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The Biological Evidence of the San-Pau-Chu People and Their Affinities

Hsiu-Man Lin

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THE BIOLOGICAL EVIDENCE OF THE SAN-PAU-CHI PEOPLE
AND THEIR AFFINITIES

By

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Dissertation
Submitted in Partial Fulfillment of the Requirement for the Degree of
Doctor of Philosophy
Anthropology

The University of New Mexico
Albuquerque, New Mexico

May 2009
@ 2009, Hsiu-Man Lin
DEDICATION

To my family,
who always stay with me during my sickness

I gratefully thank Dr. Tsang Chen-hwa for permission to use samples from the San-Pau-Chu, Wu-Chien-Tzuo South, and Nan-Kuan-Li Eeat sites. Thanks to my committee members for their supports, patient, and helpful comments. I am gratefully to Dr. Marie Lin’s team at Mackay Memorial Hospital. Also thanks to Dr. Cecil Lewis for the independent ancient DNA tests for 14 San-Pau-Chu bone samples. Last, I would like to thank friends who encouraged me in past ten years. This dissertation cannot be done without all these helps.

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ABSTRACT

Austronesian is one of the major language groups spoken. It is believed that Taiwan, Indonesian (east of Wallace line), or Bismarck Archipelago is the star point for Austronesian migrations. It is also suggested that prehistoric cultures of Taiwan were established by the ancestors of the modern Taiwanese indigenous populations, who are also Austronesian speakers. The goals of this project are to estimate biological relationships of the San-Pau-Chu (SPC) to other Asian populations (especially Polynesians) and to evaluate if Taiwan indeed plays a major role in the history of Austronesian migrations by using both dental morphological data (metric and nonmetric dental traits) and genetic evidence.

This dissertation is divided into six chapters. In chapter one, models of Austronesian dispersal are introduced. Additionally, hypotheses and significance of this study are emphasized. In chapter two, the linguistic, archaeological, osteological and genetic evidence for different models is reviewed. Chapter three provides a brief introduction to Taiwanese cultural history, and describes archaeological sites at the Tainan Scientific-
Based Industrial Park. In chapter four, a case study of dental variation from the SPC, Wu-Chien-Tsuo South (WCTS), and Nan-Kang-Li East (NKLE) sites are presented. In chapter five, genetic diversity and maternal ancestry in the SPC people is evaluated by using ancient DNA. Finally, in chapter six the biological evidence of the SPC people and their affinities is summarized.

This research is the first study in Taiwan trying to combine both morphological and genetic evidence to explore the biological nature of one prehistoric population. Because the dental morphological study and ancient DNA analyses seem to suggest a Northern Asian origin for the SPC people, it is proposed here that approximately 2,500 BP, some prehistoric Taiwanese came from mainland East Asia. However, the WCTS people, contemporaries of SPC, show a closer relatedness with the Namu from the Hawai‘i. Therefore, a multiple set of models must be considered. Studies with larger samples sizes and wider range of archaeological sites in the future will also help to gain insights.
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Chapter 1 Why Study Prehistoric Taiwanese?

Modern populations in the Pacific region are highly diverse culturally, linguistically, and biologically. How this diversity evolved among Austronesian-speaking peoples (Figure 1.1) is an intriguing mystery. Many consider this diversity to be the product of more than one migration occurring during the colonization of Oceania (Anderson et al. 1994; Burley et al. 1999; Kirch 1997, 2000; Sand 1996, 1997). Archaeological and linguistic evidence has been used to suggest that Austronesian peoples originated from either Island Southeast Asia or further East, perhaps in the Bismarck Archipelago (e.g., Ambrose 1997; Allen 1984, 1996; Bellwood 1979, 1985, 1992, 1993; Blust 1984-85; Diamond 1988; Dyen 1965, 1971; Gray et al. 2000; Kirch 1997, 2000). Studies of skeletal morphology and genetic data have offered insights into population relationships and movements; however, the ancestral homeland of Austronesian-speaking populations remains unknown and Taiwan may be its point of origin.

To access role of Taiwan in Austronesian origins, this research examines molecular and dental morphological data from three prehistoric archaeological sites in Taiwan. Molecular data include mitochondrial DNA (mtDNA) haplogroups A (HaeIII 663+/00663G), B (the 9 base pair deletion), C (Hic II 13259-), D (Alu I 5176-), F (Hinc II /Hpa I 12406-), H (Alu I 7025-), and M (Ddel 10394/AluI 10397+) and sequences of the hypervariable region I (HV1) of the mitochondrial DNA d-loop. The ancient DNA variation is compared with morphological variation drawn from metric (crown width) and nonmetric dental traits that also estimate genetic distances between ancient Taiwanese, Southeast Asians, and Oceanians. A study combining ancient DNA and dental data provides direct biological evidence of the San-Pau-Chu people, and avoids the
uncertainty that arises from using more recent Taiwanese samples that have been affected by more recent population admixture between aborigines and Han Chinese in Taiwan (http://www.apc.gov.tw/). This admixture came from two large waves of Han immigration, the first, about 350 BP during the Ching Dynasty with immigrants mostly from Fujian Province and the second major event in 1945 after World War II consisting mostly of soldiers from many parts of mainland China.

Figure 1.1 Geographical Distribution of Austronesian Speakers (in reddish-brown)

**Hypothetical Models for Austronesian Dispersals**

The presence of decorated and dentate-stamped pottery in the Pacific Island realm represents a unique cultural development, the “Lapita cultural complex,” which is
thought to correspond with the spread of Austronesian-speaking peoples. In the 1980s, two prevailing models of Lapita origins were therefore proposed. The first model posits an Asian ancestral homeland of Oceanian origins and was generally dubbed the “Express Train to Polynesian” (ETP) or “Express Train” model. J. M. Diamond (1988) was the first one to name the ETP model, but its modern version is closely associated with Peter Bellwood (1979, 1985, 1992, 1993). The ETP model suggests a population intrusion of Austronesian-speaking people into Near Oceania and a metaphor of nonstop migration (“express train”) from Island Southeast Asia through Melanesia to Polynesia.

A competing model is the “Indigenous Melanesian Origins” (IMO) model that was put forward by Jim Allen (1984). The IMO model assumes that a local social and economic center, combined with some external technologies from its immediate region, evolved into the Lapita complex (Allen 1984). Some archaeologists have advocated a more extreme view of “no need to believe in migrations at all,” arguing that this cultural complex evolved solely from indigenous populations (White et al. 1988). For instance, Ambrose (1997) uses the presence of approximately 5,000-6,000 year old pottery from sites near the north coast of Papua New Guinea as evidence that there was no influx of immigrants from Island Southeast Asia and that Melanesia was the ancestral homeland.

While the ETP and IMO models provide useful starting points in the debate, both are over-simplifications. Incorporating the aforementioned concepts, the study of the Lapita cultural complex can be seen as a continuous struggle between the phylogenetic model (Kirch and Green 1987; Bellwood et al. 1995) and the reticulate model (Bellwood 1996). Both agree that the Lapita cultural complex conceals waves of Austronesian dispersals. However, these models make different assumptions concerning commonly
shared cultural traits (Austronesian language, oceanic watercraft, horticulture, and pottery technology). The phylogenetic model assumes a common ancestry. In contrast, the reticulate model focuses on “a continuous and relatively uncoordinated shifting of linguistic, cultural, and biological boundaries through assimilation, intermarriage, borrowing and diffusion” (Bellwood 1996). In other words, the Phylogenetic model supports that there is an origin for Austronesian speakers, the reticulate model says otherwise.

The Triple-I model (Green 1991), standing for Intrusion, Innovation, and Integration, is an effort to unite both camps. Green uses different lines of archaeological evidence, such as the Lapita adze kit (the quadrangular adze and the heavy bodied portion of the Tridacna adze), as support for a body of migrants who brought genes and items of material culture to the long settled Near Oceania. The new immigrants integrated culturally and physically with the local system, and then descendants of these intruders and early settlers evolved into a unique cultural complex, the Lapita. Because this innovation arose in situ, it is less likely to have similar ceramic parallels in Island Southeast Asia. Furthermore, the complex itself is culturally heterogeneous because this culture is not only regionally differentiated in Remote Oceania but also because it has continuing contacts with culturally diverse, long resident, unrelated populations from Near Oceania (Green 1991). A related model, the “Slow Boat” model, assumes an Southeast Asian/Taiwanese origin, but the colonization in Oceania began after extensive population admixture between these immigrants and Melanesians (Kayser et al. 2000).

Terrell and Welsh proposed a “Voyaging Corridor” model, expanding on Irwin’s work (1992). They argued that the South Pacific is an interaction sphere in which “the
spatial dimensions and components of the voyaging corridor between Asia and the Pacific have undoubtedly varied with time and evolving circumstances” (Terrell et al. 1997: 556). This model emphasizes that biological and cultural interactions between populations are continuous over time and space. For instance, marriage, adoption, feasting, exchange, or friendship exists between human groups and ties individuals and societies together from time to time and from one place to another (Terrell et al. 1997).

The “Entangled Bank” model, based on Darwin's concept of the entangled bank (1859), is a related model and Terrell (1988) characterized this model as “an interlocking, expanding, sometimes contracting and ever-changing set of social, political, and economic subfields”. These two models therefore suggest there is no an ultimate origin for Austronesian speakers. Instead factors such as marriages and adoptions have influenced the biological affinities and cultures of Austronesian speakers, and genes, languages, and cultures have to be seen in a holistic point of view.

In his book “Eden in the East: The Drowned Continent of Southeast Asia,” Oppenheimer (1998) uses geology, archaeology, linguistics, genetics, and comparative mythology to present his hypotheses about Austronesian origins. Like Solheim (1984, 1996) and Meacham (1984, 1995) who see Taiwan as an isolated backwater on the periphery of Austronesian prehistory, he argues that the indigenous Taiwanese languages, which represent one of the deepest branches of Austronesian, retain many ancestral words because of the location (the extreme northern periphery) and isolation. This deep branch (Formosan) of Austronesian is found exclusively in Taiwan, while the other branch (Malayo-Polynesian) includes all other Austronesian languages and is absent from Taiwan. Furthermore, he posits that there were two sets of voyagers: one from the
Western Pacific who settled the main islands of Melanesia and the other from now so-called Eastern Indonesia who passed by the first group and settled Polynesia and Micronesia. Therefore Oppenheimer favors a “Two Train” hypothesis, which posits that the Lapita expansion would be a second migration, if there has been an Austronesian migration, by adding a better sailing technology to a pre-existing Austronesian trade network.

If brief, the aforementioned models can be differentiated as two parts. One as the ETP, IMO, Phylogenetic, Triple I, Slow Boat, and Two Train models, which support one or several Austronesian migration into Oceania; the other as the reticulate, Voyaging Corridor, and Entangled Bank models, which emphasize autochthonous origins or reticulate in situ evolutionary histories. Additionally, there is a tendency of proposed origins from east to west: ETP from Taiwan, Triple I, Phylogenetic, and Slow Boat from Taiwan or Southeast Islands, Two Train from surrounding area of the Wallace Line, and then IMO from Melanesia.

**Hypotheses**

Although phylogenetic studies of mtDNA sequences suggest that modern indigenous Taiwanese are biologically associated with the Polynesians (e.g., Sykes et al. 1995, Tajima et al. 2003, Trejaut et al. 2005), the genetics of prehistoric peoples in Taiwan have been rarely studied (Zheng 2004, Yan 2006, Chen 2007). It remains unknown whether Taiwan is the origin of Austronesian-speakers or the ancestors of the “Lapita” People. The aim of this study is to evaluate which among modern populations, including indigenous Taiwanese, are the ethnic groups most closely related to Taiwanese
prehistoric people. An additional aim is to understand if Taiwan indeed the origin of Austronesian-speaking population.

If multidimensional scaling and constructed phylogenetic trees from genetic (sequences) and dental morphological studies (crown width and nonmetric traits) produce consistent patterns, the affinities of the San-Pau-Chu people will be considered. However, if results from crown width conflicts with those from nonmetric dental traits, different statistical methods should be discussed in order to evaluate which methods have sufficient statistical power or are otherwise to be preferred in accessing the biological affinities of the San-Pau-Chu. If ancient DNA analyses cannot provide sufficient data, testing the null hypothesis for this study will then focus on dental morphological data.

If genetic and dental trait frequencies are statistically significantly correlated between the prehistoric aboriginal Taiwanese (the San-Pau-Chu people) and Polynesians relative to other groups in this region (especially Melanesian), the “out of Taiwan” models (such as Triple-I and Slow Boat) should be preferred; otherwise, an origin in Bismark Archipelago or Indonesia (east to Wallace Line) is more likely. For instance, a finding of 30% or more for haplogroup B in a substantial sample of ancient mtDNA from SPC would be required according to Tajima and colleagues (2003). Likewise, if SPC is the direct biological source of Polynesians, one would expect to find a frequency of incisor shoveling at SPC of less than 30%. Alternatively, if the Polynesian genetic and dental trait frequencies are in-between those of indigenous Taiwanese and Melanesians, intermediate models (or the possibility of selection) will be considered in future studies.
Study Area

Taiwan is circumscribed to the east by the Pacific Ocean and west by the Eurasian continent (Figure 1.2). To the north lie the Ryukyu Islands and Japan, and to the south are a series of archipelagos including the Philippines, Malaysia, and Indonesia. Taiwan consists of five island groups: the main island, Lanyu (Orchid Island), Penghu, Maju, and Jinmen. It is relatively difficult to define Taiwan as part of East Asia or Southeast Asia because of its location (about in the middle of the series of archipelagoes along mainland Asia). It is closer to the Philippines than to Japan, both in terms of geography as well as prehistory. Therefore, Taiwan is included in Southeast Asia in this project.

Relevant to the issue of ancient DNA preservation, Taiwan has both tropical and subtropical zones. Temperatures increase with decreasing altitude. The summer is long (April-November). The average winter temperature is approximately 15°C. However, the central mountains (ca. 4,000 meters) are snow-capped in the winter (Blust 1984-1985). Pollen analysis indicates that the climate of central Taiwan has changed little in the past 4,000 years (Chung et al. 1973).

Europeans and Chinese began to colonize Taiwan in the 16th and 17th centuries, respectively. The Portuguese arrived in Taiwan in 1517 and named it Ilha Formosa, but they did not colonize the island. The Dutch arrived in 1624 and established a capital at Tainan, located in southwestern Taiwan. They lost the island to Spain in an invasion two years later, but they regained control in 1641. During the 1660s, the Ming and Ching Dynasties from Mainland China overcame the Dutch, extended their influence into Taiwan, and challenged each other for control of the island. Eventually, the Ching
Dynasty made Taiwan a county of the Fujian Province around 350 years ago, which in turn triggered a flood of Chinese immigration. Until the arrival of these Han Chinese immigrants, the Austronesian-speaking Taiwanese indigenous populations comprised the majority of the island’s population (Chang et al. 1999).

