GENETIC DIVERSITY OF
HISTORIC APPLE TREES ON THE COLORADO PLATEAU
AND IMPLICATIONS FOR ITS PRESERVATION

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December 2007
ABSTRACT

Genetic Diversity of Historic Apple Trees on the Colorado Plateau
And Implications for Its Preservation

Kanin J. Routson

The diversity of landrace and heirloom fruit varieties has declining worldwide over the past century. Abandoned homesteads and orchards harbor considerable agrobiodiversity, but the extent and location of that diversity is poorly understood. I assessed the genetic diversity of 280 historic apple trees growing in 43 abandoned homestead and orchard sites in Arizona, Utah, and New Mexico using seven microsatellite markers. I compared the historic “unknowns” to 109 heirloom varieties introduced into the Southwest in the late 19\textsuperscript{th} and early 20\textsuperscript{th} centuries. Genetic results revealed 144 varieties were represented in the 280 samples. I identified 34 of these 144 varieties as named varieties. One hundred and twenty of the total samples (43\%) had DNA fingerprints indistinguishable from these 34 named varieties. The remaining 160 samples, representing 110 varieties, had unique fingerprints that did not match any of the named varieties. It is evident from the results of this study that a high diversity of historic apple varieties is represented in homestead orchards on and near the Colorado Plateau. Current policies that apply to historic fruit tree conservation, both \textit{in situ} and \textit{ex situ} have been implemented through various governmental agencies, with similar conservation initiatives also undertaken taken by private entities and grassroots organizations to begin preserving this diversity. Conservation efforts targeting orchards on the Colorado Plateau and focusing on all unique genotypes are imperative to preserve both cultural heritage and biological genetic diversity.
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INTRODUCTION

The late 19th century is often referred to as the golden years of apple growing in the United States (Calhoun 1995). Farmstead and kitchen orchards were planted with a wide variety of fruit trees to suit family needs. Apples and pears that would last all winter in the cold cellar, fast-ripening summer fruits, cider apples, cooking apples, and dessert fruits were all essential elements of the farmstead orchard. This period of American history was preceded by an era of fruit diversification, which spanned much of the 1700s and into the early 1800s. From seedling orchards planted for cider and animal feed emerged thousands of trees with high quality fruits that were worthy of naming and distribution. These trees were clonally propagated from cuttings, and traded and sold to become elements of the diverse orchards of the 19th century.

USDA pomologist W. H. Ragan undertook the task of recording the names and characteristics of every apple variety grown in the US during the 19th century. In his book, *The Nomenclature of the Apple* (1905), Ragan lists 6,654 unique named apple varieties that he found referenced in US literature between the years 1804 and 1904. In 1980, Dan Bussey began expanding upon Ragan’s register to update descriptions of known varieties and include additional apple varieties referenced in U.S. literature up to the year 1980. Over a decade after beginning this project, Bussey in *The Apple in North America* (In Prep.) lists 14,000 named US apple varieties. An estimated 86% of these pre-20th century apple varieties have gone extinct (Fowler 1990). A century later, the Red Delicious apple constitutes two-thirds of the entire apple crop in the United States. Following close behind are the Golden Delicious, Gala, and Fuji apples (Jackson 2003). In *The Fruit, Berry and Nut Inventory* (2001) Kent Whealy lists about 1,500 apple
varieties that are currently available through U.S. nurseries, many of which have been developed through modern fruit breeding. The USDA Plant Genetic Resources Unit apple collection in Geneva, NY, holds over 2,500 apple varieties from around the world, including hundreds of US heirloom varieties (Pollan 2001), many of which are no longer available through the nursery trade, nor demanded by consumers who have become accustomed to the ubiquitous Red Delicious, Gala, and Fuji apples.

Though numbers of varieties and overall percentages of varieties lost differ between species, and these numbers are at best estimates; the story is the same for all cultivated fruit crops. Commercial agriculture requires consistency of ripening time, quality retention during processing and shipping, and long storage life (Goland & Bauer 2004). This has reduced the diversity of fruit trees of the small family orchards—where diversity of ripening time, sizes, textures, and flavors were celebrated—to only a few handfuls of commercial varieties available today.

While the loss of diversity can be permanent, fruit trees have an advantage over annual crops because though the varieties are effectively lost to the public, the trees can live to remarkably old ages. In many areas, it is still possible to find regional varieties (landraces) of fruit trees that were numerous at the beginning of the 20th century (Radoslav 2001). Remnant orchards planted before the Green Revolution still survive on abandoned homesteads and old farms. Though homestead trees often persist without their original names being retained, they represent a snapshot of the diversity of fruit varieties available during the peak of fruit tree diversification, over a century ago. Nevertheless, homestead trees are a rapidly disappearing resource as old age, disease, and pressures from changes in land-use affect the remaining trees.
Surveying historic orchards to identify heirloom varieties that have disappeared from the nursery trade is a daunting task. Characterization and preservation of genetic diversity is usually based upon the physical expression of the underlying genetic variation, however morphological and taxonomic traits used to differentiate between varieties can be very inaccurate, due to the broad phenotypic variation of genotypes under environmental influences. Furthermore, many heirloom apple varieties are morphologically very similar to one another and accurate descriptions are often lacking, making conventional identification methods difficult, if not impossible for historic trees. These problems in differentiating between apple varieties are not a recent discovery: A. J. Downing in *The Fruits and Fruit Trees of America* (1852) comments, “Pomology has become an embarrassing study” (p. vii) referring to the impossibility of accurately naming and differentiating between the thousands of apple varieties available at that time.

The application of modern genetic tools to historic fruit identification may solve some of the problems that have historically plagued morphological characterization. Methods for distinguishing among similar varieties of closely related apples however, are still in their infancy and not sufficiently developed for widespread use. Molecular markers in the DNA of organisms have significant advantages over morphological traits because the DNA is less influenced by environmental factors and is capable of revealing genetic relationships and diversity not expressed in phenotypic variation. Population structures can also be identified using molecular markers; assessment of inbreeding, hybridization, drift, and the overall genetic diversity of a population can be done much more accurately with genetic data than by assessing morphological characteristics alone. Molecular mapping techniques, including DNA sequencing, allozymes, restriction
fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), variable number tandem repeats (VNTRs), and microsatellites, have become powerful and accurate tools for analyzing genetic diversity (Hammer et al. 2003).

Since the development of the polymerase chain reaction (PCR) by Kary Mullis in the mid 1980s, the use of microsatellite loci in population genetic studies has become increasingly commonplace (Balloux and Lugon-Moulin 2002). Microsatellite loci are short nucleotide sequences of up to six base pairs, repeated in tandem, head to tail, without interruption. Microsatellites have been detected in the genomes of every organism so far studied, in concentrations much higher than expected based on chance alone (Hancock et al. 1999). Microsatellites are highly polymorphic, with mutation rates of around $10^{-3}$ per locus per generation as opposed to $10^{-9}$ to $10^{-10}$ rates of point mutations (Balloux and Lugon-Moulin 2002, Jarne and Lagoda 1996). High polymorphism in microsatellite alleles makes them useful for studying closely related individuals and populations that generally show a lack of genetic variation. Mutation in microsatellite loci is generally thought to be the result of polymerase slippage during DNA replication (Jarne and Lagoda 1996). Slippage during replication can occur when the nascent DNA strand separates from the template strand. This is not a problem for non-repetitive sequences, because the nascent strand has only one way of re-annealing to the template. If the sequence is repetitive, however, the nascent strand can re-anneal out of phase. Replication of a misaligned sequence will produce a new allele either longer or shorter than the original, depending upon the direction of the slippage (Hancock 1999).

Gianfraschi et al. (1998) and Hokanson et al. (1998) were both instrumental in developing and publishing some of the original work with *Malus*-specific microsatellites
in the US. In Europe, Silfverberg-Dilworth et al. (2006) and the High-Quality Disease Resistant Apples for Sustainable Agriculture, or HiDRAS, have been at the forefront of the development of *Malus* microsatellites. HiDRAS recently (2006) published 148 new microsatellites for apples and listed all 351 currently developed microsatellites on its website.

The genetic diversity of heirloom and landrace fruit crops is beginning to gain recognition in academia. Projects are currently underway or have already been completed for using molecular markers to assess the genetic diversity in landrace apples, apricots, cherries, and others. For apples, Gayle Volk, Adam Henk, and Ann Reilley of the United States Department of Agriculture-Agricultural Research Service-National Center for Genetic Resources Preservation, USDA-ARS-NCGRP, are currently working on genotyping old English and Spanish cider apples using 19 microsatellites. Santiago Pereita-Lorenzo, Ana María Ramos-Cabrer, and María Belén Díaz-Hernández, working from Spain, have evaluated the genetic diversity of 114 Spanish apple varieties using 10 microsatellites. In comparing the local Spanish landrace varieties to 26 commercial apple varieties found in the region, Pereita-Lorenzo et al. (2006) found that Northern Spain showed a high level of genetic diversity based on observed heterozygosity. Another interesting result from their work was an observation that apparent misidentifications of varieties through genetic analysis could indicate that foreign germplasm from commercial varieties is penetrating Spain’s native apple populations; the progeny are given the names of local varieties, which makes them indistinguishable without the use of genetic analysis (Pereita-Lorenzo et al. 2006).
Given the high polymorphism associated with microsatellites, and the success of these projects using them to genotype apples, microsatellites are well suited for genotyping historic apple trees in conservation projects. In this thesis I use seven microsatellite markers to analyze the genomic DNA collected from homestead apple trees to assess the genetic diversity of historic apple trees growing on or near the Colorado Plateau. While all historic trees are of value for conservation from a cultural perspective, conservation is only warranted from a biological perspective if the trees represent genetically diverse varieties different from commonly grown apple varieties. In addition, knowledge of the genetic identities of historic trees will allow us to target specific sites of high diversity for *in situ* conservation as well as specific trees with unique genetic identities for *ex situ* conservation while allowing us to avoid over-allocating limited resources toward preserving common varieties.
CHAPTER 1

A BRIEF HISTORY OF APPLE TREES IN THE UNITED STATES

Apples traveled to the New World as pips in the pockets of European immigrants and, to a lesser extent, as young grafted trees of European varieties. Protestant settlers from across Europe, including England, Holland, Germany, France, and Scandinavia, planted apple seeds brought from Europe in seedling orchards throughout the 16th and 17th centuries (Beach 1905, Morgan 2003). Apple trees were planted at the forefront of the rapidly spreading settlement of the New World. During the “Johnny Appleseed” era of American history, Native Americans, fur traders, and missionaries carried apple pips far into the wilderness beyond the outermost settlements (Beach 1905). John Chapman was the epitome of this period, bringing bushels of seed from cider presses in the more civilized western Pennsylvania to the wilderness of Ohio, Indiana, and Illinois to supply seedling trees to new settlements in the region (Pollan 2001). Planting apples was also accomplished by Native Americans in the eastern US, but their seedling apples never became as popular as seedling peaches in Native American communities of the Colorado Plateau.

