.1957
2 15 0.8901
3.7177 0.1957

Locus 13 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 9 0.2702
7.4933 0.3944
2 10 0.7298
7.4933 0.3944

Locus 14 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 10 0.3067
8.0799 0.4253
2 9 0.6933
8.0799 0.4253

Locus 15 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 12 0.3861
9.0070 0.4741
2 7 0.6139
9.0070 0.4741

Locus 16 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 8 0.2355
6.8406 0.3600
2 11 0.7645
6.8406 0.3600

Locus 17 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 11 0.3452
8.5893 0.4521
2 8 0.6548
8.5893 0.4521

Locus 18 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 4 0.1099
3.7177 0.1957
2 15 0.8901
3.7177 0.1957

Locus 19 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 10 0.3067
8.0799 0.4253
2 9 0.6933
8.0799 0.4253

Locus 20 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 16 0.5882
9.2045 0.4844
2 3 0.4118
9.2045 0.4844

Locus 21 # obs. at locus= 19
allele: # obs: allele freq:
hets: het freq:
1 5 0.1396
4.5638 0.2402
2 14 0.8604
4.5638 0.2402

Results over all loci

Ave. sample size: 19.0000
Ave. heterozygosity: 0.3556
Ave. heterozygosity (unbiased): 0.3652
Ave. heterozygosity (direct count): 0.3556
% polymorphic loci (no criterion): 100.0000
% polymorphic loci (99% criterion): 100.0000
% polymorphic loci (95% criterion): 100.0000

La Merced Small Holders

Locus 1 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 9 0.3926
6.6772 0.4769
2 5 0.6074
6.6772 0.4769

Locus 2 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 11 0.5214
6.9872 0.4991
2 3 0.4786
6.9872 0.4991

Locus 3 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 9 0.3926
6.6772 0.4769
2 5 0.6074
6.6772 0.4769

Locus 4 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 7 0.2865
5.7240 0.4089
2 7 0.7135
5.7240 0.4089

Locus 5 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 9 0.3926
6.6772 0.4769
2 5 0.6074
6.6772 0.4769

Locus 6 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 8 0.3375
6.2603 0.4472
2 6 0.6625
6.2603 0.4472

Locus 7 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 1 0.0357
0.9643 0.0689
2 13 0.9643
0.9643 0.0689

Locus 8 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 7 0.2865
5.7240 0.4089
2 7 0.7135
5.7240 0.4089

Locus 9 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 8 0.3375
6.2603 0.4472
2 6 0.6625
6.2603 0.4472

Locus 10 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 6 0.2390
5.0922 0.3637
2 8 0.7610
5.0922 0.3637

Locus 11 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 2 0.0728
1.8900 0.1350
2 12 0.9272
1.8900 0.1350

Locus 12 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 3 0.1114
2.7724 0.1980
2 11 0.8886
2.7724 0.1980

Locus 13 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 7 0.2865
5.7240 0.4089
2 7 0.7135
5.7240 0.4089

Locus 14 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 5 0.1942
4.3819 0.3130
2 9 0.8058
4.3819 0.3130

Locus 15 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 7 0.2865
5.7240 0.4089
2 7 0.7135
5.7240 0.4089

Locus 16 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 6 0.2390
5.0922 0.3637
2 8 0.7610
5.0922 0.3637

Locus 17 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 9 0.3926
6.6772 0.4769
2 5 0.6074
6.6772 0.4769

Locus 18 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 3 0.1114
2.7724 0.1980
2 11 0.8886
2.7724 0.1980

Locus 19 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 9 0.3926
6.6772 0.4769
2 5 0.6074
6.6772 0.4769

Locus 20 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 6 0.2390
5.0922 0.3637
2 8 0.7610
5.0922 0.3637

Locus 21 # obs. at locus= 14
allele: # obs: allele freq:
hets: het freq:
1 6 0.2390
5.0922 0.3637
2 8 0.7610
5.0922 0.3637

Results over all loci

Ave. sample size: 14.0000
Ave. heterozygosity: 0.3705
Ave. heterozygosity (unbiased): 0.3843
Ave. heterozygosity (direct count): 0.3705
% polymorphic loci (no criterion): 100.0000
% polymorphic loci (99% criterion): 100.0000
% polymorphic loci (95% criterion): 95.2381