The historic records prior to the 17th century are sparse (Chang et al. 1996). However, archaeological evidence shows that there were prehistoric cultures dating to as early as 6,500 years ago that are ancestral to modern Taiwanese indigenous populations (Chang et al. 1996). All prehistoric Taiwanese sites discovered to date are presumably affiliated with the Austronesian-speaking indigenous populations (Chang et al. 1996), although this may not be true for human represented by fossilized human (Homo sapiens sapiens) cranial fragments and molars that were found in southwest Taiwan, date to 20,000-30,000 BP, and are known as Tso-Chen Man (Lien 1981a, Shikama 1976). It is unclear precisely where the Tso-Chen Man came from. The Ta-P’en-K’eng (TPK) cultural complex (2400-1900 BC) is the earliest group that is clearly associated with “coarse red ware” pottery and agriculture (Chang et al. 1996, Huang 1994, Lui 1992). To date, similar assemblages have only been found along the southeast coast of China, including the Fujian and Guangdong provinces, and northern Luzon, Philippines. Although many sites in northern Fujian are contemporaneous with TPK, archaeological evidence from Luzon seems to provide reliable dates until 3,000 BC (Bellwood 1985, cited in Chang et al. 1996).

**Archaeological Sites**

Because Taiwan’s climate varies between subtropical and tropical, and because high temperatures and humidity promote bone degradation, it is difficult to locate adequately
large human skeletal samples from prehistoric sites on Taiwan for biological comparative studies. The additional requirement for this study is that the osteological sample be drawn from a prehistoric site that dates to the time of the first appearance of the Lapita Cultural Complex further limits the sites suitable for analysis.

The Nan-Kuan Li East (NKLE), San-Pau-Chu (SPC) and Wu-Chen-Tzu South (WCTS) sites (Figure 1.3) were chosen, because they provide reasonably large series human skeletal materials with relatively good preservation and the skeletal materials have not been extensively handled by excavators or curators, which is important for ancient
DNA research because it limits the possibilities for contamination. The SPC and WCTS sites date back to 2,800-2,000 B.P., which corresponds to the Late Neolithic period of Taiwanese prehistory. However, the NKLE site dates to 4,200-4,800 BP, a time period that could make it possible to be considered ancestral to the Lapita by scholars such as Peter Bellwood (1979, 1985, 1992, 1993, 1995a, 1995b). While the SPC and NLKE sites are geographically close to each other, the WCTS site is relatively away from the SPC and NKLE sites (Figure 1.3) (Tsang et al. 2004, 2006, 2007).

The location for the center of the SPC site of this study is (120° 15’38”E, 23° 07’06”). The site extends over an expanse of 15,000 square meters (Tsang et al. 2004, 2006). The SPC site is composed of 4 cultural layers: the Ming-Ching Han Chinese Culture, Niao-Sung Culture (1800-1400 B.P.), and two layers of Ta-Hu Culture (2800-1800 B.P.) (Tsang et al. 2004, Tsang et al. 2006, Tsang et al. 2007). In the Ming-Ching Han Cultural layer, there are various sherds of china, bricks, and five human burials (Tsang et al. 2004, Tsang et al. 2006, Tsang et al. 2007). In the Nio-Sung Cultural layer, there are plain brownish sherds, stone knives, shell and bone tools, glass beads, and some disturbed human burials (Tsang et al. 2004, Tsang et al. 2006, Tsang et al. 2007). The Ta-Hu Cultural layer contains ceramics (grayish clay and sandy pottery), a few stone tools, jade artifacts (canine-shaped ornaments and rings), and a total of 131 human burials as well as few randomly dispersed trash pits (Tsang et al. 2004, Tsang et al. 2006, Tsang et al. 2007).

The center of the NKLE site falls nearly (120° 16’23”E, 23° 07’07”); the site covers 72,000 square meters (Tsang et al. 2004, 2006). The NKL East site includes three layers of TPK culture and they are at above 0.5 meter above, and above 0.1 meter above,
and below 0.3 meter below the current sea levels, respectively (Tsang et al. 2004, Tsang et al. 2006, Tsang et al. 2007). In addition, each cultural layer is approximately 10 to 20 centimeters in depth. Ceramics (dark-brown sandy, red-brown clay, and grayish-brown clay pottery), stone tools (axes, shouldered-adzes, arrows, and knives), bone and shell tools such as shell knives, ecofacts (perforated shark teeth, plant seeds, shell mounds, and complete dog remains), and 85 human burials (Tsang et al. 2004, Tsang et al. 2006, Tsang et al. 2007).

The center of the WCTS site lies at (120°16’15’’E, 23°05’26’’). The site extends over 30,000 square meters and contains three cultural layers (Tsang et al. 2004, Tsang et al. 2006, Tsang et al. 2007). The upper one belongs to the Niao-Sung culture and the lower two are from the Ta-Hu Culture (Tsang et al. 2004, Tsang et al. 2006, Tsang et al. 2007). The Niao-Sung cultural layer contains only dispersed trash pits that contained sherds, shells, plant seeds, and ecofacts such as bones of fish, pigs, and deer were found (Tsang et al. 2004, Tsang et al. 2006, Tsang et al. 2007). In the Ta-Hu cultural layer, there are grayish clay and sandy-colored pottery, bird-styled ceramics, a few stone tools, ecofacts (infrequently shell and plant seeds), and 52 human burials as well as few randomly dispersed trash pits (Tsang et al. 2004, Tsang et al. 2006, Tsang et al. 2007).

Although no radiocarbon dates are associated with the human remains, radiocarbon samples were taken from the associated living features of the sites and represent the most likely time period for these sites as a whole. These dates also agree with the stylistic features of the ceramics found in many of the burials. Further information for the archaeological context of each site is not yet available because publications about this site are still in progress.
Avenues for Future Research

This dissertation focuses on dental morphology and ancient DNA of the San-Pau-Chu people (as well as dental metric traits in the Wu-Chien-Tzou and Nan-Kuan-Li East people) in southwest Taiwan that are discussed in detail in succeeding chapters. The results of this research will highlight biological identities of Taiwan prehistoric peoples as well as their relationships with other modern or ancient populations. Additionally, the ancient DNA study of this research will provide a glimpse of the ancient DNA preservational condition for future studies. Before turning to examine the biological evidence of the San-Pau-Chu people and their affinities, the next chapter will discuss various lines of evidence such as linguistics, archaeology, and genetics.
Figure 1.3 Archaeological sites discovered in the Tainan Science-Based Industrial Park
Chapter 2 Evidence for Western Origins in Austronesian Dispersals

Although there is really no way to know what languages were spoken by the initial settlers of the Southwest Pacific region, archaeological and linguistic analyses provide indications of the most likely linguistic affiliations. According to archaeological research and linguistic reconstructions, there were two distinct stages of colonization in Melanesia and settlers are thought to have spoken languages in one of two major language groups. During the first phase at least 40,000 years ago, people began to settle in Melanesian regions, and they are thought to have spoken languages in the highly diverse Papuan language group (e.g., Dun et al. 2002, Dunn et al. 2005, Linström et al. 2007, Spriggs 1997). The second phase resulted in a secondary colonization of Near Oceania beginning about 3500 years ago (Summerhayes 2007) followed by the initial colonization of Remote Oceania.

Distribution of the Austronesian Languages

As a linguistic entity, by AD 1500, Austronesian was the most geographically dispersed language family in the world. Its range was north to Taiwan, south to New Zealand, west to Madagascar, and east to Easter Island (Bellwood et al. 1995; Lee 1997; Lynch et al. 2002). Today, it is classified under the Austroasiatic language macro-phylum and includes over 1,000 modern languages (Tryson 1995).

Hendrik Kern was the first to apply comparative linguistics to examine wide distributions of related words for sugarcane, rice, marine fauna, and the art of navigation in Austronesian languages (Kern 1889, cited in Blust 1984-85). He proposed that the
ancestral homeland of Austronesian was potentially located in Champa, Cochin-China, Cambodia and their surrounding regions. Since then, contrasting viewpoints have been advanced. Blust (1984-85, 1995), building on Haudricourt (1954), suggested that the most likely homeland of the Austronesian languages was Taiwan and that its speakers moved into the insular Pacific after several splits in accordance with the “principle of least moves.”

In contrast, Dyen (1965, 1971) believed that the primary center was in the region of New Guinea and the Bismarck Archipelago. Gray and Jordan (2000) used a parsimony analysis of an Austronesian language tree to test two competing hypotheses (the “Express Train” and the “Entangled Bank” models) for the expansion of this language group. Their results reject the entangled bank model and are compatible with the express-train model. Gary and colleagues (2009) studies 210 items (such as words for animals, kinship terms, simple verbs, colors, and numbers) of Austronesian vocabularies. Their results also place the Formosan language of Taiwan at the base of the trees, and agree with an East Asian/Taiwan origin (Gray et al. 2009).

Critiques of the comparative linguistic approach have, however, been voiced (Terrell et al. 1997). Terrell and colleagues argue that the extensive interactions in the Pacific have made the linguistic pattern uninformative because people can adopt and/or exchange languages. (Details of languages, including Austronesian, in Island Melanesia see Dunn et al. 2002, Dunn et al. 2005, Linström et al. 2005.)
Experimental voyages

Early in the nineteenth century, there were two major schools of thought concerning the direction of Austronesian migration through Polynesia. Some researchers, such as Thor Heyerdahl (1941; 1950; 1951; 1953a, 1953b; 1958; 1968), favored an initial colonization from the West; however, this hypothesis has been criticized (e.g., Sharp 1956, 1961, 1963, 1964). Heyerdahl (1950) set out to build the raft, Kon-Tiki, and claimed that the raft were able to sail from Peru across the Pacific. Sharp (1956, 1961, 1963, 1964) accepted a westerly sailing but argued that the islands were settled accidentally by explorers and that their canoes and navigation methods could not have been sufficiently accurate or reliable to choose the island destinations.

A series of experimental voyages in the canoe Hokule‘s were periodically conducted from 1976 to 1995 (Finney 1996). The data from these experiments offered evidence that traditional double canoes could sail into the wind during long-range voyages. In addition to canoe performance, the key to understanding Polynesian voyaging is in the timing of favorable seasonal wind patterns. In particular, oceanic geography in the Pacific only requires navigators to keep a running mental calculation of the distance between the target and home islands. They may then make landfall on any island within a chain of islands (Finney 1996).

Archaeological Evidence

The presence of decorated and dentate-stamped pottery (Figures 2.1-2.2) in the Pacific Island realm represents a unique cultural development, the “Lapita cultural
complex,” which is thought to correspond with the spread of Austronesian-speaking peoples. In this regard, more than 200 radiocarbon dates from the Lapita culture and a volcanic deposit directly underneath the Lapita culture on New Britain dated to 3,600 BP have helped to build a chronology of dispersals by over 3,000 kilometers (e.g., Anderson et al. 2001, Anson 1983, Kirch 2000, Summerhayes 2007).

Father Otto Meyer first reported the distinctive decorated pottery of the Lapita culture on the small island of Watom in the opening decade of the twentieth century (Meyer trans. in Anson 1983:283; cited from Kirch 1997), but their association with the origins of Polynesian culture was not recognized until the late 1940s. Later, Golson (1961, 1971) noticed unambiguous connections of Lapita pottery assemblages between islands and argued that these pottery assemblages represent ceramic series. However, rather than merely defining Lapita on a basis of pottery styles, Green offered a synthesis of Lapita as a cultural complex and noted that Lapita peoples also used lithic, shell, and bone implements. Additional elements of the complex included fishing and marine exploitation, horticultural subsistence based on a root and tree crop complex and animal husbandry of pig, dogs, and fowl.

Lapita pottery is characterized by distinct motifs typically made with small, tooth (or dentate) stamps and this pottery appears to have developed out of an earthenware ceramic tradition because it was fired at low temperature. Although a recognizable Lapita style has both contemporary representations over a wide geographic area and continuance through time, it also reflects an aspect of novelty in the complex decoration (Kirch 1997). The decorated ceramics offer many interesting aspects for theoretical interpretation, but they actually rarely make up more than 10% of a Lapita pottery assemblage. The Lapita
diaspora cannot sufficiently be explained in the simple terms of single causes and its “end” was simple a change in one aspect of material culture, specifically ceramics (Kirch 1997).

In order to refine reconstruction of Lapita dispersals, Green (1991) has geographically separated Oceania into Near Oceania and Remote Oceania (Figure 2.3). Near Oceania includes New Guinea, the Bismarck Archipelago and the Solomon Islands and represents regions colonized in the original migration of Papuan speakers. Remote Oceania extends from the Solomon chain to the more isolated archipelagoes and islands, such as Easter Island, and was colonized later, probably by Austronesian speakers. The first appearance of the Lapita ceramic tradition in the Bismarck Archipelago was as early as 1600-1400 BC (Kirch 2001).

Lapita communities cover both a large geographic region of some 4300 km from Mussau, located in the Bismarck Archipelago to Samoa located east of Melanesia, and persisted for a considerable time span of ten to fifteen centuries. Archaeologists have subdivided the Lapita world into three main geographic regions: Far Western Lapita (the Bismarcks), Western Lapita (the Solomons to New Caledonia), and Eastern Lapita (Fiji, Tonga, Samoa) (Kirch 1997).

At least three major movements occurred during the Lapita dispersal. Around 1,200 BC, the Lapita arrived at the southeastern end of Solomon Islands. At about 1100-1000 BC, this eastward expansion moved south through the Vanuatu archipelago and New Caledonia. The culture then spread to the Tonga-Samoa chain by 900-750 BC (Anderson and Clark 1999; Burley et al. 1999; Dickinson and Green 1998).
Skeletal Evidence of Austronesian Origins

Synchronic and diachronic comparisons of skeletal morphology between populations can offer insights into population relationships and movements, but locating adequately large archaeological samples in Oceania is difficult. Due to the absence of burials at many Lapita sites, only a few human skeletal samples have been excavated (Kirch 1997; Pietrusewsky 1997). At one site dated to 500 BC on Watom Island in the Bismarck Archipelago, nine in situ burials were excavated (Green et al. 1989). A morphometric study of eight partial skeletons from this site indicates a weak association between Watom people, eastern Melanesians, Polynesians, and populations of the Bismarcks (Pietrusewsky 1989). Outside the Bismarcks, skeletal remains have been analyzed from Fiji and Tonga (Kirch 1997). Comparisons of a mandible from a partial skeleton from Natunuku, Fiji, dating to approximately 1,500 BC, suggest Melanesian and Polynesian resemblances (Pietrusewsky 1985). Craniometric comparisons of a relatively well-preserved skeleton dating to 700 BC from Waya Island, Fiji, support affinities with Polynesians, Southeast Asians (including Taiwanese indigenous populations), and East Asians (Pietrusewsky 1997).

In addition, cranial morphometric and dental nonmetric studies of 32 adults from a prehistoric site, Shih-San-Hong (SSH), dating to the Bronze Age of Taiwan (AD 500-1,200), suggest a relationship to Polynesians (Chang 1993). Recently, Peitrusewsky (2005) examined a total of 2805 male crania representing 63 cranial series (comprising 7-63 crania per series) from the Pacific, East Asia, and Southeast Asia. He concluded that cranial series from Australia, Tasmania, New Guinea, and geographical Melanesia differ

20
from cranial series from East/North Asia, Southeast Asia, Polynesia and other parts of remote Oceania. Furthermore, he suggested that the Polynesians originated in eastern island Southeast Asia as proposed by Oppenheimer and Richards (2001). Nevertheless, only a handful of sites have yielded human remains and Pietrusewsky’s (2005) study is suggestive rather than conclusive due to small sample sizes.