Seedling apple trees were widely planted during the rapid westward migration of the Anglo settlers as well as by Spanish settlers moving northward from Mexico. During this same time period, Spanish and Portuguese immigrants brought apple trees to Central America and to southern North America. Spanish missionaries planted apple trees as far north as California, Arizona, and New Mexico. These trees greeted the first Anglo arrivals on the Santa Fe Trail who reached New Mexico by the 1850s (Dunmire 2004).
**Primitive Orchards**

The first orchards planted in the New World are classically known as primitive orchards (Beach 1905). They were chiefly planted from seed and used primarily for cider and livestock feed. Thousands of seedling orchards planted at the forefront of settlement transformed the primordial forests of the American wilderness, offering “visible” proof that pioneers had conquered the wilderness, while at the same time supplying them with a fresh supply of cider (Pollan 2001). Cider was indispensable to the early American farmer, and preparation for winter was incomplete without barrels of cider stored in the cellar (Beach 1905).

Apples from seedling trees were referred to as common fruit, as opposed to fancy or grafted fruits, and were typically “spitters” of poor quality. Apples are difficult to grow from seed and are not necessarily flavorful when eaten out of hand (Hedrick 1950) and thus were used for cider and livestock feed. However, because of the broad genetic variability, or heterozygosity, of the apple, seedlings would occasionally emerge that produced fruit with desirable characteristics. Such apple trees were highly sought after during the 19th century, though only occurring at odds of less than one in eighty thousand. They would bring fortune and fame to their finder (Pollan 2001), and the tree would be propagated by root divisions and grafted cuttings to become a new named variety. Literally thousands of named varieties sprung from America’s seedling cider orchards during this time.
Trees in these early orchards were pruned high to allow mowing of the grassy 
understory and to keep livestock from damaging the lower limbs (Hedrick 1950).
Incidentally, this “primitive” pruning style is a very useful trait in identifying and aging 
historic trees and in distinguishing them from 20th century trees. Commercial orchards, 
and almost all fruit trees planted after the onset of the 20th century, have been pruned in 
the modified central leader or open-wineglass forms, with the main trunk branching about 
a meter or so off the ground.

Mixed Orchards

The mixed orchards that followed the primitive orchards were small farm and 
kitchen-garden orchards that consisted of a mix of grafted and un-grafted trees of 
different species of fruits. The typical farm orchard consisted of common apples for cider 
and numerous grafted varieties to furnish the home with fresh fruit throughout the year. 
The historic means of transportation at the time were ill suited for shipping fruits, so few 
varieties were selected to withstand shipping. Apples were grown in small orchards for 
home use and to supply local markets (Beach 1905). Writing from the golden years in 
American pomology, when fruit diversity in America had reached its height, Downing 
(1852) describes America as a “young orchard”, and comments that, “He who owns a 
rood of proper land in this country, and, in the face of all the pomonal riches of the day, 
only raises crabs and choke-pears, deserves to lose the respect of all sensible men” (p. v). 
Historic orchards from this era are generally very rich in their varietal diversity, and these 
“gems” of historic orchards are more desirable for conservation, from a biodiversity 
standpoint, than the more homogenous commercial orchards that followed.
Commercial Orchards

Early commercial trade in apples existed as early as the mid 1700s, providing the metropolitan seaport of New York City with fruit for wider distribution. In 1741, a shipment of apples grown in New England was sent to the West Indies (Beach 1905). By 1861, the export of apples had grown to 68,443 bushels, valued at $39,966. These early commercial orchards were few in number and located close to well-established transportation systems around large port cities. As transportation systems improved, fruit could be transported greater distances to urban markets, and commercial orchards were able to spread to other regions. From 1850 to 1860, commercial apple production in New York State and surrounding areas grew dramatically. However, by the late 1800s, apple scab and insect pests, such as codling moth, became serious pests that sent the profitability of commercial orchards into decline. Many farmers were cutting down their commercial orchards in favor of growing more lucrative cash crops by 1890. From 1890 to 1900, notable improvements were made in methods of orchard management, including the use of cover crops, tillage, and fungal and insecticidal sprays. Also during this time, cold storage facilities increased in number, and export trade developed more extensively; this, in turn, provided wider and better markets for commercial orchards (Beach 1905).

While commercial orchards were not widely established until transportation facilities improved, homestead orchards were still widely planted through the first part of the 20th century (Beach 1905). By the 1880s, commercial nurseries like Stark Brother’s Nursery began to ship stock nationally to farmers located near railheads. The development of cheap and rapid long distance transport by steamship, train, and truck enabled commercial growers to plant orchards over much greater regions. This, in
combination with the development of cold storage facilities, enabled the rapid expansion of commercial orchards across the country (Jackson 2003). With commercially grown, picture-perfect fruit available year-round in the grocery store, the need for diverse farm and kitchen orchards went into decline, as did the diverse varieties themselves (Pollan 2001). By the mid-20th century, the planting of commercial orchards of Red Delicious was nearly ubiquitous, leaving little space for other varieties.
CHAPTER 2
PATHWAYS OF APPLE INTRODUCTION INTO THE SOUTHWEST AND IMPLICATIONS FOR COLORADO PLATEAU FRUIT DIVERSITY

Apples first came to the Southwest with Spanish missionaries in the early 17th century, arriving in New Mexico between 1604 and 1620 (Dunmire 2004). This was followed much later by an explosion of Eastern and Midwestern varieties brought by settlers who arrived with the waning of the Apache wars and the opening of the railroad and other transportation corridors near the end of the 19th century. Presumably, the Spanish and Anglo settlers introduced very different varieties. From the north, the Mormons were introducing many named cultivars of apples into Utah and northern Arizona by the 1850s. As mail-order nurseries from the Midwest began to swamp the nursery trade with color catalogs in the early 20th century, there was also a dramatic decrease in the number of varieties planted. Backyard orchardists and small commercial nurseries began buying trees predominantly from these sources. The number of varieties being planted mirrored the relatively small inventories held by a handful of large Midwest nurseries.

Spanish Introductions

Franciscan missionaries traveling up the Camino Real from Mexico City through the Chihuahuan Desert to Santa Fe, New Mexico brought with them numerous agricultural crop species from the Old World, including apples, apricots, grapes, peaches, pears, plums, and quinces. These fruits were traded from Santa Fe to the Pueblo tribes of the Southwest. According to Jett (1977), “Fray Alonso de Benavides listed peaches,
apricots, and plums as crops in New Mexico in 1629; apples and pears were also introduced during the seventeenth century”. By 1690, pomegranates, dates, figs, olives, quinces, and other fruits had been introduced in the northern Sonoran Desert, including southern Arizona, and some varieties made their way north to the Colorado Plateau. The Hopi were cultivating seedling peaches, apricots, plums, melons, onions, watermelons, and wheat traded from Franciscan missionaries by the early 17th century (Dunmire 2004). By the early 18th century, the Navajo were reportedly cultivating peaches traded from the Hopi mesas in Canyon de Chelly. In a description of his travels through Navajo country, Indian agent Henry L. Dodge noted Canyon de Chelly as "a wide rich valley" planted with “wheat, Indian corn, beans, pumpkins and melons. [The Navajo also have] fine peaches that grow abundantly and of a superior quality.” (Santa Fe Weekly Gazette 1853, as cited in Travis 2005, p. 20). When Captain John Thompson attempted to drive the Navajo (Dine’) from Canyon de Chelly in 1864, his troops destroyed hundreds of orchards on orders from New Mexico Territorial Governor James Carleton. “From 31 July to 5 August Thompson’s troops cut down some 3,200 adult peach trees and many seedlings in Monument Canyon and canyons de Chelly and del Muerto. On 7 August, 450 peach trees were destroyed in Turkey Canyon, a few more miles to the South” (Jett 1977, p. 692). While peach trees were widely planted by indigenous cultures of the Southwest from their introduction by Spaniards in the 17th century onwards, apples did not share the same popularity and were largely lacking from these orchards. This is most likely because of the difficulty in growing apples from seed and because grafting was not practiced by Pueblo cultures. Although the Tarahumara, living along these same trade routes in northern Mexico, were cultivating Spanish introductions of apples, peaches, and
quinces by the mid 18th century (Pennington 1963), mention of apples in Hopi and Navajo agriculture is largely absent until the twentieth century (Jett 1977).

Mexican campesinos planted Spanish apples from pips traded up the Camino Real from Mexico City in small gardens and orchards in and around Santa Fe, New Mexico. Josiah Gregg, a notable botanic explorer of the Southwest, wrote of the gardens and orchards of Santa Fe, New Mexico during his journey through the region in 1830: “…the settlements were thickly interspersed with vineyards, orchards, and cornfields. The orchards were planted with apples, peaches, and apricots, all seedlings and all wretchedly poor, though the grapes from Spain were delicious” (Hedrick 1950, p. 390).