Modern cranial populations are statistically similar to each other in terms of skull shape, although groupings within geographical areas can be found (Howells 1989). For instance, Pietrusewsky (1988) observed an Asian-Indonesian plus Polynesian cluster as well as Asian-to-Polynesian continuity. Brace et al. (1989) also found a Pacific cluster that comprised broad Polynesian and Micronesian groupings and that included Ainu and Jomon crania from Japan. Howells (1989) found that Australians and Melanesians generally tend to cluster apart from other Pacific and Asian groups.

Figure 2.1 Lapita potsherd (from Kirch 1997)
Additional data from the dentition are available for Asian and Asian-derived populations. Hanihara (1968) described a “Mongoloid dental complex,” characterized by a high frequency of shovel-shaped incisors, cusp 6, the protostylid, and the deflecting wrinkle. Turner (1983, 1987, 1989, and 1990) proposed a more fundamental dental division in Asia, and described Sinodonty and Sundadonty as major sets of dental traits that could dichotomize Asian populations. Sinodonts have high frequencies of specialized traits such as incisor shoveling, winged incisors, single rooted upper 1st premolar, and 3-rooted lower 3rd molar. Sundadonts have a more conservative pattern, generally characterized by the retention of primitive traits rather than the elaboration of new or rare crown variants.
Dental morphology supports a Southeast Asian origin for Polynesians and Micronesians because these three groups all possess the Sundadont pattern and form a single cluster of “Sunda-Pacific”; Australians and Melanesians fall into another cluster of “Sahul-Pacific” (Scott and Turner 1997). However, most of these dental analyses have looked at trait frequencies rather than tooth sizes with the exception in the Sahul-Pacific groups (e.g., Brace 1980, Brace and Hinton 1981). Given the abundance of data on dental dimensions, one advantage of the present study is that it collects data on both trait frequencies (via the ASU Dental Anthropology System) and tooth sizes (crown width). The latter was chosen because it is less likely to be affected by dental wear or pathology (Buikstra et al. 1994). Another advantage of using dental traits is a generally better preservational condition of teeth than bones.
Genetic Evidence

In the last twenty years, many types of genetic data, such as human leucocyte antigens (HLA), Gm polymorphisms, mitochondrial DNA (mtDNA), autosomal short tandem repeats (STR), and the nonrecombining portion of the Y chromosome, have been collected and analyzed in modern Pacific region populations. Although it is important to use caution when drawing historical interpretations based on modern patterns of variation, a substantial body of work about genetic variation has provided abundant information about the peopling of Oceania, and genetic data provide the chance to examine Austronesian speakers and the dispersals of Lapita culture.

The HLA studies suggest that the Papua New Guinea highland population is more closely related to Australian groups than other Pacific islanders, that populations in coastal Papua New Guinea and Melanesia have similar HLA distributions, and that Melanesian HLA types were not carried into eastern Polynesia (Mack et al. 2000; Serjeantson 1989). Lin and coworkers (Lin et al. 2000; Chu et al. 2001) further argue that Taiwanese indigenous populations have a closer genetic relationship with Oceanians than with Australians and highland New Guineans. For instance, HLA haplotypes A24-Cw8-B48, A24-Cw10-B60 and A24-Cw9-B61 that were common in several Taiwan indigenous samples are also observed in Maori, Papua New Guinea Highlanders, Orochons, Mongolians, Japanese, Tibetans and Thais (Lin et al. 2000). Additionally, Taiwanese indigenous peoples show a closer genetic relationship to southern Asian populations, especially to those from insular Southeast Asia (Indonesia, Philippines) where many high frequency HLA alleles specific for the Taiwanese indigenous peoples are also seen (Lin et al. 2005). However, all HLA studies have a potentially major
disadvantage because these loci are under selection (Black et al. 1997).

Early studies of the distributions of Gm polymorphisms and their resistance to malaria have also offered an explanation of the colonization of Oceania (Kelley 1990; Clark et al. 1993). The Gm hypothesis suggests that Austronesian speakers introduced this marker into Melanesia. Given a genetic advantage in the malarial environment on coastal lowlands of Near Oceania, Austronesian-speaking populations quickly dispersed throughout Oceania.

Surveys indicate that the mtDNA 9 base pair (9-bp) deletion serves as a marker of Polynesian affinities with Asians. The 9-bp deletion occurs at moderate to high frequencies from Taiwan south through the Philippines and is nearly fixed in Polynesia. For instance, previous studies have shown a gradual cline of 9-bp deletion frequencies from Maoris (98%), western Polynesian (89%), eastern Polynesian (80%), and Melanesian (41%), to almost complete absence in the New Guinea Highland groups (Murry-Mcintosh et al. 1998; Merriwether et al. 1999; Kaestle et al. 2000). This 9-bp deletion is present in Austronesian- and many non-Austronesian-speaking groups; however, this deletion haplotype, associated with three polymorphisms at nucleotide positions 16217, 16247, and 16261 in the mtDNA control region, is almost restricted to Polynesians (Merriwether et al 1999). As a result, one haplotype is called the “Polynesian motif” (Melton et al. 1995; Redd et al. 1995). Studies from Oceanic and Asian populations also suggest an origin in Island Southeast Asia and a colonization route along the north coast of New Guinea (Lum and Cann 1998; Lum et al. 2000). Additionally, Friedlaender and colleagues (2008) suggest a close relationship between Polynesians and Asian/Taiwanese Aboriginal populations, but only a weak association with any
Melanesian groups according to the mtDNA.

Phylogenetic analyses of mtDNA sequences (Trejaut et al. 2005; Friedlaender 2007) defines a new subclade B4a1a, a motif of three coding region mutations at nucleotides 6719, 12239, and 15746, that provides a unique link between indigenous Taiwanese, Polynesians, and Micronesians and endorses mid-Holocene population movements originating in Taiwan (Bellwood et al. 1995; Cox 2005) or Wallacea (Oppenheimer et al. 2001). In addition, four lineages of macrohaplogroup M, which have independent branches distributed across Asia, East Africa, Australia, and Near Oceania, were identified in Near Oceania, and three out of these four lineages are limited to certain locations within Northern Island Melanesia: M27 is most common and diverse in Bougainville, M28 in the interior of east New Britain, and M29 in southern New Ireland and east New Britain (Merriwether et al. 2005). Meanwhile, Soares and coworker (2008) focused on haplogroup E. They found E2b is frequent in Taiwan and, thus, they suggested that there was a potential subsequent dispersals from Island Southeast Asia to Taiwan.

Using mitochondrial and autosomal STR polymorphism data, Lum and colleagues (1998a) argued that mtDNA types were more diverse than autosomal STR loci. Their results also indicated that most Remote Oceanic populations clustered together and were related to both Asia and Near Oceania in terms of the nuclear markers. However, mtDNA types in Polynesia and Micronesia are most closely related to Island Southeast Asian types. Lum et al. (1998b) further suggested potential sex-biased gene flow in the Austronesian migrations.
Investigations of the Y chromosome have been recently initiated and provide additional information. Studies of 19 biallelic polymorphisms on the nonrecombining portion of the Y chromosome suggest that both Taiwanese indigenous populations and Remote Oceanic populations can trace their ancestry back to Southeast Asia (Deka et al. 2000; Sue et al. 2000). Kayser et al. (2000) found a unique DYS390.3-/RPS4Y711T haplotype (DYS390 deletion with the RPS4Y711T mutation) in Cook Islanders, Melanesians, and Indonesians. They also proposed that all Polynesian Y chromosomes can be traced back to Melanesia, where an extensive population admixture between populations in Island Southeast Asia and Melanesia occurred before the colonization of Oceania. In addition, a base substitution at the 92R7 locus of Y chromosomal lineages indicates recent admixture events between European and Polynesians (Hurles et al. 1998).

Kayser and coworkers (2008) state that O-M110 is the predominant nonrecombining Y-chromosome (NRY) haplogroup of Asian origin in the Admiralties and likely originated in Taiwan (with frequencies of 34.1% in Taiwan, 12.8% in Philippines, 2.5-9.7% in Island Southeast Asia, and completely absent from Mainland East and Southeast Asia as well as the western parts of Island Southeast Asia). Thus, O-M110 provides a direct Y chromosome evidence for a Taiwanese origin of the Austronesian expansion. On the other hand, K-P79, another NRY haplogroup that can be assigned to a specific regional source within northern Island Melanesia is the first direct evidence of Melanesian NRY haplogroup in Polynesia (Kayser et al. 2008). Therefore, Kayser and colleagues (2008) claimed that Polynesians have a dual genetic origin, 79% East Asian origin and a 21% Melanesian origin. To sum up, genetic evidence suggests not only a high East Asian (from mtDNA, matrilineal practice) but also a considerable Melanesian
component (from Y-chromosome, patrilineal practice), reflecting sex-biased admixture, but also reflected in cultural elements and language in Polynesian history (Kayser et al. 2008, Kayser et al. 2008).

Recent study (Moodley et al. 2009) by analyzing the distribution of a bacterial parasite of humans, *Helicobacter Pylori* (specifically hspMaori), showed a higher genetic diversity in Taiwanese hspMaori than Pacific non-Taiwanese hspMaori. Additionally, the haplotypes of the later form a single clade, which originates from one of the clades amongst indigenous Taiwanese haplotypes.

In general, molecular evidence from modern populations in the Pacific region indicates that populations in Island Southeast Asia and in the Melanesian Bismarck Archipelago have contributed genes to Polynesians. It is consistent with hypotheses supporting a non-local population spread into Remote Oceania from Island Southeast Asia. In addition, differential sex-biased gene flow may have occurred during the colonization of this considerable area. It also tends to agree with the Triple-I model or the “Slow Boat” model.

**Genetic Evidence from Indigenous Taiwanese Populations**

From the estimated coalescent times for two clusters (C2 and C4), the mtDNA lineages leading to them were inferred to have been introduced into Taiwan approximately 11,000-26,000 years ago, suggesting ancient immigrations of the two mtDNA lineages and a long history of the aboriginal people in Taiwan (Tajima et al. 2003). MtDNA sequence types of aboriginal Taiwanese populations carry only one (T16217C in cluster C7) or two (T16217C and C16261T in C8) of the three “Polynesian
Motif” mutations along with the 9-bp deletion. However, there is no A16247G mutation among the aboriginal Taiwanese (Tajima et al. 2003).

The lack of the Polynesian motif in the aboriginal Taiwanese groups agrees with previous findings (Melton et al. 1995, 1998; Sykes et al. 1995), indicating that the motif itself is unlikely to have arisen in Taiwan. A Neighbor-Joining tree indicated that the three population clusters, including the north, south, and middle-east coast of aboriginal Taiwanese, remained largely intact, except the Ami (Tajima et al. 2003). Tajima and colleagues also found that nine aboriginal groups clustered more distantly with Taiwan Han and Thai (bootstrap value 63%). However, this still creates two separate sub-clusters that include populations from Taiwan, consisting of the aboriginal Taiwanese and Taiwanese Han-Thai, respectively. The genetic difference between aboriginal Taiwanese and Taiwanese Han at the population level is consistent with those in the phylogenetic analyses undertaken with HLA loci (Lin et al. 2000; Chu et al. 2001); however, it is yet indefinite that this difference results from recent admixture between indigenous Taiwanese and Han Chinese, selection, or both.

Four mtDNA haplogroups, including B, E, R9, and M7 (Figure 2.4), in indigenous Taiwanese account for more than 90% of mtDNA variation, which differs from that observed in China (where these haplogroups comprised less than 40% of the variation) (Trejaut et al. 2005). Four haplogroups B (including subhaplogroups B4b and B5a2, which is associated with 16266G), E, F (specifically F4b which is associated with 10097C-16218-16311), and M (specifically M7b, associated with 16086-16129-16324), characterized more than 80% of the mtDNA variation observed in the Atayal, Saiaiat, and Bunun populations that occupy in north and central Taiwan; Subhaplogroups B4a, D5,
F3b (characterized by 16220C as well as the lack of a transition at nucleotide 16335 that was seen in R9c), M7c, and N9a comprised 72.2% of the mtDNA variation of the population of south and southeast Taiwan (Trejaut et al. 2005).

**Ancient mtDNA studies**

Ancient mtDNA samples dating from 500 BC to 1,600 AD from Melanesian archipelagos (Watom and Vanuatu), southern Remote Oceania (Fiji, Samoa, and Tonga), and northern Remote Oceania (Chatham Islands, Society Islands, Hawaii, Caroline Islands, Yap, and Marianas) indicate that the earliest inhabitants of southern Remote Oceania may have originated in Melanesia (Hagelberg 1993). Unfortunately, the sample sizes for Hagelberg’s study are small, only 1-4 individuals per site. A subsequent ancient DNA study of Easter Island people indicated that their DNA samples present typical Polynesian markers, including the three characteristic mtDNA HVI substitutions and the 9-bp deletion (Hagelberg et al. 1994).

There are also few published studies regarding the ancient mtDNA sequence study of Taiwanese archaeological sites in past few years. For instance, Zheng (2004) analyzed a total of 22 human osteological samples, including bones and teeth from 16 individuals, and found five out of those 22 samples with ancient DNA. From these five samples from five different individuals, Zheng concluded that the Wu-Shan-Tou people are most closely related to the modern Bunun (Zheng 2004). Later, Chen et al. (2007) employed the same materials and stated that the closeness between the prehistoric Wu-Shan-Tou people and the modern Bunun could be because the Bunun once lived at the site of the Wu-Shan-Tou archaeological site. Furthermore, the relative remote relationship between
the prehistoric Wu-Shan-Tou people and the Siraya could be because the Wu-Shan-Tou people were replaced by another population approximately 2,500 years ago or because admixture between the Siraya and the Han Chinese in the past several hundred years has generated genetic dissimilarities between the modern Siraya and the prehistoric Wu-Shan-Tou people (Chen et al. 2007).

Figure 2.4 Relationships between haplogroups B, D, E, F, M, and R9 (from Treajaut et al. 2005).

Another study (Yan 2006) of three Iron-Age archaeological sites, the Fan-Zai-Yuan, Lu-Liao, and Hui-Lai-Lee sites, in central Taiwan suggests that prehistoric populations such as the Fan-Zai-Yuan people and the Lu-Liao people are closely related to the Atayal and Bunun, respectively. Surprisingly, there is no connection or haplotype-sharing
between the Hui-Lai-Lee people and any modern Taiwanese indigenous population. Instead, one out of three collected human osteological samples that was successfully sequenced found a M9a haplotype present in the Hui-Lai-Lee people, and the authors concludes a maternal relationship between the Hui-Lai-Lee people and Northern Asians, especially Tibetan people (Yan 2006). However, this suggestive result comes from a total sample size of three and only one positive PCR amplification.

In addition to human remains, animal bones could also be indicators of population migration since humans transported animals such as rats and pigs as food items (e.g., Matisoo-Smith 2002, 2004; Larson et al. 2007). Two case studies of modern and archaeological populations of *Rattus exulans* from both Chatham Island and New Zealand provide pictures regarding the relationship between the archaeological and the extant populations (Matisoo-Smith 2002). The Chatham Island material was chosen specifically because analyses of extant Chatham Island rats showed that they were a monophyletic group within Polynesian rats, with limited mtDNA variability. Fifteen of the extant rat sequences from the Chatham Island material possessed a single nucleotide polymorphism not found in any other Polynesian *R. exulans* population (Matisoo-Smith et al. 1998), and showed little other variation, either with each other or when compared to other modern sequences. This limited mtDNA variation is unusual when compared to other *R. exulans* populations in Polynesia (Matisoo-Smith 2002). On the other hand, given that *R. exulans* is not native to New Zealand, and could only reach the archipelago with humans (Matisoo-Smith et al. 1998), multiple prehistoric introductions of rats to New Zealand (Sutton 1994; Murray-MacIntosh et al. 1998) were revealed by a high level of variation (34 identified haplotypes out of 50 collected samples).
An additional study (Matisoo-Smith 2004) of the rats used mtDNA from both archaeological and modern samples collected from island Southeast Asia and the Pacific to construct phylogenies based on ~240 base pairs of the D-loop from samples. Three major haplogroups were identified and a Neighbor-Joining tree including sequences from these three haplogroups shows a clear geographic patterning: haplogroup I solely from Southeast Asian samples (Philippines, Borneo, and Sulawesi), haplogroup II from both Southeast Asian and Oceanic samples, and haplogroup III from Remote Oceanic samples (except Halmahera). Therefore, multifaceted models incorporating a more complex view of the Lapita intrusion, especially Green’s VC Triple-I model was supported (Matisoo-Smith 2004). Unfortunately, this study did not include Taiwanese rats.

By using mtDNA from 781 modern and ancient Sus specimens, Larson and colleagues (2007) provided evidence regarding two human-mediated dispersals of domestic pig (Sus scrofa) through Island Southeast Asia into Oceania: one is associated with Lapita and later Polynesian migrations and links modern and archeological Javan, Sumatran, Wallacean, and Oceanic pigs with mainland Southeast Asian pigs; the other links mainland East Asian pigs (potentially from peninsular Southeast Asia) to western Micronesia, Taiwan, and the Philippines. Also, these so-called “wild” pigs within this region are most likely feral descendants of domestic pigs introduced by early agriculturalists. Of these dispersal routes, the Lapita and later Polynesian dispersals into Oceania appear to be exclusively associated with Pacific Clade pigs (Larson et al. 2007).

In conclusion, it is most likely that admixtures of groups of people happened in Near Oceania before they migrated to Polynesia with potential backward dispersals to Southeast Asia. Additionally, various social customs, such as matril-/patri-linear practice,
may affect the directions of dispersals and result in sex-biased gene flow that presents in modern Austronesian speakers.
Chapter 3 Cultural History of Taiwan

This chapter provides brief overviews of Taiwanese archaeology and of indigenous Taiwanese today. Human fossils from Taiwan date to as early as 20,000-30,000 years ago, but agricultural implements and ceramic pottery first appear only 5,000-6,000 years ago. After the introduction of food production, continuous prehistoric cultural complexes occur throughout Taiwan. The historic period begins approximately 300-500 years ago.

Three sites included in this study, San-Pau-Chu (SPC), Nan-Kuan Li East (NKLE), and Wu-Chien-Tsuo South (WCTS), are briefly introduced below. The SPC samples were used for both ancient DNA and dental morphological studies because they were handled relatively little by either excavators or curators, diminishing the opportunity for contamination. The NKLE site is generally believed to be a representative of the earliest ceramic (cord-marked) cultural complex (Tsang et al. 2004, 2006, 2007), which is considered by some to be ancestral to the Lapita culture (Bellwood 1979, 1985, 1992, 1993, 1995a, 1995b). The SPC and WCTS sites correspond to late Neolithic of Taiwanese prehistory and postdate the appearance of Lapita cultural complex by about 500 years. With dental comparisons of these three sites along with ancient DNA study of the SPC site, this project can potentially provide insight into Taiwanese, Austronesian migration, and Lapita dispersals.

Introduction

Prehistoric and historic periods in Taiwan are divided by 1624 A.D, the date of Dutch colonization, however, signs of human existence in Taiwan occur as early as the
late Paleolithic Age. The earliest human fossils are from Tainan Prefecture and are referred to as Tso-Chen Man (*Homo sapiens sapiens*) (Figure 3.1). The fossils are dated to between 20,000 and 30,000 years ago (Lien 1981a, Shikama 1976). There are no cultural remains in conjunction with these fossils.

Prehistoric times include the Paleolithic, Neolithic, and Iron Age periods. The earliest known cultural complex belongs to the Chang-Pin culture that existed until approximately 6,000 to 5,000 years ago (e.g., Liu 1992, 1993, Tsang 2000). It is generally assumed that the prehistoric cultures since the Neolithic Age were made by Austronesian speakers (Chang and Ward 1996). However, there may be other populations that lived in Taiwan before or along with Austronesian-speaking peoples.

Historic times began with Dutch colonization (1624-1662), followed by Ming (1662-1683) and Ching (1683-1895) Dynasties’ control from mainland China, Japanese colonization (1895-1945), and then independence led by the national government of the Republic of China (R.O.C.).

**Archaeological Chronology of Taiwan**

More than one thousand archaeological sites have been found in Taiwan and can be grouped into at least 17 prehistoric cultural complexes that are primarily based on pottery styles (e.g., Liu 1992, Sun et al. 1975, Tsang 2000). These cultures include the Ta-Pen-Keng (recently also called Da-Ben-Keng) (e.g., Chang 1969, Huang 1994, Liu 1962, Tsang 1992, Yang 1961) culture in the early Neolithic, the Chih-Shan-Yen (e.g., Huang Shih-Chiang 1981, Huang, Tai-Xiang 1994, Liu 1992, Tsang 2000, Wang 1980), Yuan-Shan (e.g., Chang 1964, Chang and Sun 1957, Huang 1994, Kono 1937, Lien Chai-Mei
Figure 3.1 City and River Distribution in Taiwan (a map drawn by the Institute of Ethnology, Academia Sinica, Taipei, 1972).

Liu Yi-Chang 1992, Peirusewsky et al. 2001, Tsang and Liu 2001a, Tsang et al. 2001, Yang 1961), Fan-Tsu-Yuan (e.g., Sun 1962), Ta-Chiu-Yuan (e.g., Chiu 1991), Niao-Sung (e.g., Wu et al. 1979, Chen 1977, Huang 1982), Chi-Pu (e.g., Guo 2005), and Kuei-Shan (e.g., Huang et al. 1987, Li Kuang-Chou et al. 1985, Li Kung-Ti 1989, 1993, 2001, Sun et al. 1992) cultures in the Iron Age. Table 3.1 presents an outline of the culture history of prehistoric Taiwan.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Time Period</th>
<th>Location</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Tso-Chen Man)</td>
<td>Paleolithic</td>
<td>southwest Taiwan</td>
<td>30,000-20,000 years</td>
</tr>
<tr>
<td>Chang-Pin</td>
<td>Paleolithic</td>
<td>eastern Taiwan</td>
<td>-6,500 years ago</td>
</tr>
<tr>
<td>Ta-Peng-Keng</td>
<td>Early Neolithic</td>
<td>all parts of Taiwan</td>
<td>5,000 years ago</td>
</tr>
<tr>
<td>Chih-Shan-Yen</td>
<td>Middle Neolithic</td>
<td>northern Taiwan</td>
<td>4,000-2,700 years ago</td>
</tr>
<tr>
<td>Yuan-Shan</td>
<td>Middle Neolithic</td>
<td>northern Taiwan</td>
<td>4,200-2,500 years ago</td>
</tr>
<tr>
<td>Niu-Ma-Tou</td>
<td>Middle Neolithic</td>
<td>central-west Taiwan</td>
<td>5,000-3,500 years ago</td>
</tr>
<tr>
<td>Niu-Chou-Tz</td>
<td>Middle Neolithic</td>
<td>southwest Taiwan</td>
<td>5,000-3,500 years ago</td>
</tr>
<tr>
<td>Fu-Shan</td>
<td>Middle Neolithic</td>
<td>eastern Taiwan</td>
<td>5,000-3,500 years ago</td>
</tr>
<tr>
<td>Botanical Garden</td>
<td>Late Neolithic</td>
<td>northern Taiwan</td>
<td>2,500-2,000 years ago</td>
</tr>
<tr>
<td>Ying-Pu</td>
<td>Late Neolithic</td>
<td>central-west Taiwan</td>
<td>3,500-2,000 years ago</td>
</tr>
<tr>
<td>Ta-Hu</td>
<td>Late Neolithic</td>
<td>southwest Taiwan</td>
<td>3,500-2,000 years ago</td>
</tr>
<tr>
<td>Pei-Nan</td>
<td>Late Neolithic</td>
<td>eastern Taiwan</td>
<td>3,500-2,000 years ago</td>
</tr>
<tr>
<td>Chi-Lin</td>
<td>Late Neolithic</td>
<td>eastern Taiwan</td>
<td>3,500-2,000 years ago</td>
</tr>
<tr>
<td>Shih-San-Hang</td>
<td>Iron Age</td>
<td>northern Taiwan</td>
<td>1,900-500 years ago</td>
</tr>
<tr>
<td>Fan-Tsu-Yuan</td>
<td>Iron Age</td>
<td>central-west Taiwan</td>
<td>1,900-500 years ago</td>
</tr>
<tr>
<td>Ta-Chiu-Yuan</td>
<td>Iron Age</td>
<td>central-west Taiwan</td>
<td>1,900-500 years ago</td>
</tr>
<tr>
<td>Niao-Sung</td>
<td>Iron Age</td>
<td>southwest Taiwan</td>
<td>1,900-500 years ago</td>
</tr>
<tr>
<td>Kuei-Shan</td>
<td>Iron Age</td>
<td>southern Taiwan</td>
<td>1,900-500 years ago</td>
</tr>
<tr>
<td>Chi-Pu</td>
<td>Iron Age</td>
<td>eastern Taiwan</td>
<td>1,900-500 years ago</td>
</tr>
</tbody>
</table>

Table 3.1 Chronology of archaeological cultures in Taiwan

**The Early Neolithic Age**

The defining characteristic of the Ta-Peng-Keng culture (5,000 years ago) is coarse cord-marked ceramic potsherds (Figure 3.2) that were first found at the Ta-Peng-Keng
site in the Taipei Prefecture and the Feng-Pi-Tou site in the Kaohsiung Prefecture by Chang Kwang-Chih (Chang 1969). Since 1969, sites belonging to this culture have been discovered along the northern, eastern, and southwestern coasts of Taiwan as well as in the Taipei Basin and on the Pang-Hu Islands (Tsang 1992).

Figure 3.2 Coarse cord-marked sherds from the Kuo-Yeh Site, Peng-Pu (from Tsang 2000)

The Middle Neolithic Age

There were several regional variations of Taiwan prehistoric cultures in the Middle Neolithic between 5,000 to 3,000 years ago. These include the Chih-Shan-Yen (Figure 3.3) and Yuan-Shan (Figure 3.4) cultures in the northern Taiwan (e.g., Chang 1964, Chang and Sun 1957, Huang Shih-Chiang 1981, 1994, Huang Tai-Xiang 1994, Kono 1937, Miyahara 1926, Lien Chai-Mei 1988, Liu 1992, Ryuzo 1911, Shi 1954, Sun 1954, Tsang 2000, Wang 1980), the Niu-Ma-Tou culture in the west-central part of the island
(e.g., Liu 1955), the Niu-Chou-Tz culture in the south (e.g., Huang 1977, Li 1992, Liu 1977, Naoichi 1954), and the Fu-Shan cultures in the east (e.g., Li and Yeh 1995).

Figure 3.3 Sherds from the Chih-Shan-Yen site (from Huang 1994)

Figure 3.4 Sherds from the Yuan-Shan (right) site (from Huang 1994)
The characteristic of this time period is fine Cord-Marked and Plain Ware pottery, which have much better quality of pottery and are made from fine clay. Rice imprints were found in some potsherds. Another feature is abundant sea shells (shell midden) in this cultural layer.

_The Late Neolithic Age_

As in the Middle Neolithic Age, each region in the Late Neolithic has its own special characteristic cultures. For instance, the Botanical Garden culture was found in the north (e.g., Guo 2002, Huang 1994, Liu 1992, Tsang 2000), the Ying-Pu culture (Figure 3.5) in the west-central (e.g., Ho and Liu 2006, Ke 1964, Sun and Lien Chai-Mei 1975, Anonymous 1965), the Ta-Hu culture in the south (e.g., Chen 1980), and the Pei-Nan and Chi-Lin cultures in the east (e.g., Huang 1993, Li 1987, 2002, Lien 1981b, 1982, 1990, 1992, Lien and Sun 1986, Liu 1990, Sun 1967, 1988, Sun and Lien 1984, 1985, Tan et al. 1998, Wang 1984, Yang 1997, Yen 1987, 2005).

Little is yet known about the Botanical Garden culture (e.g., Guo 2002, Huang 1994, Liu 1992, Tsang 2000), however, its pottery tends to be fine and thick-walled, brown or light brown in color, and is decorated with checked impressions (e.g., Tsang 2000). The characteristic of the Middle Neolithic culture in the rest of the western part of Taiwan, including the Ta-Hu culture (e.g., Chen 1980, Tsang 2000), is grayish-black pottery, which is of very fine quality, thin and highly polished, and decorated with wavy patterns.

During the same time period, a large cemetery of stone cist coffins (Figure 3.6), later called the Peinan site, was discovered because of a course change in the East Coast
Railway line at Peinan near the Taitung City in the east Taiwan in 1980. The Pei-Nan site, gives its name to the Pei-Nan culture, is the largest village from this time period found so far in Taiwan (e.g., Huang 1993, Li 1987, 2002, Lien 1981b, 1982, 1990, 1992, Lien and Sun 1986, Liu 1990, Sun 1988, Sun and Lien 1984, 1985, Tan et al. 1998, Wang 1984, Yang 1997, Yen 1987, 2005). Rich remains and well-preserved artifacts (e.g., Lien 1981b, 1982, 1990, 1992, Lien and Sun 1986, Sun and Lien 1984, 1985) were recovered within the coffins, including jars, pots (Figure 3.7), spindle whorls, cups, stone or jade ornaments (Figure 3.8) (e.g., Hung 2004, Hung et al. 2006, Hung et al. 2007, Lien 1990, Tan et al. 1998, Yang 1997), arrowheads, spearheads (e.g., Huang, Xin-Kai 1993, Liu Ke-Hong 1990), and adzes, however, artifacts are relatively fragmentary in areas outside the coffins. Architectural remains (e.g., Lien and Sun 1986) were also discovered, such as stone pillars and paved living floors (Figure 3.9).
The fascinating aspect of Chi-Lin Culture (e.g., Sun 1967, Tsang 2000) is the discovery of large carved stone sarcophagi, walls (Figure 3.10), monoliths, statues, wheels, pillars, and perforated stone disks instead of sandy paste and undecorated pottery.