Late 17th and early 18th century Jesuit missionaries, including Padre Francisco Eusebio Kino, and Franciscan missionaries planted many species of fruits in mission gardens throughout the Primería Alta. The lower elevations of the northern Sonoran Desert were better suited for dates, figs, pomegranates, and quinces than temperate fruits; nevertheless, low-chill varieties of apples and pears were introduced as well, and some made their way north to the Colorado Plateau.

**Mormon Pioneers**

The 19th century saw the settlement of Utah by Mormon pioneers. Brigham Young, traveling with a group of Mormon settlers from Illinois, founded Salt Lake City in 1847, and quickly began transforming the desert into “an agricultural paradise”. According to Hedrick (1950, p. 391), “By 1860, pome and drupe fruits were being grown in temperate valleys of the whole state”. An excerpt from C. H. Oliphant’s autobiography, published in Hedrick (1950, p. 392), the Salt Lake City pioneer describes
his efforts to introduce cultivated fruits into Utah and the northern edge of the Colorado Plateau:

Soon after my arrival in Salt Lake City, the spirit was upon me to labor for the introduction of fruit into Utah. I sent East for scions to engraft on seedlings trees which had been produced in the country. This proved a failure. The mail that winter was left along the route at way stations. The next season it was gathered up by ox teams and my scions arrived several months after the season had passed when they could be used. The spring after my arrival, in 1854... I went into the business of raising trees and introducing good varieties of fruit as fast as my circumstances would permit... Lorenzo Dow Young, having been back to the states, had made an effort to bring a number of choice varieties of fruit across the plains in a growing condition in a wagon... We succeeded in getting several varieties through, but lost all but one, and that proved to be the Rawles Jannet. This was the first introduction of this choice variety of apples into Utah.

Other Anglo Settlers

Anglo explorers, miners, and ranchers began settling the Colorado Plateau in the mid 19th century, but their agricultural introductions were largely held at bay by Apache warriors until the late 19th century. With the waning of the Apache wars and the completion of the Santa Fe Trail, the Southwest was opened to a flood of settlers who brought with them numerous varieties of fruits and agricultural crops. Many of the extant historic orchards in the Southwest date to this time period. Small kitchen orchards planted
for homesteads and ranches still adorn the landscape, as do smaller commercial orchards planted to supply mining boomtowns.

**USDA Introductions**

Following the onset of the USDA Plant Introduction Program (1887), agricultural experiment stations were established by the University of Arizona in the farming communities of Prescott, Snowflake, and Sulfur Springs, Arizona, to introduce and test drought-adapted crops for dryland subsistence farming in Arizona. Similar farms were located in New Mexico and Texas. While in operation, from 1887 to 1917, when they were outdated by the wide-scale replacement of dryland agriculture by groundwater pumping to irrigate fields, these experiment stations had brought 177 apple varieties into Arizona, New Mexico, and Texas (Bussey, D. 2006, pers. comm., 14 March). The Prescott station, which was located seven miles north of town, planted the apple varieties King David, Rome Beauty, White Winter Pearmain, Arkansas Black, and Gravenstein in the year 1912 (McOmie 1918). No fruit tree experiments were planted at the Snow Flake Experimental Farm (McOmie 1918).

**Stark Brother’s Nursery**

Discussions with longtime residents of the farming communities of Torrey and Caneville Utah (Routson and Nabhan 2007), revealed that Stark Brother’s Nursery of St. Louis, MO was one of the main sources of fruit trees for central Utah residents, and likely for other farmers throughout the Southwest, from at least the 1930s. James Heart Stark’s nursery venture began in 1816 as a small nursery along the banks of the Mississippi River less than 50 miles north of St Louis in the town of Louisiana (Terry 1966). A generation
later, James Stark’s sons, Clarence, Edgar, and W.P., transformed the small family-run nursery into one of the nation’s largest and most effective commercial nurseries. The newly completed transcontinental railroad passed near the Stark Brother’s Nursery, allowing for speedy and convenient shipping to the newly settled West. By 1887, full-page adds for Stark Brother’s Nursery could be found in nearly all of the country’s most popular newspapers and magazines. In 1896, Stark Brother’s Nursery released one of the first full-color catalogs of fruit trees in the US, offering fifty different apple varieties, in addition to peaches, apricots, plums, pears, and cherries. This color catalog and its hyperbolic descriptions of new varieties as miracles would prove to be the pivotal element in Stark’s national success. Another factor adding to Stark’s popularity on the Colorado Plateau became evident in 1887, when Clarence Stark was diagnosed with tuberculosis and was advised by his doctor to recuperate in the dry, sunny climes of the Southwest (Terry 1966). Clarence Stark relocated to the Denver, Colorado area where he began planting extensive orchards, and making trips to other areas of the Southwest to promote the Stark Brother’s Nursery (Routson & Nabhan 2007, Terry 1966). He apparently visited Fruita, Utah, advising Mormon farmers on their varietal choices.

This rich history of fruit introductions into the Southwest, beginning as early as 400 years ago with Spanish colonist Don Juan de Oñate in 1598 (Seefeldt 2005), and with substantial contributions to by Mormon pioneers and other Anglo settlers during the 19th century, and then overwhelmed in the early 20th century by USDA plant introductions, and later by Stark Brother’s nursery, is reflected in the handful of withering historic orchards that persist across the Colorado Plateau. Apples of all sizes, shapes, and colors
and producing an equal number of flavors, textures, and specific uses, are still found in small numbers, part of a diminishing heritage conserved in these historic orchards.

While sufficient time has passed for the apple, *Malus domestica* to adapt to the Southwest environment and for unique-local genotypes to emerge, it is unclear whether any evolutionary adaptations have emerged in apple varieties grown on the Colorado Plateau. Such varieties could possibly have emerged from seedling trees grown from the original Spanish introductions, but subsequent orchards planted by Anglo settlers from the 19th century onwards consist almost entirely of grafted Eastern and Midwestern varieties and have had little time to diversify. Irrespective, varieties introduced from all sources may represent important agrobiodiversity and cultural adaptations, and thus are of potential interest for conservation.
CHAPTER 3
GENETIC DIVERSITY OF HISTORIC APPLE TREES IN THE SOUTHWEST

The primary purpose of this project was to assess the genetic diversity of historic fruit trees surviving on the Colorado Plateau to determine if they are important to conserve in situ, for their unique or diverse genetic qualities. The results may suggest where in situ conservation efforts should be directed, not just for apples, but possibly for other fruit species as well.

Genomic DNA from leaf samples of 280 historic apple trees collected from 43 sites in Arizona, Utah, and New Mexico were analyzed using seven microsatellite markers. The historic “unknowns” were compared to 109 heirloom varieties introduced into the Southwest in the late 19th century by USDA agriculture experiment stations and the Stark Brother’s Nursery. The historic trees that could not be matched to any of the known varieties should be considered of interest for further study as potentially rare varieties that have vanished from the nursery trade. From these trees, unique and desert-adapted varieties should be identified for preservation.

RESEARCH HYPOTHESES

In this study I test whether historic apple trees planted in orchards on and near the Colorado Plateau can be identified to variety using microsatellite analysis to determine if high levels of varietal richness persist in these orchards.
MATERIALS AND METHODS

Field Sampling

I collected leaf samples from 280 historic trees from 43 historic orchard sites* in Arizona, New Mexico, and Utah located on or near the Colorado Plateau. This was not a random sample, but “stratified” or targeted to places with presumed antique orchards, and to trees of visually differing morphologies.

* One of the collection sites (Dixon Library, NM) was from a small orchard of young trees grafted from historic trees from the greater Santa Fe area, making the total number of collection sites unknown.

Figure 1
Distribution of Sample Sites On and Near the Colorado Plateau: Arizona (25 sites), New Mexico (15 sites), and Utah (3 sites).
The main technical objective of this study was to assess the genetic diversity of historic fruit trees on the Colorado Plateau and surrounding areas, and to see if samples could be identified to named varieties using genomic DNA. Since funding and time were limited, and because most of the thousands of extant apple trees in historic orchards are common varieties, I tried to collect leaf samples only from trees that were visibly different varieties. However, this was not always possible, especially for trees without fruit. In addition to avoiding sampling trees of the same variety, I avoided collecting leaf samples from young trees. Ideally I would have liked to sample only from trees planted prior to the 1920s, around the time when numerous apple varieties began to disappear from nursery catalogs. However, since I was visually determining the age cutoff, I chose to sample only from visibly “historic” trees, based upon size and overall apparent age of the trees.

I recorded GPS locations for the sample trees using a Garmin eTrex-Vista handheld unit (Olathe, KS) in UTM (Nad1983) coordinates, and I also nailed small aluminum tags to the trees with a numerical identifier for later identification of the sample trees. The samples I collected from trees in New Mexico were located using GPS but were not marked with aluminum tags.

For the first set of DNA collections in the 2006 growing season, I collected four to five healthy leaves from each tree and placed them in Whirl-Pak Bags® partially filled with Drieright®. Once the leaves had thoroughly dried, I transferred them to paper coin envelopes. For the later specimens that I collected, I skipped the Drieright® and Whirl-Pak® Bags step, placing the leaves directly in the paper coin envelopes. The leaves dried
flatter and equally well this way, and saved several steps. I also collected and photographed fruit samples from trees that bore fruit.

For the second set of leaf samples in the 2007 growing season, I collected 10-12 leaves, avoiding old leaves and leaves with ‘rust’ whenever possible. These samples were stored in plastic zip-lock bags and sent fresh to the Plant Cytometry Services (Schijndel, The Netherlands) to determine ploidy using flow cytometry, and to the USDA-ARS-NCGRP in Fort Collins, CO, where the DNA lab work was done. For each sample, roughly 50mg of fresh leaf tissue was put into a 96-well plate to be frozen at -20º C for extraction. The remaining leaves were frozen at -80º C and stored at the USDA-ARS-NCGRP facility in Fort Collins. Re-sampling the trees with aluminum tags proved very easy; however, relocating some of the trees in New Mexico with only GPS locations proved difficult in a few situations in which the orchard trees were densely spaced.