Figure 3.6 Peinan Cist Coffins (from http://www.nmp.gov.tw/).

Figure 3.7 Peinan Pottery (from National Museum of Prehistory: A Commemorative Edition).
Figure 3.8 Peinan Jade Objects (from National Museum of Prehistory: A Commemorative Edition).

Figure 3.9 One constant, in situ excavation at the Peinan Cultural Park (from National Museum of Prehistory: A Commemorative Edition)
The Iron Age

In Taiwan, the beginning of the manufacture and use of iron began around the birth of Christ (e.g., Liu 1992, 1993, Tsang 2000). During the prehistoric Iron Age, regional cultures continued to flourish all over Taiwan, including the Shih-San-Hang culture in the north (e.g., Chang 1993, Huang 1994, Lin Jia-Wei 1996, Lin Hsiu-Man 1997, Liu Chin-Hsin 2005, Liu Yi-Chang 1992, Peitrusewsky et al. 2001, Tsang and Liu 2001a, Tsang et al. 2001, Yang 1961), the Fan-Tsu-Yuan and Ta-Chiu-Yuan cultures in the west-central region (e.g., Chiu 1991, Sun 1962), the Niao-Sung culture in the south (e.g., Chen 1977, Huang 1982, Wu and Lee 1979), and the Chi-Pu culture in the east (e.g., Guo 2005). A newly excavated site at Kuei-Shan in the Pingtung Prefecture reveals unique features in comparison with aforementioned cultures stepping into the Iron Age and may represent a culture of its own (e.g., Huang et al. 1987, Li Kuang-Chou et al. 1985, Sun et al. 1992).
In addition, this is the time period that local groups in Taiwan or indigenous Taiwanese initiated closer contacts and interactions with the Han Chinese, which sowed the seeds for their later assimilation into a dominant Han culture.

Similar to the Peinan site, the Shih-San-Hang site (e.g., Huang 1994, Lin Jia-Wei 1996, Lin Hsiu-Man 1997, Liu Yi-Chang 1992, Peitrusewsky et al. 2001, Tsang and Liu 2001a, Tsang et al. 2001, Yang 1961) was explored in the course of a large-scale rescue excavation necessitated by the construction of the Pali Sewage Plant. Because the pottery from the Shih-San-Hang site consists mostly of reddish-brown and hard sherds decorated with geometrical patterns (Figure 3.11-3.12), this site is a characteristic of the Shih-San-Hang culture.

An iron workshop (Figure 3.13) was found, showing that the Shih-San-Hang people had begun to engage in iron production and to use iron tools for subsistence tools and weapons (e.g., Liu 1992, Tsang 2000, Tsang and Liu 2001). Evidence for trade between Taiwan and mainland China can be also seen from coins (Figure 3.14) dated to the Tang and Sung Dynasties (e.g., Liu 1992, Tsang 2000, Tsang and Liu 2001).

Two hundred and ninety-one skeletons were discovered from the Shih-San-Hang site (Figure 3.15). The Shih-San-Hang people seldom had caries and abscess according to Chang’s (1993) study of 17 adult males and 15 females (1993). She also suggested that the Shih-San-Hang people may have chewed betel nuts due to the severe dental wear patterns and may have used their teeth as tools because of the abnormal wear patterns on their molars. Enamel hypoplasias occurred in some individuals, potentially indicating malnutrition or disease in childhood (Chang 1993, Liu 2005). Osteoarthritis is the most
predominant pathology, and traumas, such as fractures, were rarely observed (Chang 1993).

Figure 3.11. Sherds from the Shih-San-Hang site (from Tsang 2000).

Figure 3.12. Pottery from the Shih-San-Hang site (from Tsang 2000).
Figure 3.13 The Iron Smelting Furnace at the Shih-San-Hang site (from Tsang 2000).

Figure 3.14 Chinese coins from the Shih-San-Hang site (from Tsang 2000).
Figure 3.15 Shih-San-Hang Burials (from Tsang 2000).

Pottery of the Kui-Shan culture (e.g., Huang et al. 1987, Li Kuang-Chou et al. 1985, Li Kun-Xiu 2006, Sun et al. 1992, Tsang 2000) was distinguished by a unique and distinctive decorative style (Figure 3.16), which is impressed human figures or “J”-shaped designs. It is believed by several scholars that the Kui-Shan culture belong to late Peinan culture and might be the origin of the modern Paiwan (Li 2006).

A Microcosm of southwest prehistory in the Tainan Science-Based Industrial Park

The 1,000 hectare Tainan Science-Based Industrial Park is located among the Xin-Hua, Shan-Hua, An-Ding Counties in the Tainan Prefecture. Because of the development of this area since 1995, a series of rescue archaeological excavations have been conducted. Before November 2007, 60 prehistoric sites have been discovered in the park
and surrounding area, and thirty-one sites have subsequently been excavated during the course of construction (Tsang et al. 2007).

Figure 3.16 Decorated potsherds from the Kui-Shan Site (from Li et al. 1985).

According to changes of depositional environment in the southwestern coastal plain of Taiwan (e.g., Lin 1963; Sun 1964, 1970; Chen 1993; You 2003), a marine transgression commenced in the area of the Tainan Scientific-Based Industrial Park, beginning more than 10,000 years ago. The sea level in this area reached its highest point at around 6,500 years ago. After the glacial retreat, declining ice melt and tectonic activities of the Pacific plate (Sun 1970) combined to cause a gradual drop in sea level. The land of the Nan-Kuan-Li East site rose above the sea level beginning around 4,500 years ago. The land of the San-Pau-Chu site outstripped the sea level between 4,500 years and 3,500 years ago. The land of the Wu-Chien-Tzou South site lifted from the sea level between 3,500 and 2,000 years ago.

The cultures present in the sites in the park that have been excavated represent a microcosm of Taiwan’s prehistory (Figure 3.17) (Tsang et al. 2004, 2007, Tsang et al.)
Two sites, Nan-Kuan-Li and Nan-Kuan-Li East, yielded remains of the Ta-Pen-Keng (TPK) culture (4,800-4,200 B.P.), the You-Hsian-Fang site belongs to the Niu-Chou-Tz culture (3,800-3,300 B.P.), 26 sites, such as San-Pau-Chu and Wu-Chien-Tsuo South, document the presence of the Ta-Hu Culture (3,300-1,800 B.P.), 20 sites, including Tao-Ye, belong to the Niao-Sung culture (1,800-500 B.P.), five sites, among them Wu-Chien-Tsuo North, date to the Siraya culture (500-250 B.P.), and, finally, ten sites record the presence of recent Han Chinese Culture (250 B.P.-present) (Tsang et al. 2004, 2007, Tsang et al. 2006). Information on types of sites represent is still unavailable. Future publications of the results of the archeological excavations should shed light on these questions.

**Indigenous Taiwanese Today**

Although Taiwan and its peoples were historically recorded, it is not until the Ching Dynasty that indigenous Taiwanese began to be separated into the “Plains Tribes” (Ping-Pu Tsu) and the “Mountain Tribes” (Kao-Shan Tsu) according to their locations (Lin 2003). Furthermore, in order to respond to rights of indigenous populations, an official institute, Council of Indigenous Peoples, was established in 1996 (http://www.apc.gov.tw/).

Currently, there are 14 officially recognized indigenous populations, nine of them are classified as Mountain Tribes. These are Amis (with largest population size), Atayal, Bunun, Paiwan, Puyuma, Rukai, Saisiyat, Tsou, Yami (the most isolated population and also called Tao) (Lin 2003, http://www.apc.gov.tw/chinese/indexMain.jsp, http://www.dmtip.gov.tw/). Five more tribes have been identified as indigenous
Taiwanese since 2001, including the Thao (with the least population size and previously identified as Plain Tribes of the Tsou), Kavalan (originally considered a part of the Amis before 2002), Taroko (originally included as a sub-tribe of the Atayal before 2004), Sakizaya (originally viewed as one of the branches of the Amis before 2007) and Seediq (originally classified as one of the branches of the Amis before 2007) (Lin 2003, http://www.apc.gov.tw/chinese/indexMain.jsp, http://www.dmtip.gov.tw/).

Many populations, such as Saisiyat, Atayal, Rukai, Ami, Thao, Tsuo, Bunun, and Ketagalan, believe in ancestor spirits and each tribe has its own festival for the spirits, such as *maljeveq* (native pronunciation) in Paiwan (personal communication with IV Mei-Hui), *angayaw, mulaliyaban*, and *basibas/mangamangayaw* (only for males) (personal communication with Agilasay Lin) in Peinan as well as *mugamut* (only for females) (personal communication with Nuo-Ling Lin) in Peinan. Additionally, the distinctive social structures of each tribe still exist today. To give an example, the Amis retains a matrilineal society (http://www.dmtip.gov.tw).
Figure 3.17 Culture(s) at each site in the Tainan Science-Based Industrial Park (revised from the Brochure of “Journey of Archaeology, Exhibition at the Tainan Science-Based Industrial Park,” National Museum of Prehistory).

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Chapter 4: Dental Variations of the Prehistoric Peoples in Southwest Taiwan

Biological anthropological studies that emphasized the description and measurements of living populations, in particular indigenous peoples in Taiwan, began in the late nineteen century, but systematic research on living indigenous Taiwanese did not begin in earnest until the establishment of the medical school at the Taipei Emperor University (tai-ho-ku de-i-ko-ku dai-gan-ku or たいほくていこくだいがく) in 1936. Since then, many studies have been conducted. Research has varied in focuses from studies of living populations to investigations of skeletal remains from archaeological sites. However, relatively few studies were done after the 1960s. Before 1993, only one systematic study on 32 human skeletons from the Shih-San-Hang (SSH) site was performed (Chang 1993), but the skeletal material has received considerably more attention since then.

Samples for this study were obtained from series of rescue excavations in southwestern Taiwan. Because of the construction at the Tainan Science-Based Industrial Park in the Tainan Prefecture, many archaeological excavations had been conducted in order to protect cultural resources found in the Park and surrounding regions. These efforts have uncovered sixty archeological sites, including the San-Pau-Chu (SPC), Wu-Chien-Tsuo South (WCTS), and Nan-Kang-Li East (NKLE) sites. Approximately half of the sites have been systematically partially or fully excavated. Six cultural phases (the TPK, Niu-Chou-Tz, Ta-Hu, Niao-Sung, Siraya, and Han Chinese Cultures) were
identified, ranging from 4,800 BP to the present (Tsang et al. 2004, 2006; Tsang et al. 2007).

Although there is no evidence to infer who developed these culture and where they came from, this Park is located within the distribution of modern Siraya populations (one of the Ping-Pu Tsu, or so-called Plains Tribes) who speak one of the Austronesian languages (Li 1996). Additionally, five sites that were defined as the Siraya culture (500-250 B.P.), such as Wu-Chien-Tsuo North, were found in this Park (Tsang et al. 2004, 2006; Tsang et al. 2007). The objective of this chapter is, therefore, to attempt to examine the biological relationships among the human remains from SPC, NKLE, WCTS, prehistoric and modern Southeast and Northern Asians, and modern Austronesian speakers in the Pacific by means of dental size and morphology (crown width and nonmetric traits). The relationships between these populations will provide insights into the place of Taiwan in Austronesian migrations.

**Materials and Methods**

*The SPC, WCTS, and NKLE Samples in General*

A total of 131, 52, and 85 human skeletons were discovered from the SPC, WCTS, and NKLE sites, respectively. In the SPC, WCTS, and NKLE sites, 58, 28, and 66 individuals were separately examined for primary inventory, dental metric and/or non-metric traits, or both depending on the presence of the tooth. The age of individuals range from infants to middle adulthood based on dental development (Ubelaker 1989), epiphseal fusions (Ubelaker 1989), long bone length for immature individuals (Ubelaker 1989), and Suchey and Brooks pubic symphyseal Age System (Suchey and Brooks 1990,
Suchey and Katz 1986) as well as Todd pubic symphyseal Age System (Todd 1921a, 1921b). The sexes of the individuals were estimated from the cranium (Acsádi and Nemeskéri 1970) and os coxae (Buikstra and Mielke 1985, Phenice 1969), but the sex of many individuals could not be determined because of combination of their burial positions and current storage condition (i.e. covered by silicon and polyester). At times, this combination of features obscured pelvic and cranial morphology (Figures 4.1-4.4).

Pathological conditions such as osteoarthritis (Figures 4.5-4.6), fractures (Figure 4.7), and variations such as the presence of a squatting facet (Figure 4.8) on tibiae were observed in many of these individuals. Vertebral osteophytosis is the most common pathology. Eleven out of forty-five (24.44%) SPC people have osteophytic lipping on their epiphyseal rings of lumbar vertebrae (L2-5). Additionally, there are a number of traumatic lesions that suggest inter-individual conflicts.

Figure 4.1 Pubic of SPC F11 II B3 with poor preservation.
Figure 4.2 Pubic morphology of SPC G12 II B2 in current storage condition.

Figure 4.3 Sex of SPC F16 II B3 from the cranium is undetermined because of the combination between the burial position and current storage condition.
Two individuals had stone spears in their rib cages: one from SPC (G16 II B1, approximately 30 years old, probably male) (Figure 4.9), the other is from NKLE (F5 B11, 25-35 years old, undetermined sex). Clear cuts from cervical to lumbar vertebrae in one (K17 II B1, 23-35 years old, probably male) of the SPC people also suggest the presence of violence. K17 II B1 bears one cut from C6-7 to T1-4 and another from T11-12 to L1-5, and likely extending to sacrum- S1-2 (Figure 4.10). Unlike a potential case of head-hunting in the Wu-Chien-Tsuo Site (B3, age and sex are not determined) (Figure 4.11), which belongs to a Niao-Sung Culture dated to 1400-1000 before present (Tsang et al 2004, 2006; Tsang et al. 2007), neither an unusual burial position nor pathological condition was found in these individuals from SPC. However, two of the individuals with traces of trauma (4.88%) from SPC (G1 II B1 and K17 II B1 with systematic locations at T0-1P7-8 and T0P0-1) are both most likely male. Do these cases of violent trauma
indicate a simple inter-individual conflict event or evidence of more systematic raiding or war (Keeley 1996, Golitko and Keeley 2006)? Further studies from this site, the surrounding area, and similar time periods as well as burial distribution will be done in the future to answer this question.

Figure 4.5 SPC I17 II B4 with Osteoarthritis.
Figure 4.6 WCTS N12 B7 with osteophytes on the spinous processes of T5 (curved spicules), T6 and T7 (fusion present).

Figure 4.7 WCTS N15 B1 with fused humerus, radius, and ulna.
Figure 4.8 SPC H18 II B4 with a squatting facet on the tibia.

Figure 4.9 SPC G16 II B1 with a stone spear transversing the rib cage.
Figure 4.10 SPC K17 II B1 has thoracic and lumbar vertebrae with clear cuts.

Figure 4.11 WCT B3 was decapitated.
Principals for Observations and Measurement of the Dental Morphology

Because of the practice of tooth extraction (Figure 4.12), the prevalence of severe dental attrition (Figure 4.13), and the current storage condition\(^1\) for these human remains, the numbers of each tooth scored for non-metric traits and measurement of crown width vary. To assess intra-observer error, studies of the measurement error present in both discrete and continuant traits were conducted. The error statistics for intra-observer errors were based on the similarity between scores and measurements from two different measuring sessions by the same observer.