Known heirloom varieties were obtained from the USDA-ARS-PGRU (Plant Genetic Resources Unit), Geneva, NY. Varieties that were not available through the Geneva facilities were obtained from Lee Calhoun of Calhoun’s Nursery in Pittsboro, NC, Ram Fishman of Greenmantle Nursery in Garberville, CA, and Gordon Tooley of Tooley’s Trees in Truchas, New Mexico. Leaf samples were processed in the same manner as the historic unknown samples.

**Microsatellite Analysis**

The genetic analysis of historic apple trees of the Southwest and 109 known varieties introduced into the Southwest was done with the kind technical guidance of Gayle Volk, Adam Henk, and Ann Reilley of the USDA-ARS-NCGRP lab in Fort Collins, CO, following procedures outlined in Volk et al. (2005). We extracted genomic
DNA from the leaf samples of 280 historic trees using Qiagen DNeasy 96 plant kits (Qiagen, Valencia, CA). Two separate sets of DNA were extracted from each sample and ran independently through PCR and Gel analysis to verify the end results. *Malus*-specific microsatellites were amplified using unlinked primers (GD12, GD15, GD96, GD100, GD142, GD147, & GD162) as described by Hokansen et al. (1998) and by Hemmat et al. (2003).

**Table 1**

*Malus*-Specific GD Microsatellites Used In This Study.

<table>
<thead>
<tr>
<th>Name</th>
<th>Repeat type</th>
<th>Expected Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD12</td>
<td>(CT)$_{32}$</td>
<td>136-210</td>
</tr>
<tr>
<td>GD15</td>
<td>(AGC)$_{5}$</td>
<td>139-156</td>
</tr>
<tr>
<td>GD96</td>
<td>(TC)$_{22}$</td>
<td>125-209</td>
</tr>
<tr>
<td>GD100</td>
<td>(GA)$_{12}$</td>
<td>200-242</td>
</tr>
<tr>
<td>GD142</td>
<td>(TC)$_{19}$</td>
<td>116-175</td>
</tr>
<tr>
<td>GD147</td>
<td>(AG)$_{7}$</td>
<td>113-175</td>
</tr>
<tr>
<td>GD162</td>
<td>(GA)$_{23}$</td>
<td>196-278</td>
</tr>
</tbody>
</table>

Forward primers were labeled with either IRD700 or IRD800 infrared fluorescent dyes obtained from MWG-Biotech (High Point, NC). Unlabeled reverse primers were obtained from IDT (Coralville, IA).

**Table 2**

Sequences For The Seven *Malus*-specific GD Primers Used In This Study.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Forward Sequence</th>
<th>Reverse Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD12</td>
<td>TTGAGGTGTCTCCCATGGGA</td>
<td>CTAACGAAAGCGCACTTCTT</td>
</tr>
<tr>
<td>GD15</td>
<td>CCGAGCGAGCAAGGATCC</td>
<td>ACTCCATCATCGAGTGTT</td>
</tr>
<tr>
<td>GD96</td>
<td>CGGCGCCAAATCACCT</td>
<td>GCCAGCCTCTATGTTCCCAGA</td>
</tr>
<tr>
<td>GD100</td>
<td>ACAGCAAGGTGTGGGGTAAAGGT</td>
<td>TGGCGGACAAAGGAAAAAAAAGTG</td>
</tr>
<tr>
<td>GD142</td>
<td>GGCACCCAAAGCCCTAA</td>
<td>GGAACCTACGACAGCAAGTTACA</td>
</tr>
<tr>
<td>GD147</td>
<td>TCCGCCATTTCTCTGC</td>
<td>AAAACCGCTGCTGCTAAC</td>
</tr>
<tr>
<td>GD162</td>
<td>GAGGCAAGTGACAAAAAGATG</td>
<td>AAAATGTAACAAACCAGCTCCAGT</td>
</tr>
</tbody>
</table>

Polymerase chain reactions (PCR) were carried out for all samples and known varieties in 15uL total volume. Each 15 ul reaction contained: 0.3ul GoTaq® Flexi Taq Polymerase (Promega, Madison, WI) (5u/ul), 3ul Promega 5x Colorless GoTaq® Flexi
Buffer (10mM Tris-HCl, 50mMKCl, and 0.5% Triton X-100), 1.5ul of 0.25mM MgCl$_2$, and 1.5ul of 0.25mM dNTPs. Forward and reverse primers were added to a final concentration of 0.25 pM/rxn except for GD12 at 0.3 pM/rxn and GD100 at 0.5pM/rxn. Genomic DNA, isolated as described above, was added at 0.5-5 ng/ reaction. Reaction volumes were adjusted to 15 ul using sterile distilled H$_2$O. PCR reactions were multiplexed, with primers GD12 and GD100 run together, GD142, GD147, GD162 together, and GD15 and GD96 run together. PCR was carried out using MJ Research (Reno, NV) PTC 200 Thermocycler. Amplifications were done using touchdown PCR, where the thermocycler reduced the annealing temperature 1º every cycle, starting at 63ºC and ending at 54ºC, followed by an annealing temperature of 55ºC for 18 cycles, and ending with a 2 minute 72ºC extension.

PCR products were diluted 1:1 with a loading buffer of formamide bromophenol blue loading buffer, and were denatured at 95ºC for 5 minutes. Denatured products were diluted 1:10 with additional loading buffer. Diluted products were loaded on gels (6.5% LI-COR KB Plus acrylamide: LI-COR, Lincoln, NB), and run in 1x TBE buffer (89mM Tris, 89mM boric acid, and 20mM EDTA) for 1 hour, 45 minutes at 1500V, 40W, 40mA, and 45ºC in a LI-COR 4200 DNA Sequencer. Digital images of the gels collected by LI-COR Saga Generation2 software were manually analyzed using Saga software. Each allele at each locus was manually scored in Saga before being compared to the duplicate sample. Samples that did not match, or samples where one of the duplicates did not amplify were rerun, from PCR amplification through gel running and analysis. For two samples, the duplicate sample did not match the original, suggesting different DNA source material. This may have been a result of leaves being collected from vegetative
rootstock and from the grafted tree, or a result of human error. Both samples were discarded from the study. Ploidy was determined using flow cytometry by Gerard Geenen of the Plant Cytometry Services, Schijndel, The Netherlands.

**Microsatellite Data Analysis**

Identical genotypes and samples indistinguishable from named historic varieties were calculated manually in Microsoft Excel 2004 for Mac, version 11.3.7. Allele frequencies, average and expected heterozygosities were computed using GenAlEx version 6 (Peakall and Smouse 2006) available online: [http://www.anu.edu.au/BoZo/GenAlEx/](http://www.anu.edu.au/BoZo/GenAlEx/).

Population genetic structure within the samples of historic apple trees was assessed using STRUCTRE version 2.2 (Pritchard et al. 2000) available [http://pritch.bsd.uchicago.edu/software](http://pritch.bsd.uchicago.edu/software). The program uses a Bayesian approach to introduce Hardy-Weinberg population structure to minimize disequilibrium (Evanno et al. 2005). Burnin length was set for 75,000 runs, and a run length of 200,000 steps. The authors (Pritchard et al. 2007) recommend burnin and run lengths of 10,000 to 100,000 as typically sufficient to obtain good estimates of parameter values. The program was run for K=1-15 possible populations, and the appropriate number of populations (K) was estimated using the informal method of graphing Log Pr(X|K). Pr(K) is typically very small, effectively zero for K less than the appropriate value, and plateaus for larger values of K. The smallest value of K after Pr(K) levels off, is often the correct value of K (Pritchard et al. 2007)

Nei’s genetic distance (1983) was computed using Microsatellite Analyzer (MSA) 4.05 (Dieringer and Schlötterer 2003) available online: [http://i122server.vu-wien.ac.at](http://i122server.vu-wien.ac.at)
Nearest neighbor phylogenetic dendrograms were computed using *Phylip* 3.67 (Felsenstein 2005) available online: 
([http://evolution.genetics.washington.edu/phylip.html](http://evolution.genetics.washington.edu/phylip.html)) and drawn using *HyperTree* (Bingham and Sudarsanam 2000) available online: 
([http://kinase.com/tools/HyperTree.html](http://kinase.com/tools/HyperTree.html)). PCA ordinations were preformed using PC-ORD version 4.0.

**RESULTS**

Genetic analysis revealed that 120 of the 280 sample historic trees were indistinguishable from the fingerprints of 34 named varieties (Figure 2). The remaining 160 samples did not match any of the known varieties. The 160 sample ‘unknowns’ had represented 110 unique varieties based on unique fingerprints. Of the 280 samples there were a total of 144 (51%) distinct varieties, including both named and unknown varieties (Table 3).

**Table 3**

<table>
<thead>
<tr>
<th>Known and Unknown Samples and Numbers of Different Varieties Found In This Study.</th>
<th>Number of Samples</th>
<th>Number of Varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Named Varieties</td>
<td>120</td>
<td>34</td>
</tr>
<tr>
<td>Unknown Varieties</td>
<td>160</td>
<td>110</td>
</tr>
<tr>
<td>Total</td>
<td>280</td>
<td>144</td>
</tr>
</tbody>
</table>
Sample Trees Matching 34 Known Varieties: varieties with an “**” match all but one allele in microsatellite GD100 for all or most of the samples. These one or two base pair shifts may or may not indicate the variety is morphologically distinguishable from the actual named variety. Samples labeled with “***” are samples that positively matched more than one variety (see Table 4).

Of the 34 known varieties, only five well known varieties (Ben Davis, Delicious, Grimes Golden, Jonathan, and Winesap) were relatively common in orchards around the Colorado Plateau with more than four samples each keying to these varieties. A number of trees matched genotypes of named varieties at all but one or two alleles for the locus GD100. It is unclear from the results of this study whether these one or two base pair shifts result in morphologically different varieties. Genetic results also revealed that several of the named varieties had identical fingerprints (Table 4) indicating either labeling error or that they are just synonyms of the same variety. Named varieties Winter
Banana and White Winter Pearmain also had identical genotypes, but this is most likely a result of a mislabeled tree at the USDA.

**Table 4**

<table>
<thead>
<tr>
<th>Variety</th>
<th>Apparent Synonym</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Strawberry</td>
<td>Yates</td>
</tr>
<tr>
<td>Fameuse</td>
<td>Canada Red</td>
</tr>
<tr>
<td>Maiden Blush</td>
<td>Chenango Strawberry</td>
</tr>
</tbody>
</table>

Ploidy results (Table 5) revealed 24 of the 280 samples (8.6%) were triploid (3x = 51) with an extra set of chromosomes fused onto the normal diploid (2x = 34) genotype. Triploids arise spontaneously in 2×-by-2× crosses, and typically have larger fruit than diploid apple trees (Ferree & Warrington 2003). From field observations, many of the triploids in this study appear to be late ripening, large-fruited winter apples.

**Table 5**

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>ploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td>256</td>
<td>2x</td>
</tr>
<tr>
<td>24</td>
<td>3x</td>
</tr>
</tbody>
</table>

Population structure for historic apple trees growing on or near the Colorado Plateau was analyzed using the program *STRUCTRE* version 2.2 (Pritchard et al. 2000). I used an informal approach to determine possible number of populations (K) as described by Pritchard et al. (2007) and Evanno et al. (2005), based upon the Log Pr(X|K) (figure 3). The graph of lnP(D)|K shows a possible plateau at K=5 possible populations, as
described by both Pritchard et al. (2007) and Evanno et al. (2005) as the most likely value of the real K. However, the variance, \( V(\ln P(D)) \), is excessively high for all possible values of K greater than one, and increases in magnitude with increasing K, suggesting no population structure exists in historic apple trees on or near the Colorado Plateau. This outcome is not surprising, considering that apple trees planted in the region during the late 19th and early 20th centuries were almost exclusively grafted trees of named varieties. Apple varieties are single genotypes with desirable fruiting characteristics that are clonally propagated. Hardy-Weinberg population structure does not apply to trees selected and propagated in this way.

Figure 3

Natural Log Probability (lnP(D)) for K Possible Populations of Historic Apple Trees on the Colorado Plateau. The add-hock value of K is located where the graph plateaus and begins to level off, in this case, possible at K=5. However, the excessively high variance \( V(\ln P(D)) \) associated with the lnP(D) suggests no Hardy-Weinberg population structure exists for historic apple trees growing on or near the Colorado Plateau.

High levels of observed heterozygosity (Table 6) in historic apple trees on and near the Colorado Plateau indicate high levels of genetic diversity. Heterozygosity was calculated from a sample size of 144 individuals, representing each of the 144 distinct
varieties found growing on and near the Colorado Plateau. During PCR and gel analysis, GD100 was often highly overloaded in comparison to GD12, which was multiplexed on the same gel. This resulted in 6% missing data at GD100 where we were unable to determine the allele size from the gels. Heterozygosity (Table 6) and allele frequencies (Appendix III) should be interpreted with dubiety for this allele. The number of alleles (Na), number of effective alleles (Ne), and the fixation index (F) for the samples are also reported in Table 6, though they are of limited value for interpretation since historic apple trees on and near the Colorado Plateau do not comprise a genetic population. For this same reason, F-statistics were not calculated for samples.

Table 6

Sample size (N), number of alleles (Na), number of effective alleles (Ne), observed (Ho) and expected (He) heterozygosity, and fixation index (F) calculated for samples. Only samples with unique genotypes were used (as opposed to all 280 samples, see table 3.0). GD100 had 6% missing data for samples—which may affect numbers for this allele.

<table>
<thead>
<tr>
<th>Locus</th>
<th>N</th>
<th>Na</th>
<th>Ne</th>
<th>Ho</th>
<th>He</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD12</td>
<td>144</td>
<td>11.000</td>
<td>4.018</td>
<td>0.833</td>
<td>0.751</td>
<td>-0.109</td>
</tr>
<tr>
<td>GD15</td>
<td>144</td>
<td>2.000</td>
<td>1.021</td>
<td>0.021</td>
<td>0.021</td>
<td>-0.011</td>
</tr>
<tr>
<td>GD96</td>
<td>144</td>
<td>14.000</td>
<td>6.818</td>
<td>0.875</td>
<td>0.853</td>
<td>-0.025</td>
</tr>
<tr>
<td>GD100</td>
<td>134</td>
<td>9.000</td>
<td>5.386</td>
<td>0.485</td>
<td>0.814</td>
<td>0.404</td>
</tr>
<tr>
<td>GD142</td>
<td>144</td>
<td>13.000</td>
<td>7.352</td>
<td>0.924</td>
<td>0.864</td>
<td>-0.069</td>
</tr>
<tr>
<td>GD147</td>
<td>144</td>
<td>12.000</td>
<td>5.471</td>
<td>0.868</td>
<td>0.817</td>
<td>-0.062</td>
</tr>
<tr>
<td>GD162</td>
<td>144</td>
<td>13.000</td>
<td>5.795</td>
<td>0.896</td>
<td>0.827</td>
<td>-0.083</td>
</tr>
</tbody>
</table>

Nei’s genetic distance (1983) was computed using Microsatellite Analyzer (MSA) 4.05 (Dieringer and Schlötterer 2003). Nearest neighbor phylogenetic dendrograms were computed using Phylip 3.67 (Felsenstein 2005), and drawn using HyperTree (Bingham and Sudarsanam 2000) for the known varieties (Figure 5) and for the samples (Figure 6). These dendrograms show the genetic relatedness of known varieties with respect to each other, and unknown varieties with respect to each other, and with respect to named varieties found in the samples.
Figure 4
Nearest Neighbor Dendrogram of Named Varieties Using Nei’s (1983) Genetic Distance.
Figure 5
Nearest Neighbor Dendrogram of Samples Using Nei’s (1983) Genetic Distance.
For the dendrogram of samples (Figure 5), the state from which the samples were collected is attached as a prefix to the sample number to show possible associations of geographic origin to genotype. This was also repeated in PCA ordinations (Figure 7). The dendrogram displays no clusters as uniquely belonging to a single state.

Principle Components Analysis (PCA) was performed on both the known varieties and the samples as another method for viewing genetic difference between apple varieties. Again, both known varieties (Figure 6) and samples (Figure 7) were analyzed to see differences and similarities between known varieties and between unknown samples and known varieties.

In both the PCA ordination of the named varieties and the PCA ordination of the unknown varieties, there are two distinct clusters of varieties that appear to be the result of the presence of two larger alleles at marker GD12 (182 and 190 base pairs) instead of the more common 148 to 162 base pair-length alleles.
Figure 6
PCA Ordination of Named Varieties Introduced Into the Southwest During the Late 19th and Early 20th Centuries.

Samples that could be keyed to named varieties based on their genetic fingerprints were labeled with variety names. Unknown samples were left with their numerical identifiers and the state from which the sample originated appended to the end of the number. Relations between all samples were analyzed genetically and geographically by
the state identification tags. Geographic separation of samples would indicate that
different sources of apple trees were used in different regions. Historic apple trees from
New Mexico may have been originally introduced from Spain to the region via 16th and
17th century Spanish explorers. Geographic differentiation could also imply different
apple preferences associated with different regions. Either way, there was no apparent
.genetic separation by geographic origin among samples. The historic trees across the
Colorado Plateau might all share the same recent parents, or the parent diversity could
have been obscured by small sample size.
Figure 7

PCA Ordination of Samples. The state from which each sample was obtained is appended to the end of the numerical sample name. There is no evidence of samples differing by state: either samples originating from different sources do not show substantial genetic differences, all of the sampled trees are of a common origin, or most of the trees are from a common origin, and too few samples originate from different sources to be identified through PCA.

Morphological traits (Table 7) of fruit size, shape, color, ripening time, and color of the flesh in addition to species (eating apple or crab apple) were recorded from photos of the fruit (Appendix IV) and field notes for 120 samples that produced fruit either
during the 2006 or 2007 growing season that I was able to photograph. Samples were grouped into clusters (Figure 8) using Ward’s distance, based upon fruit morphology in JMP version 5.12 statistical software.

Table 7

<table>
<thead>
<tr>
<th>Trait Categories</th>
<th>Species</th>
<th>Size</th>
<th>Shape</th>
<th>Color</th>
<th>Flesh</th>
<th>Season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>M. domestica</em></td>
<td>S/M/L</td>
<td>R/RC/C</td>
<td>R/RC/YG/YG/Y</td>
<td>YW/YW/Y</td>
<td>E/M/ML/L</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A nearest-neighbor dendrogram based on Nei’s (1983) genetic distances (Figure 9) was computed for the 120 samples with morphological data. The genetic clusters were color-coded and the individuals within each genetic cluster were coded with the same colors in the morphological dendrogram to compare morphological classification with genetic classification. Names of samples as identified by genetic analysis were included in both dendrograms. Not only did the morphological tree show no association with the genetic tree, but the morphological tree also failed to group individuals of the same variety into the same clusters in many situations. The results of the morphological tree should be regarded with a degree of dubiety because of possible sampling and observer error (morphological observations were based off of a photo of a single fruit). Even so, the results strongly indicate a high degree of plasticity in morphological traits within varieties.
Figure 8
Cluster Analysis Using Ward’s Distance of Samples Based on Fruit Morphology Data. Names and color-codes are based on a genetic-distance dendrogram of the same samples (Figure 9) using nearest-neighbor (Nei’s 1983) genetic distance.
Figure 9
Nearest-Neighbor Dendrogram (Nei’s 1983) of Samples with Fruit Morphology Data. Color-coded branches match the colored samples in the fruit morphology dendrogram (Figure 8).
The seven GD microsatellites effectively differentiated between samples and named varieties in this study, except in cases where the samples are presumed to be of the same variety or a closely-related sport as indicated by Hokanson et al. (1998), and in cases where known samples were given more than one name (or have been misnamed). The high observed heterozygosity of these microsatellites make the probability of different genotypes appearing the same very slight and so, this low probability will be disregarded for the rest of this discussion. Half of the samples were different varieties of apples, 144 in total. One hundred and ten of these are unknown varieties. These unknown varieties could be regionally unique varieties, varieties extinct from the nursery trade, or extant varieties originally from other regions that have not been recorded as being introduced into this region.