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\(^1\) Most of the skeletal remains from these sites are collectively stored in a climate-controlled room at one location in the Park. They are still preserved in silicon jackets and have not been extracted because of the large amounts of samples that were discovered and because of a lack of research space to study them.
Nonmetric Traits

Fifteen non-metric dental traits\(^2\) were scored (Tables 4.1-4.4) following the Arizona State University (ASU) dental anthropology system (Turner et al. 1991) and the Dental Visual Recoding Forms\(^3\) (Buikstra et al. 1994). Observations of each trait were made twice independently. For the study of intra-observer error, Spearman’s tests of shoveling in the incisors (Table 4.5), double-shoveling incisors (Table 4.6), Carabelli’s Cusp of maxillary 1\(^{st}\) molar, hypocone and cusp 5 of maxillary molars (Table 4.7), were performed in the JMP statistical program.

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\(^2\) The fifteen non-metric traits are winging, incisor shoveling, incisor double-shoveling, lower canine distal accessory ridge (dar), 1st premolar root number, hypocone, metaconule, Carabelli’s cusp, protostylid, cusp 5, cusp 6, cusp 7, molar root number, enamel extension, and peg-shaped tooth.

\(^3\) Recorded dental data include dental inventory, development, occlusal surface wear, dental caries, abscesses, dental calculus, premortem dental modifications.
Data from both sides were recorded. However, if data from one side were missing, the other side was scored and used. Data were originally recorded by degrees of expression, then these scores were transformed into binary variables (1 for present, 0 for absent) (Appendix I) and analyzed as frequency data. Advantages of binary data include utility for global comparisons (Scott and Turner 1997) and avoidance of over-estimating statistical differences (Sokal and Rohlf 1995, Madrigal 1998), specifically the reduction of type I (alpha) errors (Sokal and Rohlf 1995, Madrigal 1998).

Table 4.1 Numbers of incisors scored for non-metric traits in the SPC site.

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<th>L. I¹</th>
<th>L. I²</th>
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<td>Shoveling (0-7)</td>
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<td>17</td>
<td>19</td>
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<tr>
<td>Double-shoveling (0-6)</td>
<td>21</td>
<td>18</td>
<td>22</td>
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Table 4.2. Numbers of maxillary 1st premolar scored for non-metric traits in the SPC site.

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<td>Root Number</td>
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Table 4.3 Numbers of maxillary molars scored for non-metric traits in the SPC site.

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<th>L. M²</th>
<th>L. M³</th>
<th>R. M¹</th>
<th>R. M²</th>
<th>R. M³</th>
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<td>Hypocone (0-6)</td>
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<td>14</td>
<td>7</td>
<td>16</td>
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<td>6</td>
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<tr>
<td>Metaconule (0-5)</td>
<td>14</td>
<td>15</td>
<td>7</td>
<td>16</td>
<td>13</td>
<td>6</td>
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</tbody>
</table>

Table 4.4 Numbers of mandibular molars scored for non-metric traits in the SPC site.

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<th>L. M₂</th>
<th>L. M₃</th>
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<th>R. M₂</th>
<th>R. M₃</th>
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<td>10</td>
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<td>16</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Cusp 5 (0-5)</td>
<td>13</td>
<td>21</td>
<td>6</td>
<td>17</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>Cusp 6 (0-5)</td>
<td>13</td>
<td>11</td>
<td>6</td>
<td>17</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>Cusp 7 (0-4)</td>
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<td>6</td>
<td>17</td>
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<td>8</td>
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Table 4.5 Numbers for Kappa and Spearman’s tests for shoveling.

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<th>Shoveling U I²</th>
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<tr>
<td></td>
<td>22</td>
<td>19</td>
<td>19</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 4.6 Numbers for Kappa and Spearman’s tests for double-shoveling.

<table>
<thead>
<tr>
<th></th>
<th>Double-shoveling U I¹</th>
<th>Double-shoveling U I²</th>
<th>Double-shoveling L I¹</th>
<th>Double-shoveling L I²</th>
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</thead>
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<tr>
<td></td>
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</table>
Table 4.7 Numbers for Kappa and Spearman’s tests for Carabelli’s Cusp, cusp 5, and hypocone.

<table>
<thead>
<tr>
<th>Carabelli's Cusp M1</th>
<th>Cusp 5 M1</th>
<th>Cusp 5 M2</th>
<th>Hypocone M1</th>
<th>Hypocone M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>21</td>
<td>17</td>
<td>18</td>
<td>20</td>
</tr>
</tbody>
</table>

Metric Traits

Crown widths (buccolingual diameters) are defined as the widest diameter of the tooth and were measured perpendicular to the mesiodistal plane using a dial or digital caliper accurate to 0.01 mm (Buikstra et al. 1994). Although crown length (or mesiodistal diameter) and crown height were taken when possible, both dimensions are more likely to be affected by dental attrition (e.g., Buikstra et al. 1994, Hillson 1996, Mayhall 2000) or pathology (e.g., Buikstra et al. 1994, Hillson 1996) and were not included in this study (Appendix II).

All teeth were measured (Tables 4.8-4.9) in order to avoid missing potential data of skeletal remains from archaeological sites. Additionally, all measurements were pooled by tooth because sexes of individuals in these three sites could not always be determined (Appendix III) in the current storage condition. Depending on preservation, either the left or right side of the maxillary and mandibular teeth was used. If both were present, the mean of these two measurements were taken to prevent unequal sizes of left and right antimeres. This procedure should maximize sample size and eliminate the redundancy inherent in separate analyses of teeth from the right and left side. Measurements of all samples were made twice at different times in order to test intra-observer errors. Repeated measure ANOVA for each tooth were used for the error statistics, with statistic significance set at $\alpha = 0.05$. The average precision for each tooth was also calculated in
order to evaluate the difference between the measurements in millimeter using the 2nd measurement as a percentage of the first measurement.

<table>
<thead>
<tr>
<th></th>
<th>I₁</th>
<th>I₂</th>
<th>C</th>
<th>Pm₁</th>
<th>Pm₂</th>
<th>M₁</th>
<th>M₂</th>
<th>M₃</th>
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</thead>
<tbody>
<tr>
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<td>41</td>
<td>37</td>
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<td>27</td>
<td>16</td>
</tr>
<tr>
<td>WCTS</td>
<td>15</td>
<td>2</td>
<td>5</td>
<td>13</td>
<td>10</td>
<td>12</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>NKLE</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>14</td>
<td>16</td>
<td>20</td>
<td>20</td>
<td>9</td>
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<tr>
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<td>36</td>
<td>68</td>
<td>63</td>
<td>62</td>
<td>53</td>
<td>27</td>
</tr>
</tbody>
</table>

Table 4.8 Numbers of maxillary teeth measured for crown width.

<table>
<thead>
<tr>
<th></th>
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<th>I₂</th>
<th>C</th>
<th>Pm₁</th>
<th>Pm₂</th>
<th>M₁</th>
<th>M₂</th>
<th>M₃</th>
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<tbody>
<tr>
<td>SPC</td>
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<td>41</td>
<td>42</td>
<td>37</td>
<td>32</td>
<td>26</td>
<td>13</td>
</tr>
<tr>
<td>WCTS</td>
<td>11</td>
<td>15</td>
<td>15</td>
<td>11</td>
<td>14</td>
<td>9</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>NKLE</td>
<td>5</td>
<td>6</td>
<td>11</td>
<td>17</td>
<td>19</td>
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<td>67</td>
<td>70</td>
<td>70</td>
<td>60</td>
<td>50</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 4.9 Numbers of mandibular teeth measured for crown width.

**Comparative Populations**

Previous published studies of modern and prehistoric populations for non-metric dental traits (all pooled data: Chang 1993, Hanihara et al. 1975, Hanihara 1977, Hanihara 1992, Scott et al. 1997, Turner et al. 1977) and crown width (Brace and Vitzthum 1984; Hanihara 1979: males only; Houghton 1978; Houghton 1980; Katayama et al. 1988; Kean and Houghton 1990: males only; Snow 1974) (Tables 4.10-4.11) (also see Appendix IV for details) were used for comparisons. For example, Chang (1993) used metric and non-metric traits of 17 males and 15 females to study paleopathology and population relationships from the prehistoric archaeological site in north Taiwan, Shih-San-Hang, dated to the Bronze Age. Samples from Scott and Turner (1997) provide comparative data for worldwide populations from prehistoric time periods, such as the Jomon people, to the present.
The analyzing of dental data could be divided into three levels. First is to detect variation within the SPC people in order to remove outlier from this study to avoiding that the outliers may skew the overall tooth sizes. Second is to detect variations amongst the SPC, WCTS, and NKLE sites in order to evaluate the possibility to increase my sample sizes for comparison. The third is to apply various measurements to estimate population variations between the SPC people, the NKLE people, the WCTS people, prehistoric and modern Southeast and Northern Asians, and modern Austronesian speakers in the Pacific.

Z-scores were calculated and used to screen for outliers in crown width. If any measurement was larger than +2.5 S.D. or below -2.5 S.D. for that tooth, the observation was treated as an outlier and excluded from this study. Next, pair-wise t-tests of each tooth at $\alpha = 0.05$ level were employed to investigate if there is crown width variation amongst the SPC, WCTS, and NKLE people; if no significant variation between crown width was detected among these three sites, then all measured teeth were combined and referred to the same group in order to increase the sample size of this study for population comparisons.

Factor analyses and Multidimensional scaling (MDS) analysis of the maxillary and mandibular crown width were performed using SPSS 16.0 for Windows (2007 SPSS Inc.). Factor analysis is one of the analytical methods for data reduction (Gorsuch 1983); it reduces numbers of variables by computing new, uncorrelated new axes that are based on variance-covariance structure of variables. These new axes, called principal components, are constrained to uncorrelated and represent the main sources of variance in the data.
The first principle component explains the most variance, as gauged by its eigenvalue, of any of the component; the reminder of the component follow suit in sequential order.

MDS is a technique for information visualization in order to explore similarities or dissimilarities in data (Cox et al. 2001, Kruskal et al. 1978). In this project, the factor analysis was used to evaluate how populations are related to each other by identification of group of crown width and MDS was used to visualize similarities in dental crown widths between populations.

<table>
<thead>
<tr>
<th>Maxillary teeth</th>
<th>I1</th>
<th>I2</th>
<th>C</th>
<th>PM1</th>
<th>PM2</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPC+NKLE</td>
<td>31</td>
<td>24</td>
<td>30</td>
<td>55</td>
<td>52</td>
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<td>46</td>
<td>22</td>
</tr>
<tr>
<td>WCTS</td>
<td>14</td>
<td>2</td>
<td>5</td>
<td>12</td>
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<td>10</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Japanese</td>
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<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Ainu</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>N/A</td>
<td>N/A</td>
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<td>N/A</td>
<td>N/A</td>
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<tr>
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</tr>
<tr>
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<td>78</td>
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</table>

This study
This study
Kazuro Hanihara (1979)
Kazuro Hanihara (1980)
Kazuro Hanihara (1981)
Kazuro Hanihara (1982)
Kazuro Hanihara (1983)
Kazuro Hanihara (1984)
Kean & Houghton (1990)
Snow (1974)
Houghton (1980)
Katayama & Tagaya (1988)
Houghton (1978)
Brace & Vitzthum (1984)
Brace & Vitzthum (1985)
Brace & Vitzthum (1986)

Table 4.10 Sample sizes of crown width for maxillary teeth
Different estimations of genetic distance, including NEI72 and Balakrishnan & Sanghvi’s $B^2$, were calculated in NTSYSPC 2.0 (Exeter Software, Setauket, New York) using frequency data of non-metric traits to explore relationships between groups. Clustering analyses, such as Neighbor-Joining (NJ) (Saitou et al. 1987) and Sequential Agglomerative, Hierarchical, and Nonoverlapping (SAHN) trees were constructed from NEI72 and Balakrishnan & Sanghvi’s $B^2$ distances in NTSYSPC 2.0. These proxies for genetic distances were calculated and used because they measure dissimilarity between populations and can construct a family tree of all populations. Nei72 is a mathematical measure that is used to approximate the degree of genetic separation (differentiation) between populations. There are several differences between the NJ and SAHN trees. The NJ algorithm does not make the simplifying assumption of equal evolutionary rates in all groups in a cluster. Instead, NJ uses a more complex distance formula that helps to control for the effects of unequal evolutionary rates to gauge which operational taxonomic units (OTUs) in the analysis should initially be grouped together as nearest neighbors, then uses the internal node between these neighbors to recalculate distances (using the same complex algorithm) between the neighbors and the remaining OTUs. NJ trees are unrooted although a root may be placed at the midpoint of the network (or by another method) to facilitate visualization and interpretation. NJ trees are also statistically consistent under many models of evolution. Therefore, a NJ tree is highly likely to produce an accurate representation of relationships given sufficient data. SAHN, as an un-weighted pair group method, assumes equal evolutionary rates. It averages distances between other OTUs and each pair of OTUs that form a cluster and assumes. This process of agglomerative averaging can lead to an inaccurate, distorted portrayal of
relationships if the evolutionary rates were not constant or, less specifically, if there is marked variation in the distances of individual OTUs already grouped in a cluster to other OTUs outside the cluster. Cluster analysis can visualize objects into clusters (groups) so that objects (i.e. populations) from the same cluster are more similar to each other than objects from different clusters (Aldenderfer et al. 1984).

<table>
<thead>
<tr>
<th>Mandibular teeth</th>
<th>I1</th>
<th>I2</th>
<th>C</th>
<th>PM1</th>
<th>PM2</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPC+NKLE</td>
<td>31</td>
<td>36</td>
<td>51</td>
<td>60</td>
<td>57</td>
<td>50</td>
<td>42</td>
<td>15</td>
</tr>
<tr>
<td>WCTS</td>
<td>11</td>
<td>16</td>
<td>15</td>
<td>10</td>
<td>14</td>
<td>9</td>
<td>20</td>
<td>4</td>
</tr>
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<td>20</td>
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<td>29</td>
<td>34</td>
<td>33</td>
<td>36</td>
<td>35</td>
<td>17</td>
</tr>
<tr>
<td>Mokapu, Hawai'i</td>
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<td>7</td>
<td>7</td>
<td>7</td>
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<td>7</td>
</tr>
<tr>
<td>Niah Cave, Mesolithic</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Niah Cave, Neolithic</td>
<td>6</td>
<td>8</td>
<td>17</td>
<td>12</td>
<td>15</td>
<td>16</td>
<td>18</td>
<td>16</td>
</tr>
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<td>Niah Cave, Modern</td>
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<td>118</td>
<td>148</td>
<td>152</td>
<td>97</td>
<td>151</td>
<td>118</td>
</tr>
</tbody>
</table>

Table 4.11 Sample sizes of crown width for mandibular teeth

The points of agreement between NJ and SAHN dendrograms were assessed using the Consens subroutine of NTSYSPC 2.0. The Consens subroutine (Figure 4.22) in NTSYSpc 2.02 was also used to compare tree plots in order to evaluate if there is no
common structure between the different distance measures (Nei72 vs. Balakrishnan & Sanghvi’s B²).