The 144 varieties determined in my 280 samples—in addition to the high levels of observed heterozygosity between samples—indicate that a high diversity of apple varieties was cultivated on the Colorado Plateau during the late 19th and early 20th centuries. For the purposes of this study, all samples that could not be identified to variety should be considered of value for future conservation efforts. This number could become smaller as more named varieties are genotyped using the seven microsatellites, and compared back to the unknown samples for more positive matches. Since most of the common varieties of apples in the United States during the late 19th and early 20th centuries were included in the named varieties fingerprinted in this study, many of the apple varieties found that did not match these named varieties may be rare or unique genotypes that are no longer propagated anywhere else.
PCA ordinations and Nei’s genetic dendrograms show the genetic relatedness of both named and unknown apple varieties found in this study. Targeting apple varieties with the greatest genetic distance from common named varieties is one strategy for prioritizing which unknown varieties should be preserved to ensure that the broad genetic diversity within the region is conserved. However, due to the low number of microsatellites used in this study, the actual level of diversity may not be readily apparent, potentially making this strategy less effective for implementing conservation than would be an overarching plan to preserve all 110 unique unknown varieties.

No genotype-to-geographic site correlation could initially be found for these samples. This does not preclude samples in different regions having different origins and introduction pathways, as apple trees could have been traded across the entire region from different original sources. However, it is more likely that my samples came primarily from the Eastern and Midwestern United States due to the high number of trees introduced to this region by settlers and nurseries from those regions. If trees from earlier Spanish introductions and subsequent local varieties are present in my samples, they are few in number, or by some chance they were broadly distributed across the region, and among my sample sites, currently making it difficult to identify them based on geographic origin. Cross-pollination of seedling apples of Spanish origin with Midwestern and Eastern varieties may also act to obscure particular (single or monophyletic) origins. As Gayle Volk et al. at the USDA-ARS-NCGRP obtain data from a Spanish cider apple project in which they are involved, there may emerge new opportunities to positively identify trees of Spanish origin.
When I attempted to correlate preliminary fruit morphological data with genetic data to distinguish between varieties, I initially found that identifications based upon fruit morphology alone yields highly inconsistent results. Aside from sampling biases and human error, high phenotypic plasticity influenced morphological characteristics of fruit size, shape, and color appear to vary considerably within varieties. These results indicate that accurate characterization of historic varieties based on fruit morphology alone is not very likely, at least in the context of this study. Accuracy of fruit morphology may increase if data are recorded for multiple fruits from a single sample tree over multiple seasons, ensuing that the fruit sampled are at the peak of their maturity. However, an assessment of this nature would require a large time commitment with unknown success rates. Furthermore, even if varieties could accurately be classified into groups by morphological characteristics, it would still not be possible to differentiate between potentially rare genotypes with morphological traits similar to those of common varieties. This said, there might be genetic adaptations that exist in local varieties that are indistinguishable from those in named varieties still available in the nursery industry, due to the limited number of alleles sampled in genetic fingerprints. Conservationists and land managers interested in differentiating between historic apple varieties should rely on genetic fingerprinting for broadly characterizing genetic diversity, but should understand the limits of such techniques, and may wish to record morphological data as well.
CHAPTER 4
IN SITU AND EX SITU POLICY AND CONSERVATION STRATEGIES FOR AGROBIODIVERSITY

Genetic conservation historically relied on two different strategies that have been supported by governmental and non-governmental organizations. One of these two strategies is the *ex situ* (offsite) conservation that involves seed banks, zoos, botanical gardens, and other collections expressly made for maintaining genetic diversity and for plant breeding. The number of *ex situ* germplasm collections has grown rapidly since the early 1970s, when there were globally fewer than ten large seed banks, holding perhaps no more than a half million accessions. Now, more than 1,500 national, regional, or international genebanks are recorded in the FAO World Information and Early Warning System (WIEWS) database on Plant Genetic Resources for Food and Agriculture (PGRFA), and over 5 million accessions are stored worldwide in *ex situ* germplasm collections. Apples are stored at over 180 of these repositories (WIEWS website, accessed 11/14/2007).

As global development continues to displace subsistence farming and traditional crops, and gene banks become the sole proprietors of much of our agricultural biodiversity, we are beginning to see the inadequacies of *ex situ* conservation. *Ex situ* conservation fails to preserve much of the traditional horticultural, cultural, and culinary knowledge associated with the crops. Furthermore, *ex situ* gene repositories are most accessible to academic researchers and plant breeders, and are seldom of much benefit to the subsistence farmer from whom the material was originally obtained.
The idea of in situ conservation has become recognized as a means to overcome the shortfalls of ex situ conservation. According to U.S. Congress Office of Technology Assessment, OTA (1987), in situ conservation is done by “Maintaining plants, animals, and microbes onsite—in their natural environments—as the most effective way to conserve a broad range of diversity” (p. 6). Agricultural resources conserved on-farm, in fields, orchards and hedgerows, within their centers of origin, allow for continued evolution and adaptation to changing environments, as well as the conservation of cultural knowledge and uses associated with the crops, which are not possible in ex situ collections. Furthermore, employing a diversity of local varieties, in theory, should help protect traditional farmers from catastrophic crop losses. In addition, locally adapted varieties require fewer chemical inputs than high-yielding commercial cultivars, allowing farmers to spend less on pesticides, fertilizers, and spray equipment (U.S. Congress OTA 1986).

Both ex situ and in situ conservation strategies have their own particular benefits and drawbacks, and perhaps conservation is best achieved through a combination of the two strategies. As concluded by OTA (1987), establishing and maintaining links between in situ and ex situ management systems are essential to increasing the efficiency and overall effectiveness of efforts to maintain diversity.
Table 8

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<td>Increasing human intervention</td>
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Table 9

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<td>Maintain:</td>
<td>Maintain:</td>
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<tr>
<td>- A reservoir or library of genetic resources</td>
<td>- Genetic interaction between semi-domesticated species and wild relatives</td>
<td>- Breeding material that cannot be stored in genebanks</td>
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<tr>
<td>- Evolutionary potential</td>
<td>- Wild populations for sustainable harvesting</td>
<td>- Field research and development on new varieties and breeds</td>
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<tr>
<td>- Functioning of various ecological processes</td>
<td>- Viable populations of threatened species</td>
<td>- Offsite cultivation and propagation</td>
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<tr>
<td>- Vast majority of known and unknown species</td>
<td>- Species that provide important indirect benefits (e.g. for pollination or pest control)</td>
<td>- Captive breeding stock of populations threatened in the wild</td>
</tr>
<tr>
<td>- Representatives of unique natural ecosystems</td>
<td>- “Keystone” species with important ecosystem support or regulating function</td>
<td>- Ready access to wild species for research, education and display</td>
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</tbody>
</table>

A combined strategy of *in situ* and *ex situ* conservation efforts is likely to be the most effective option for conserving homestead fruit trees of the Colorado Plateau.
Orchards with high numbers of unique varieties should be targeted for in situ conservation, while at the same time all potentially unique or rare varieties should be propagated ex situ in a conservation orchard. In addition to ensure against losses of varieties due to drought and old age, an ex situ collection will allow greater access to the varieties for propagation efforts to reintroduce these varieties back into our communities.

FORMAL FEDERAL POLICY VERSUS GRASSROOTS CONSERVATION

Some federal agencies have made a commitment to put conservation policies into practice. For example, the National Park Service (NPS) has historic preservation policies in place that allow for preservation of the cultural resources as well as genetic resources of historic sites to be preserved. Historic orchard preservation and restoration workshops were conducted by the NPS and collaborating agencies:

1. Sleeping Bear Dunes National Lakeshore with the Olmsted Center for Landscape Preservation (2001);

2. Capitol Reef National Park in collaboration with Center for Sustainable Environments, Northern Arizona University (2006);

3. Orchard management plans have been completed for the Capitol Reef historic orchards and the John Mirror historic orchards.

The Conservation Study Institute of the NPS produced the publication, Stewardship Begins with People (Diamant et al. 2007) as an atlas of local collaborative projects involving the Park Service and communities to preserve authentic traditional cultures as well as significant cultural landscapes (Diamant et al. 2007). Preservation
projects from 24 national parks throughout the nation are described in this atlas.

Designation of National Heritage Areas (described in a later section) is another formal policy mechanism to encourage voluntary stewardship and cultural resource preservation through federal funding incentives. In addition to collaborative cultural preservation projects, the NPS has in place an agreement with the USDA genetic repositories to store off-site unique germplasm, which includes unique varieties of heirloom fruits.

The Bureau of Land Management and USDA Forest Service on the other hand, lack formal historic preservation policies and have no agreements with genebanks to store genetic material (other than a few conifers) ex situ, making conservation on these federal lands more difficult to achieve via traditional methods. However, the USDA Forest Service has established Zoological-Botanical Areas to conserve some native (wild) crop relatives in situ.

Insufficient federal and state land management policies have motivated some grassroots organizations to conserve historic orchards and their genetic resources through grassroots and collaborative stewardship approaches. Although still voluntary, these initiatives engage private landowners, nongovernmental organizations, and federal agencies in collaborative conservation efforts for cultural and biological resources such as historic apple trees.

Grassroots Organizations

A number of grassroots nonprofit organizations have arisen in recent decades in response to the inadequacy of governmental support to curb the loss of biodiversity. Grassroots organizations dedicated to the preservation of endangered agricultural crop
varieties include Biodiversity International, The SAVE Foundation, Genetic Resources Action International (GRAIN), Global Diversity Trust, Seed Savers Exchange, Native/Seeds/SEARCH, North American Fruit Explorers (NAFEX) and others.

In a policy assessment of the effectiveness of grassroots biodiversity organizations, the U.S. Congress, Office of Technology Assessment (1986, p. 49) determined that

Grassroots activities are a vital part of U.S. efforts to maintain biological diversity. They supplement those efforts by undertaking activities for which they are uniquely suited or especially willing, but they are unable to replace the government’s broader responsibilities for maintaining biological diversity.

The report goes on to say that (p. 50):

The strength of grassroots organizations lies in a shared commitment to preserve resources which are perceived as valuable and threatened. The local focus and flexibility of many grassroots groups can make them particularly responsive to many issues. These organizations, however, can be vulnerable due to limited or unstable funding, and dependence on the enthusiasm of a single individual or small group.

Grassroots organizations are an immensely important factor in the preservation of crop genetic resources in the U. S. and around the world, often including those of apples. In consultation with and in combination with government-supported *ex situ* germplasm repositories, these grassroots organizations are working to preserve much of the world’s agricultural biodiversity using both *in situ* and *ex situ* strategies. According to the US Congress OTA (1986, p. 52), “Networking organizations that coordinate the activities of
the individuals and small grassroots groups can enhance the contributions to the maintenance of biological diversity”. In short, governmental and nongovernmental efforts often compliment one-another just as in situ and ex situ efforts do.

**SPECIFIC GOVERNMENT-BASED POLICY STRATEGIES FOR IN SITU PRESERVATION**

**National Heritage Areas**

The NPS National Heritage Area program supports conservation through grassroots organization and voluntary (private and civic) preservation initiatives. Regions designated as National Heritage Areas achieve conservation through bringing in federal support for heritage education, restoration, and preservation for voluntary efforts by private landowners and tribal residents within the Heritage areas (Santa Cruz Valley National Heritage Area Feasibility Study Summary 2005). Furthermore, National Heritage Areas are managed at a local level and because preservation is voluntary, designation of a region as a National Heritage Area does not negatively affect property rights of landowners living within the designated area who do not wish to participate.

For regions designated as National Heritage Areas, federal funding is available for the preservation of cultural resources, including historic orchard preservation. This is another option for private landowner collaborative stewardship of our cultural resources in cooperation with the federal government. In other regions, such as the Silos and Smokestack region of Iowa, have provided support to the Seed Savers Exchange Heritage Farm for conservation education through agritourism.
The National Register of Historic Places

The National Register of Historic Places, enabled by the National Historic Preservation Act of 1966, is a federal policy that can be used to help preserve historic orchards within the United States. The National Historic Preservation Act enables the protection of historically significant properties approved by the Secretary of Interior (36 CFR §60 (2004)): “The National Historic Preservation Act of 1966, 80 Stat. 915, 16 U.S.C. 470 et seq., as amended, authorizes the Secretary of the Interior to expand and maintain a National Register of districts, sites, buildings, structures, and objects significant in American history, architecture, archeology, engineering and culture.”

Historic orchards are considered by the 1966 National Historic Preservation Act to be of heritage value that can be registered in the National Register under the category of agriculture/subsistence and subcategory of an agricultural field, which include pastures, vineyards, orchards, wheatfields, crop marks, stone alignments, terraces, and hedgerows (McClelland 1997).

On the Colorado Plateau, the historic orchards at Capitol Reef National Park and the historic orchards at Slide Rock State Park are both listed in the National Register of Historic Places. The Slide Rock historic orchards have benefited directly from this designation through a heritage grant received by the Center for Sustainable Environments to help restore the historic orchards. In addition to mapping, tagging, identifying to variety, and assessing the condition of the historic trees, cuttings from the historic trees were propagating onto new rootstock through grafting to preserve the genetics of the historic trees. A new orchard of the newly grafted trees will be planted at the park to
ensure the continued survival of the Slide Rock apple varieties once the original trees have perished.

Placing an historic orchard on the National Register of Historic Places is one means available to the landowner for drawing attention to the value of historic orchards. However, the National Register was more designed and is most appropriate for the preservation of historical architectural resources, which include *inter alia*, historic structures and buildings, landmarks and sites. Such constructed resources do degrade with time, but are not prone to true extinction, as are biotic entities. Consequently the National Register of Historic Places is limited in its capacity to preserve the genetic resources of historic orchards. Nevertheless, property owners with historic orchards can use the register to qualify for funds with which to maintain and upkeep their orchards and re-propagate the historic varieties. Registered historic orchards may also be less prone to destruction from changes in land use than non-registered orchards. Aside from this however, the National Register offers little else for preserving heirloom fruit varieties, for such properties can still be sold or developed.

The National Register of Historic Places offers landowners with significant historic properties—including historic orchards—incentives for the continued preservation of the historic resource. Listing orchards in the National Register of Historic Places will not necessarily ensure the survival of historic varieties, but may be a valuable first step towards their conservation.
COLLABORATIVE CONSERVATION PROJECTS BASED ON THE COLORADO PLATEAU AND IN THE GREATER SOUTHWEST

There are a number of collaborative projects underway in the Southwest to preserve and restore the cultural heritage of desert-adapted heirloom fruits. These projects include restoring the Hopi peach orchards by the Natwani Coalition and Center for Sustainable Environments; Renewing America’s Food Traditions collaboratively managed by Slow Food USA; the Southwest Regis-TREE begun by Native Seeds/SEARCH; and the Kino Fruit Trees Project of the Arizona Sonora Desert Museum and Tumacacori National Historic Park.

Hopi Orchard Restoration

Over many years, Hopi elders have requested assistance in restoring historic orchards and propagating heirloom fruits. The first effort of this kind was initiated by the Meals for Millions Foundation office in Tucson, Arizona upon the request of Hopi farmers in the 1980s. Subsequently, the late Ferrol Sekakuku a former tribal governor initiated peach orchard restoration below Second Mesa in 2004. In the spring of 2005, Center for Sustainable Environments and the Natwani Coalition held a grafting workshop on Second Mesa in an effort to restore the Hopi Peach through propagating cuttings from historic trees onto young rootstock. Though the success of these efforts have thus far been limited in restoring historic peach orchards at Hopi, they will hopefully inspire future projects and successes. Historic orchard preservation workshops will not only serve to educate people on basic horticultural skills, but will bring awareness to the value of the disappearing resource of the genetic diversity represented in historic orchards.
Renewing America’s Food Traditions (RAFT)

RAFT is a collaborative of nonprofit food, agriculture, conservation, and educational organizations that, beginning in 2004, have been working to rescue the endangered traditional foods and culinary knowledge of America. Organizations currently involved in RAFT include: American Livestock Breeds Conservancy, Center for Sustainable Environments at Northern Arizona University, Chefs Collaborative, Cultural Conservancy, Native Seed/SEARCH, Seed Savers Exchange, and Slow Food USA. RAFT has not only conducted the only survey of agrobiodiversity of the nation, but has ranked these foods in order of endangerment and risk of extinction. Apples are the second most endangered food crop in America according to RAFT surveys, with 180 varieties listed as endangered or threatened. Endangered foods on the RAFT list are foods that were once common in regional cuisine, but have virtually vanished from the region, as well as from nursery sources. The RAFT project works to identify these endangered foods and begin restoring them into our diets, and in doing so, rescue the crops themselves. It lists the most delicious and recoverable ones on the Slow Food Ark of taste. Beginning in 2008, the organization will sponsor heirloom fruit tree conservation workshops in two regions of North America (RAFT website, accessed 8/12/2007).

Southwest Regis-TREE

The Southwest Regis-Tree is an expansion of the Arizona Regis-Tree begun in 1992. Kevin Dahl and Gary Paul Nabhan of the Native Seeds/SEARCH staff started the Arizona Regis-TREE in the early 1990s when they became concerned about declines in historic orchards throughout Arizona. They wished to pioneer in the US an in situ
conservation strategy based on a model from Daniel Querol’s Nicaraguan project, Güises Montaña Experimental (Inter Pares 2002). This register of historic orchards aids researchers in locating and preserving historic orchards. From 1994-1996 Native Seeds/SEARCH field intern, Michael Hathaway traveled throughout the state listing historic orchards. In addition, many landowners within the state who had historic orchards on their property registered their orchards during this time. From the late 1990s through 2005, the Regis-TREE was at Prescott College and was incorporated into Andrew Millison’s M.A. thesis and Kanin Routson’s undergraduate research. In 2005, the Regis-TREE was brought to CSE where it was revived; uploaded to the worldwide web; and expanded into the Southwest Regis-TREE to include the historic orchards sampled in this thesis in New Mexico and Utah. The Southwest Regis-TREE will likely be relocated to Native Seeds/SEARCH in the next year or two where there are plans to begin a conservation orchard to begin preserving the genetic heritage of our historic orchards in the Southwest (Southwest Regis-TREE website, accessed 12/8/2007).

**Kino Fruit Trees Project**

The Kino Fruit Trees Project began as a collaborative project between Jesús García from the Arizona-Sonora Desert Museum, Robert Emanuel from the University of Arizona, and David Yubeta of Tumacácori National Historical Park. Its goals are to research, locate, propagate, and re-establish historically accurate fruit trees introduced by late 17th and early 18th century Jesuit missionaries such as Padre Francisco Eusebio Kino. Cuttings collected from historic fruit trees during the project were rooted or grafted and in June 2007, planted out in a small conservation orchard located on site of the original 5-acre mission orchard in Tumacácori National Park. The site is adjacent to the
De Anza National Historic Trail, and lies near the center of the Upper Santa Cruz watershed proposed as a new National Heritage Area. In October, 2007, the US House of Representatives approved designation of the areas as a future National Heritage Area.