Cedric A. B. Smith’s Mean measures of divergence (MMD) were also calculated for discontinuous (nonmetric) traits (Konigsberg 2006). It is often assumed that MMD provides a good measure of degrees of phenetic similarity in dental traits (Guatelli-Steinberg et al. 2001, Irish 2000). However, MMD is vulnerable to small sample sizes (Harris and Sjøvold 2004). Despite this problem, MMD remains a standard metric in dental anthropology (Turner and Eder 2006) and thus is used alongside other techniques in the present study.

Results

The intra-observer errors for both discrete (Appendix V) and metric (Appendix VI) traits indicate good agreement (Table 4.12 for Spearman’s test of intra-observer control in nonmetric traits)⁴. Less agreement was observed in shoveling for mandibular lateral incisors (0.4198) as well as in double shoveling for maxillary central incisors (0.5914) and mandibular central incisors (0.4485). These phenomena could result from small sample sizes (equal to or less than 15) for mandibular incisors. However, small sample size cannot explain the unexpected poor agreement for maxillary central incisors (n = 23). The average precisions of crown width are almost identical between the 1st and the 2nd measurements (Table 4.13). If the intra-observer errors for dental observations or measurements of one tooth were statistically significantly different, then the values for that trait or measurement would not be included.

---

⁴ No error control study was conducted for traits such as metaconule, protostylid, and cusps 6, which were not present in any tooth or only was present in only one tooth.
Nonmetric Traits

Among non-metric traits, all observable SPC individuals (n = 30) had shoveling on the central or/and lateral incisor(s). 93.1% of the observable SPC individuals (n = 29) had double-shoveling on the central or/and lateral incisors. A quarter of the individuals from SPC in whom the trait could be scored (n = 24) had two roots on the first premolar.

<table>
<thead>
<tr>
<th>Shoveling</th>
<th>Double Shoveling</th>
</tr>
</thead>
<tbody>
<tr>
<td>I¹</td>
<td>I²</td>
</tr>
<tr>
<td>Spearman's Test</td>
<td>0.7135</td>
</tr>
</tbody>
</table>

Carabelli's Cusp

<table>
<thead>
<tr>
<th>Hypocone</th>
<th>Cusp 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>M¹</td>
<td>M²</td>
</tr>
<tr>
<td>Spearman's Test</td>
<td>0.723</td>
</tr>
</tbody>
</table>

Table 4.12 Results for Spearman’s tests

For maxillary teeth, almost two thirds (62.96% of n = 27) of the individuals at SPC in whom the trait could be scored had a hypocone on their maxillary molars. One (4.76% of n = 21) has a metaconule on the 1st molar (J16 II B3). Only three SPC individuals out of 28 (10.71%) in whom the feature could be scored had the Carabelli’s trait (Figure 4.14), in which the highest frequencies are in European (75-85% of individuals), then African, Asians and Native Americans, and rarest in Pacific Islands (35-45%) (Kolakowski et al. 1980, Scott 1980).

For mandibular teeth, none (0 of n = 25) had a protostylid, one (4.55% of n = 22) of the individuals at SPC in whom the trait could be observed had a cusp 6 (H16 II B11), and one (4.71% of n = 24) had a cusp 7 (H15 II B8). Ten out of seventeen (58.82%) individuals had a cusp 5. Eighty-four percent (21 out of n = 25) of the SPC people had an enamel extension (Figure 4.15) or enamel pearl on one or more maxillary and mandibular molars.
Metric Traits

Between zero to three teeth were excluded as outliers for each type of tooth from the measurements (crown width) of the SPC, WCTS, and NKLE sites after z-scores with a threshold of 2.5 were applied (Tables 4.14-4.15). T-test results showed that the crown width of the SPC and NKLE people were not significantly different, with the exception of lateral incisor, which could not be adequately tested because of the small sample size (n=1) from the NKLE.

<table>
<thead>
<tr>
<th>Maxillary</th>
<th>I1</th>
<th>I2</th>
<th>C</th>
<th>Pm3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision</td>
<td>n average</td>
<td>n average</td>
<td>n average</td>
<td>n average</td>
</tr>
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<td>27 0.9963</td>
<td>41 1.0021</td>
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<th>M2r</th>
<th>M3</th>
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<td>n average</td>
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<tr>
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<td>30 1.0067</td>
<td>27 0.9996</td>
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<tr>
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<tr>
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<td>10 1.0065</td>
<td>12 1.0006</td>
<td>6 0.9953</td>
<td>2 1.0078</td>
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<table>
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<td>n average</td>
<td>n average</td>
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<tr>
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<td>43 1.0064</td>
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<tr>
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<td>6 1.0033</td>
<td>11 0.9835</td>
<td>17 0.9970</td>
</tr>
<tr>
<td>WCTS</td>
<td>11 0.9981</td>
<td>16 0.9974</td>
<td>15 1.0087</td>
<td>11 0.9946</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Maxillary</th>
<th>Pm4</th>
<th>M1</th>
<th>M2r</th>
<th>M3</th>
</tr>
</thead>
<tbody>
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<td>Precision</td>
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<td>n average</td>
<td>n average</td>
<td>n average</td>
</tr>
<tr>
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<td>26 0.9966</td>
<td>13 1.0010</td>
</tr>
<tr>
<td>NKLE</td>
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<td>19 0.9983</td>
<td>16 0.9865</td>
<td>2 1.0178</td>
</tr>
<tr>
<td>WCTS</td>
<td>14 1.0074</td>
<td>9 0.9972</td>
<td>9 0.9908</td>
<td>4 1.0109</td>
</tr>
</tbody>
</table>

Table 4.13 Average precision of each type of tooth for SPC, NKLE, and WCTS sites
The tests indicated that significant differences exist in the crown width of the maxillary canine (SPC/NKLE vs. WCTS), 1st premolar (SPC/NKLE vs. WCTS), and 2nd premolar (SPC vs. WCTS), mandibular central incisor (NKLE vs. WCTS), lateral incisor (SPC vs. WCTS), canine (SPC vs. WCTS), 2nd premolar (SPC vs. WCTS), 2nd molar (SPC vs. WCTS), and 3rd molar (SPC vs. WCTS). Most of these differences separate SPC/NKLE (as one group) from the WCTS people. As a result, after the tests, the SPC samples were pooled with the NKLE samples, but separated from the WCTS samples (Tables 4.16–4.17).

<table>
<thead>
<tr>
<th>Number</th>
<th>I²</th>
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<th>C</th>
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<td>2</td>
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<td>14</td>
<td>16</td>
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</table>

Table 4.14 Numbers of teeth for T-test for maxillary teeth from each site.

<table>
<thead>
<tr>
<th>Number</th>
<th>I²</th>
<th>I²</th>
<th>C</th>
<th>Pm²</th>
<th>Pm²</th>
<th>M²</th>
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<tbody>
<tr>
<td>SPC</td>
<td>26</td>
<td>30</td>
<td>40</td>
<td>43</td>
<td>38</td>
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<td>26</td>
<td>13</td>
</tr>
<tr>
<td>WCTS</td>
<td>11</td>
<td>16</td>
<td>15</td>
<td>10</td>
<td>14</td>
<td>9</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>NKLE</td>
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<td>6</td>
<td>11</td>
<td>17</td>
<td>19</td>
<td>18</td>
<td>16</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 4.15 Numbers of teeth for T-test for mandibular teeth from each site.

Population Comparisons

Populations were compared by Factor Analyses, using factor extraction by Principal Component Analysis (PCA). Table 4.18 presents the variances explained by the first six components (a total of 15 components). Over 99% of the variance was explained by the first two components for both maxillary and mandibular crown width. Among population
compared, WCTS, Japanese, Ainu, and Namu have smaller maxillary crown widths according to their principle component scored (all have first PC scores < 0.98).

MDS plots from distance matrixes generated from maxillary and mandibular crown width (Figures 4.16-4.17) produce two different pictures of the SPC affinities: the former suggests that populations of Tonga and modern Niah Cave are closely related to the SPC and NKLE people, but later states that the SPC-NKLE people are most related to Japanese and the Australian Aborigines.

The NJ trees constructed by NEI72 and Balakrishnan & Sanghvi’s B² from the maxillary and mandibular nonmetric traits reveal a certain degree of similarities. The trees form from both NEI72 (Figure 20) and Balakrishnan & Sanghvi’s B² distances based on maxillary discrete traits shows a close relationship between the SPC-NKLE group and the Shih-Sang-Hong (SSH) people, followed by Japanese, and then Pima. China-Mongolia (CM) and Recent Japanese (RJ) also form a sub-cluster, showing their close affinities, as noted previously by Matsumura (2006). The Ainu sample was close to the SPC-NKLE and SSH group in the output of Nei72, but not in the Balakrishnan & Sanghvi’s B² distances.

<table>
<thead>
<tr>
<th>T-test</th>
<th>I₁</th>
<th>I₂</th>
<th>C</th>
<th>Pm₁</th>
<th>Pm₂</th>
<th>M₁</th>
<th>M₂</th>
<th>M₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPC: WCTS</td>
<td>0.548</td>
<td>0.100</td>
<td>0.006</td>
<td>0.001</td>
<td>0.002</td>
<td>0.451</td>
<td>0.639</td>
<td>0.804</td>
</tr>
<tr>
<td>SPC: NKLE</td>
<td>0.533</td>
<td>—</td>
<td>0.814</td>
<td>0.805</td>
<td>0.324</td>
<td>0.931</td>
<td>0.076</td>
<td>0.197</td>
</tr>
<tr>
<td>WCTS: NKLE</td>
<td>0.850</td>
<td>—</td>
<td>0.030</td>
<td>0.031</td>
<td>0.117</td>
<td>0.575</td>
<td>0.225</td>
<td>0.668</td>
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Table 4.16 T-test results for maxillary teeth.

<table>
<thead>
<tr>
<th>T-test</th>
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<th>I₂</th>
<th>C</th>
<th>Pm₁</th>
<th>Pm₂</th>
<th>M₁</th>
<th>M₂</th>
<th>M₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPC: WCTS</td>
<td>0.137</td>
<td>0.020</td>
<td>0.000</td>
<td>0.244</td>
<td>0.038</td>
<td>0.173</td>
<td>0.028</td>
<td>0.046</td>
</tr>
<tr>
<td>SPC: NKLE</td>
<td>0.137</td>
<td>0.950</td>
<td>0.097</td>
<td>0.343</td>
<td>0.311</td>
<td>0.391</td>
<td>0.954</td>
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</tr>
<tr>
<td>WCTS: NKLE</td>
<td>0.033</td>
<td>0.147</td>
<td>0.190</td>
<td>0.180</td>
<td>0.325</td>
<td>0.093</td>
<td>0.058</td>
<td>0.105</td>
</tr>
</tbody>
</table>

Table 4.17 T-test results for mandibular teeth.
SAHN was also applied to visualize the biological affinities between these populations. In the SAHN tree, SPC was grouped together with SSH/Japanese/Ainu/Pima and with Eastern Polynesian (EP)/Oahu/Hawai‘i and CM and RJ based on Nei72 distances (Figure 4.20) for discrete traits. However, SPC was an outgroup when Balakrishnan & Sanghvi’s B² distances were used for discrete traits in the SAHN tree subroutine (Figure 4.21).

In the present analysis, it appears that the NJ trees are more reliable than SAHN clusters. The NJ trees appear to be more reliable because they correctly represent relationships visible in univariate frequency data. For example, the SPC people tend to have generally Sinodont dental morphology as demonstrated by a 100% frequency of incisor shoveling and a high frequency (84%) of enamel extension. NJ trees cluster SPC
with other Sinodont populations but the SAHN trees do not reliably do so. Why the NJ trees appear to be more reliable in this analysis than SAHN trees is uncertain, but it may well have to do with unequal evolutionary rates or markedly different population sizes in the groups entered into the analysis as OTUs. It is possible, for example, that the ancestors of the SPC people had a small population size and had drifted relatively rapidly.

The SAHN cluster of Balankrishan & Sanghvi’s $B^2$ distance appears to be the least reliable of the trees. It shows that the SPC people are very distance from all other populations, including another ancient Taiwanese population, the Shih-San-Hang people. However, in that tree, the other clusters such as CM-RJ and SSH-Japanese are still consistent with the trees constructed by NJ of Nei 72 and Balankrishan & Sanghvi’s $B^2$ distance and SAHN clustering of Nei 72 distances.

The Consens subroutine (Figure 4.22) in NTSYSpc 2.02 was also used to compare tree plots and it shows there are four major clusters. The first cluster was formed by the SPC/SSH/Japanese. The SPC samples were clustered with the SSH samples, and then sub-clustered with the Japanese samples. The second cluster consisted of Eskimo, North and South American Indian (NSAI), Northeast Siberia (NS), and American Arctic (AA). The third cluster is South African/New Guinean/ Melanesians, each with equal distance. The components for the last cluster are CM and RJ. In brief, it seems to indicate that the SPC and SSH peoples distinctly resemble Japanese (Sinodonts). The results of the Consens clustering subroutine should be taken with caution because, for the reasons mentioned above, the NJ trees are likely to be more reliable than the SAHN clusters. The Consens subroutine merely displays relationships that are present in 100% of the trees it
summaries. Nevertheless, the few clusters that merge in this subroutine are consistent with aforementioned nonmetric results.

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<th>Extraction Sums of Squared Loadings</th>
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<td>.071</td>
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<tr>
<td>6</td>
<td>.003</td>
<td>.021</td>
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Table 4.18 Factor Analyses for crown width.

The MMD results from 5 traits (shoveling on maxillary central incisor, Carabelli’s cusp, 3-cusped maxillary 2nd molar, 4-cusped mandibular 1st molar and 4-cusped mandibular 2nd molar) of 17 populations was not applied in this study because all MMD values between the SPC people and other 16 populations are all negative, which may indicate small sample sizes of the SPC samples (n = 13-28). However, MMD results from 4 traits (shoveling on maxillary central incisor, Carabelli’s cusp, cusp 6 on the
mandibular 1st molar, and cusp 7 on the mandibular 1st molar) of 24 populations suggest the sample sizes are adequate. Seven out of 24 comparative populations have positive results (n for SPC = 19-28) between the SPC people and other 23 populations, including SPC to Recent Japan (0.1339), then to China-Mongolia (0.2531), Pima (0.6789), North and South American Indian (0.8547), Northeast Siberia (1.1307), South Siberia (1.4751), and Eskimo (1.7696). Because sample sizes seems not to be the only factor here (Eskimo only have samples sizes from 21 to 30, but North and South American Indians have sample sizes from 1368 to 2756), it is suggested that the SPC people were most likely to represent Sinodonty in dental morphology, at least with respect to the traits characteristic of Sinodonty (especially incisor shoveling) that could be scored for this study.

**Simulation Test**

A limitation of this study is the overall sample size, especially for nonmetric traits. This issue has been emphasized by MMD values shown in this study and in which some MMDs had negative values. Although negative values could be adjusted and re-set to zero on a trait-by-trait basis, it is not recommended because this creates another problem of over-estimation of the population differences (Harris and Sjøvold 2004). A randomization test (sample size = 100, bootstrap = 100) was therefore applied to test the effect by a small sample size equivalent to that of SPC.