Background knowledge of the genetic identities of historic fruit trees in the region will greatly increase the effectiveness of these conservation projects by allowing for targeted *in situ* and *ex situ* strategies to conserve historic fruit trees. Basic knowledge of locations of historic fruit trees is essential for their conservation, but for apples and many other fruit crops there are literally thousands of extant historic trees in abandoned orchards scattered across the Colorado Plateau. With limited access to funding, it is impossible to conserve all of these trees, and there would be no point in doing so when the majority of these trees are composed of a handful of common varieties. Genetic fingerprinting is an ideal method for identifying historic trees with unique genetic characteristics, and further greatly reduces the number of historic trees and orchards to direct funding towards their conservation.

**OTHER STRATEGIES FOR CONSERVING HEIRLOOM FRUITS**

**Niche Markets**

Conservation projects are likely to be insufficient in the long run to fully restore the diversity of heritage fruits, because they will always be subject to funding shortages, and changes in conservation priority. Reintroducing them back into American agriculture and diets will better ensure against their loss. Niche markets may be one effective way of achieving this reintroduction. Goland and Bauer (2004) studied the utility of niche local markets in the preservation of heirloom varieties of apples. Through multidimensional
scaling analysis of pilesorts of names of heirloom apple varieties compiled by Ohio orchardists based on salient characteristics of taste, use, and marketability of the fruits, they found a strong correlation between local markets and the preservation of heirloom varieties. According to Goland and Bauer (2004 p. 228):

Demands of the conventional commercial market with regards to transportation, appearance, and storage exclude the old varieties. On the other hand, there is a strong congruence between sales in local markets and the production of old varieties. Local markets, such as roadside stands and ‘pick-your-own’ operations, play an important role in the preservation of heirloom varieties of apples.

When selecting apple varieties for local markets, Goland and Bauer (2004) found that Ohio apple growers were much more likely to choose varieties based upon flavor and versatility than on the characteristics favored by commercial apple marketing in chain groceries. They concluded that local markets help to preserve apple diversity by giving growers a greater freedom to choose varieties that may not meet the standards for commercial markets, but may have higher flavor or may be historically important to the region.

**Preservation Workshops and Conferences**

To further bring awareness to the need to preserve historic orchards for their valuable cultural and genetic resources, Gary Nabhan and Kanin Routson of the NAU Center for Sustainable Environments presented at the 2006 Land Trust Alliance Conference, Nashville TN, at the 2006 Natural Areas Conference, Flagstaff, AZ, and at the Arizona Historical Society in Tucson, AZ in 2007. These conferences, as well as
other venues in the future, help bring awareness to the scientific, land stewardship, and genetic conservation communities throughout the country that will hopefully spark further field research and similar conservation projects in other regions throughout the country.
CONCLUSION

This project was an initial assessment of historic apple trees growing on the Colorado Plateau to determine if sufficient genetic diversity exists within the region to validate conservation efforts from a biological perspective. Historic fruit trees are currently of value for conservation based solely on their cultural importance, though different preservation strategies would be implemented if historic fruit trees were not genetically diverse or unique to the region and hence not in danger of extinction.

The results of this study indicate that historic orchards on the Colorado Plateau and surrounding areas hold a high level of genetic diversity of historic and landrace fruit varieties not necessarily identified elsewhere, and thus have merit for preservation from both biological and cultural perspectives. All 110 varieties that did not match known heirloom varieties fingerprinted in this study should be conserved based on their potential to be rare or unique genotypes, and based upon potential regional adaptations. Additional genetic fingerprinting of known varieties may make it possible to identify more of the varieties, and more extensive field observations will make it possible to further prioritize trees based upon unique or desirable morphological traits. In addition, coring trees to determine exact age and collecting drought and disease stress data will provide more information to prioritize trees for conservation. Older trees are more likely to be rare or unique varieties than trees planted after 1930.
RECOMMENDATIONS

To effectively conserve local landraces and historic fruit varieties from extinction, immediate action is called for to identify important heritage trees, and through a mix of *in situ* and *ex situ* conservation remove immediate risks of extinction. Conservation strategies should include:

1. Identify orchards with high levels of genetic diversity of fruit varieties for *in situ* conservation and unique genotypes for *ex situ* conservation

2. Collaborate with governmental agencies, grassroots organizations, and private landowners for both *in situ* and *ex situ* conservation projects to propagate historic trees onto new rootstocks in conservation orchards and to replace historic trees as they die, and to collect dormant cuttings from unique genotypes for *ex situ* preservation in USDA germplasm repositories.

3. Integrate fruits back into our lives through public awareness projects, workshops, and through niche markets such as farmers’ markets and nurseries to distribute heirloom fruits and trees to the public.

This study identified 110 unique unknown varieties of merit for conservation (Step 1), however, the 280 trees sampled in this project were but a fraction of the historic trees growing within the region, and broader and more intensive sampling efforts will be essential for conserving historic fruit tree diversity in the region.

Governmental heritage preservation programs such as the NPS National Heritage Areas Program and the National Register of Historic places are useful tools for preserving historic orchards and fruit trees on eligible historic properties (Step 2). The
NPS National Heritage Area program will be of great service for *in situ* historic orchard preservation in regions designated as National Heritage Areas. In addition, the National Register of Historic Places is a valuable tool available to landowners for *in situ* preservation of historic orchards on eligible historic properties. Listing an orchard in the National Register of Historic Places, or achieving National Heritage area status for a region will not necessarily ensure the survival of historic varieties, but are important first steps towards their conservation. This said, few historic orchards are likely to fall into an area with National Heritage designation or meet the criteria of listing a property in the National Register of Historic Places. For orchards not protected by either of these designations, options are limited for effective long-term *in situ* conservation.

For *ex situ* conservation, the collaboration between the NPS and the USDA germplasm repositories is a valuable asset for the NPS that should be fully utilized for preserving unique varieties of historic fruits located on NPS lands. A similar agreement should be enacted between the USFS and the USDA germplasm repositories for *ex situ* conservation of unique historic fruit varieties growing on USFS lands. For unique germplasm located on private lands, no existing collaboration agreement exists for *ex situ* conservation either. Conservation orchards established by a local project may be best suited for *ex situ* conservation of genetic diversity of historic fruit trees growing on private and reservation lands.

Local conservation projects such as the Southwest Regis-TREE, RAFT, and the Kino Fruit Trees Project by nonprofit organizations fill the gaps between governmental conservation, private landowners, and the general public to ensure the success of conservation efforts as a whole. Conservation projects that solely focus on preserving
heirloom fruits may be more likely to have a larger impact on preserving heirloom fruits across the Colorado Plateau than unlinked, site-specific conservation projects by the NPS. Nonprofits and even Universities can work together to expand public awareness of the need for conserving our heritage of heirloom fruits.

The collaboration of governmental agencies with nonprofits and private landowners will be essential in accomplishing the task of conserving heritage fruits of the Colorado Plateau, but it is still likely to be insufficient to fully restore our heritage of fruit tree diversity. To fully conserve the diversity of our agricultural crops and ensure against more tragic losses of agrobiodiversity, we need to restore the diversity of these varieties into our food system (Step 3). If we do not value diversity in the food we eat, we are unlikely to ever fully safeguard these heirloom fruits from present and future risks.
LITERATURE CITED


Pennington, C. P. 1963. The Tarahumar of Mexico, Their Environment and Material Culture. University of Utah Press, Salt Lake City, UT.


### APPENDIX I

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66
**APPENDIX II**

**APPLE VARIETIES IDENTIFIED IN UNKNOWN SAMPLES.**

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APPENDIX IV

ALLELIC PATTERNS AND FREQUENCIES IN SAMPLES

Figure 10
Allelic Patterns Across Samples.

Table 5 (duplicated from page 30 for convenience)

<table>
<thead>
<tr>
<th>Locus</th>
<th>N</th>
<th>Na</th>
<th>Ne</th>
<th>I</th>
<th>Ho</th>
<th>He</th>
<th>F</th>
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<tbody>
<tr>
<td>GD12</td>
<td>144</td>
<td>11.000</td>
<td>4.018</td>
<td>1.713</td>
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<td>GD96</td>
<td>144</td>
<td>14.000</td>
<td>6.818</td>
<td>2.117</td>
<td>0.875</td>
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<td>GD100</td>
<td>134</td>
<td>9.000</td>
<td>5.386</td>
<td>1.852</td>
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<td>0.814</td>
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<td>2.176</td>
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<td>0.827</td>
<td>-0.083</td>
</tr>
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</table>

Sample size (N), number of alleles (Na), number of effective alleles (Ne), information index (I), observed (Ho) and expected (He) heterozygosity, and fixation index (F) calculated for samples. Only samples with unique genotypes were used (as opposed to all 280 samples, see table 3.0). GD100 had 6% missing data for samples—which may affect numbers for this allele.
Figure 11
Allele Frequencies for Seven Loci in Samples.
APPENDIX V

PHOTOS OF SELECTED SAMPLES

Figure 12
Kr-004: Westfield Seek No Further
Figure 13
KR-016: Unknown Variety
Figure 14
KR-028: Unknown Variety
Figure 15
KR-043: Jonathan
Figure 16
KR-131: Unknown Variety
Figure 17
KR-133: White Astrachan
Figure 18
KR-142: Tolman Sweet

Figure 19
KR-160: Unknown Variety
Figure 20
KR-169: Unknown Variety

Figure 21
KR-170: Unknown Variety
Figure 22
KR-173: Slide Rock Heritage Tree, Arkansas Black
Figure 23
KR-273: Unknown Variety

Figure 24
KR-278: Unknown Variety
**Figure 25**
KR-283: Unknown Variety

**Figure 26**
KR-286: Unknown Variety