In this test, four traits (shoveling on maxillary central incisors, Carabelli’s cusp, Cusp 7 on the mandibular 1st molar, and Cusp 6 on the mandibular 1st molar) were chosen because data for them could be obtained for 24 populations and thus they form the basis for the MMDs evaluated in this study. Next, 100 individuals per trait, with each trait
assigned a frequency matching that observed at SPC (100% for shoveling, 10% for Carabelli’s cusp, 5% for Cusp 7, and 0% for Cusp 6) were separately set up and then randomly chosen for 100 times. Each time, the same numbers of individuals were randomly selected without replacement from the 100 simulated individuals to produce draws of 19, 28, 20, and 19 observations (in order to match the sample sizes for the teeth of SPC) for shoveling, Carabelli’s Cusp, Cusp 7, and Cusp 6, respectively.

Figure 4.16 Multidimensional Scaling (PROXISCAL) of maxillary crown width.

The resampling protocol produced one mode for shoveling (all present at 100%) and Cusp 6 (all absent), six modes for Carabelli’s Cusp (absent to five out of 28 with Carabelli’s Cusp), and five modes for Cusp7 (absent to four out of 28 with Carabelli’s Cusp). From these results, thirty combinations (Table 4.19) were produced using four
traits (shoveling, Carabelli’s cusp, Cusp 6, and Cusp 7). Four out of 30 combinations (13.44%) exactly resemble the SPC frequencies for all traits. On a trait-by-trait basis, for shoveling incisor 100% of the samples of 19 individuals had a 100% frequency of shoveling, for Carabelli’s Cusp 32% of the samples of 28 individuals had a 10.7% frequency of Carabelli’s Cusp, for Cusp 7 42% of the samples of 20 samples had a 5% frequency of Cusp 7, and for Cusp 6 100% of the samples of 19 individuals had a 100% frequency of Cusp 6.

Figure 4.17 Multidimensional Scaling (PROXISCAL) of mandibular crown width

An attempt to assess the impact of other traits that distinguish Sinodonts from Sundadonts was applied to Table 4.19. These samples always have 100% incisor shoveling, which suggests a Sinodont affinity. All the resampled groups have a 0%
frequency of Cusp 6. Generally, Cusp 6 has a frequency between 30-60% in both Sinodonts and Sundadonts; South Siberians (a Sinodont population) are an exception (Turner and Scott 1996). The SPC dentations are, therefore, unusual relative to both the “average” Sinodont and Sundadont population in the low frequency of Cusp 6. In the simulation, 96.03% of the samples had Cusp 7 frequency between 0-15% and Carabelli’s frequency between 3.71-17.86%. Neither of these traits discriminates well between Sinodonts and Sundadonts. The other traits that distinguish Sinodonts from Sundadonts (winging on maxillary central incisors, 1-rooted 1st maxillary premolar, and 3-rooted 1st mandibular molar (Turner and Scott 1996)) were not simulated, in part due to a lack of data for some or many of the comparative populations. Nevertheless, the overall impression that the SPC samples resemble Sinodonts, albeit with an unusually low frequency of Cusp 6, seem to be amply supported.

Discussions and Conclusion

Many studies have suggested that genes are a major controlling factor in tooth development and dental morphology (e.g., Biggerstaff 1979; Dixon and Steward 1976; Gabriel 1948; Garn 1977; Kraus and Furr 1953; Kraus 1957; Krogman 1960; Moorrees 1962; Nakata 1983; Osborne 1963, 1967; Tobias 1955; Townsend et al. 1994; Witkop 1960), while environmental factors influence trait expression to some extent (Scott and Turner 1997). The later is especially true for traits expressed within the same field, such as shoveling at maxillary lateral incisors (Scott et al. 1997). Therefore, this study incorporates crown width (continuous) and nonmetric (discrete) traits, both of which
reflect the genetic background of dental morphology and to avoid potential environmental noise.

Although the SPC sample is from the same time period of the WCTS samples and almost 2000 years younger than the NKLE samples, it is interesting to observe the SPC sample clusters with the NKLE samples. Additionally, crown widths of the SPC-NKLE cluster are consistent larger than those of the WCTS samples except for mandibular central incisors.

Brace and others (e.g., Brace 1967, Brace and Mahler 1971) have described worldwide trends in dental reduction that began as soon as people obtained pottery and have continued to present. This trend raises new questions: why would shrinkage in tooth size have acted on the WCTS samples but not on the SPC samples, given that both sites date to the same cultural complex, the Ta-Hu Culture (2,800-2,000 BP)? If they are indeed synchronous, do they are in fact sample two spatial frames (one earlier and one later), or perhaps do they just represent two different ethnic groups or at least people of partially different biological origin? If they sample two populations, does it mean that the WCTS people were newcomers who borrowed cultural elements of the Ta-Hu Culture from nearby regions such as the SPC site? It is conceivable that the differences in crown width found between SPC and WCTS are due to more males in the sample from SPC, but the disparity between the sex ratios would have to be large. Of the skeletons whose sex could be estimated with confidence at SPC ,there are 13 females and 5 males while at WCTS the numbers are 11 females and 3 males; the other skeletons are of ambiguous sex or juvenile. These numbers do not lend support to the notion of unequal sex ratios between the sites (females are overrepresented in both sites to a roughly similar degree). It is
more likely that the difference in tooth size indicates a biological difference, but this and other questions can only be resolved in future studies.

In terms of biological affinities, Principal Component Analysis of mandibular crown width and genetic matrixes (especially the NJ trees, which seems to be the most reliable for these data) from dental nonmetric traits tend to tell a similar story that the SPC-NKLE people are closely related to Sinodont populations such as Japanese. Surprisingly, the SPC-NKLE cluster is also highly correlated to the SSH people, who were considered to be biologically related to Polynesians (Sundadonts) because some have an atypical Rocking jaw (which is common in Polynesians) and also share some cranial traits (Chang 1993). Additionally, the multidimensional scaling of maxillary crown width reveals biological closeness between the SPC-NKLE cluster with populations of Tonga and modern Niah Cave. Does this potentially imply biological admixture for the group of the SPC-NKLE people? Future study of cranial and infra-cranial morphology will help to answer this question.

In brief, the dentition of the SPC people tends to be large in size as well as clearly Sinodont, or at least similar to Northeast Asians, in morphology. These features seem to indicate the SPC people have little to do with Austronesian speakers in Polynesia and suggest the probable importance of a Melanesian origin or admixture for modern Polynesians. However, the SPC people might still have provided a few contributions to the gene pool of the Polynesians if one considers maxillary crown size and a few similarities in nonmetric traits. As Hanihara (2008) noted, “the complex population history of East/Northeast Asian rather than the simple Southeast Asia model of East/Northeast Asia origin.” Therefore, multifaceted models, such as a Triple-I model,
may also be necessary to explain the unexpectedly complex biological mosaic known so far in prehistoric Taiwan and any influence the island’s aboriginal inhabitants may have had on the spread of Austronesian speakers into the Pacific.
Figure 4.18. Neighbor-Joining tree Nei 72 distance based on nonmetric traits, NSAI: North and South American Indian; NES: Northeast Siberia; AA: American Arctic; CM: China-Mongolia; RJ: Recent Japan; KH: Khosian; SA: South Africa; NG: New Guinea; ML: Melanesian; AU: Australia; MI: Micronesia; SEE: Southeast Asia (Early); SER: Southeast Asia (Recent); PO: Polynesian; SS: South Siberia.
Figure 4.19 Neighbor-Joining tree of B&S distance from nonmetric traits (abbreviation as shown in Figure 4.16).
Figure 4.20 SAHN cluster of by Nei72 distances of nonmetric traits (abbreviation as shown in Figure 4.16).
Figure 4.21 SAHN cluster of B & S distances based on nonmetric traits (abbreviation as shown in Figure 4.16).
Figure 4.22 Comparisons between B & S and Neighbor-Joining (METHOD: STRICT) (abbreviation as shown in Figure 4.16).
all with present for shoveling on maxillary central incisor (n=19) and absent for cusp 6 on mandibular 1st molar (n=19)

<table>
<thead>
<tr>
<th>Cusp 7 (n=20)</th>
<th>38% (0 out of 20)</th>
<th>42% (1 out of 20)</th>
<th>14% (2 out of 20)</th>
<th>5% (3 out of 20)</th>
<th>1% (4 out of 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carabelli’s cusp (n=28)</td>
<td>3% (0 out of 28)</td>
<td>1.14%</td>
<td>1.26%</td>
<td>0.42%</td>
<td>0.15%</td>
</tr>
<tr>
<td></td>
<td>8% (1 out of 28 = 3.71%)</td>
<td>3.04%</td>
<td>3.36%</td>
<td>1.12%</td>
<td>0.40%</td>
</tr>
<tr>
<td></td>
<td>26% (2 out of 28 = 7.14%)</td>
<td>9.88%</td>
<td>10.92%</td>
<td>3.64%</td>
<td>1.30%</td>
</tr>
<tr>
<td></td>
<td>32% (3 out of 28 = 10.71%)</td>
<td>12.16%</td>
<td>13.44%</td>
<td>4.48%</td>
<td>1.60%</td>
</tr>
<tr>
<td></td>
<td>18% (4 out of 28 = 14.29%)</td>
<td>6.84%</td>
<td>7.56%</td>
<td>2.52%</td>
<td>0.90%</td>
</tr>
<tr>
<td></td>
<td>13% (5 out of 28 = 17.86%)</td>
<td>4.94%</td>
<td>5.46%</td>
<td>1.82%</td>
<td>0.65%</td>
</tr>
</tbody>
</table>

Table 4.19 Randomization test for 4 traits of 24 populations (n = 100, bootstrap = 200).

In this test, four traits (including shoveling on maxillary central incisors, Carabelli’s cusp, Cusp 7 on the mandibular 1st molar, and Cusp 6 on the mandibular 1st molar) were chosen. One hundred individuals per trait, in which each trait had a frequency that matched SPC were separately set up and then were randomly drawn for 100 times. Each time, the same numbers of individuals were independently sampled without replacement from 100 individuals to produce draws of 19, 28, 20, and 19 individuals (in order to match the sample sizes for the teeth of SPC) for shoveling, Carabelli’s Cusp, Cusp 7, and Cusp 6, respectively. Each column in this table represents the chance to get a specific combination of crown traits. For instance, the chance to get a combination of teeth without shoveling, cusp 6, cusp 7, and Carabelli’s is 1.14% as shown in the left, upper corner (the first column). Finally, these traits do not suffice to characterize populations as either Sinodont or Sundadont, although Sinodonts tend to have a high frequency of shoveled incisors, like the people from SPC.
Chapter 5: Mitochondrial Diversity and Maternal Ancestry in the San-Pau-Chu People

Because of a higher copy number per cell, it is easier to recover mitochondrial DNA (mtDNA) information from ancient remains than the nuclear DNA (e.g., Binladen et al. 2006, Poinar et al. 2003, Stone and Stoneking 1998). In addition, mtDNA has a maternal inheritance pattern and the lack of recombination, which facilitate population studies. Another advantage of using mtDNA is that it has been widely studied in various modern populations around the world (e.g., Chen et al. 2007, Friedlander 2007, Hagelberg 1993, Hagelberg et al. 1994, Kivisild 2002, Merriwether et al. 2005, Lum and Trejaut et al. 2005; Stone and Stoneking 1998, Yan 2006, Zheng 2004).

A variety of genetic studies of mtDNA in Taiwan indigenous peoples have been conducted in the past decade (e.g., Sykes et al. 1995, Lin et al. 2000, Lin et al. 2005, Tajima et al. 2003, Trejaut et al. 2005, Yan 2006, Zhang 2004), and they have identified substantial haplogroup frequency variation. For instance, 80% of the people from populations in north and central Taiwan (Atayal, Saiaiat, and Bunun) belong to mtDNA haplogroups B4b, B5a2, E, F4b, and M7b; populations from north-central Taiwan (Tsou and Saisiat) are highest in B5a2; 72.2% of the members of populations from south and southeast Taiwan are characterized by haplogroups B4a, D5, F3b, M7c, and N9a; Amis and three southernmost populations of indigenous Taiwan are restricted to D5 lineages (Trejaut et al. 2005). Additionally, Tajima and colleagues (2003) examined mtDNA mutations characteristic of the “Polynesian Motif” which are located at nucleotides 16217, 16247, and 16261 (Melton et al. 1995, Redd et al. 1995) in indigenous Taiwanese and they found that they carry one (T16217C in cluster C7) or two (T16217C and C16261T
in C8) “Polynesian Motif” mutations, but the A16247G mutation has not been observed among these peoples.

The objective of this chapter is to evaluate genetic relationships between prehistoric Taiwanese (the San-Pau-Chu people), modern indigenous Taiwanese, Austronesian-speaking populations, and populations around the world. The relationships between these populations will shed light on the affinities of the San-Pau-Chu (SPC) people and on the place of Taiwan in Austronesian migrations. The hypothesis to be tested is that the SPC people have polymorphisms at nucleotides 16217, 16247, and 16261 in the hypervariable region 1 as well as medium to high frequencies (30% or more) of the 9 base pair deletion. Asian ancestral haplogroups (such as A and M) and characteristic haplogroups of indigenous Taiwanese (such as B, D, F, M, and R9) will also be analyzed in order to understand the role of the SPC in Taiwanese prehistory and to see if Taiwan was indeed the first stop for Proto-Austronesian speaking populations. In brief, samples from the SPC site in the Tainan Science-Based Industrial Park of southwest Taiwan were collected. Markers (Figure 5.1) for mtDNA haplogroups A (HaeIII 663+/00663G), B (the 9 base pair deletion), C (Hinc II 13259-), D (Alu I 5176-), F (Hinc II/Hpa I 12406-), H (Alu I 7025-), and M (DdeI 10394/AluI 10397+) as well as sequences of the hypervariable region I (HV1) were applied.

Materials

A total of 108 samples, including 23 long bones and 85 teeth, were collected from 41 individuals (samples from 18 out of 41 individuals were collected twice) for ancient DNA analyses. Initially, 23 long bone samples from 23 different individuals were
collected for the pilot study, and later 1-4 tooth samples per individual were collected from 37 different individuals, including 18 previously sampled individuals and 19 new individuals from the San–Pau-Chu (SPC) site at the Tainan Prefecture in southwest Taiwan. Published (Table 5.1) and unpublished (Hunley and Healy) (Table 5.2) mtDNA data derived from sizable populations (n>20) were used for comparisons.

Laboratory Methods

The preparation of ancient DNA (aDNA) samples prior to analysis includes grinding the tooth root or long bone into fine powder, extracting DNA from the powder, applying
PCR to amplify DNA, using NuSieve agarose to visualize the DNA, and then examining restriction sites, or length variants, or alternatively cloning the DNA, and then sequencing the DNA. All extractions and pre-PCR steps were done in dedicated aDNA facilities, including aDNA laboratories at the University of New Mexico (UNM) (2001-2003, 2006 June-December), Arizona State University (ASU) (March-May 2007), and Mackay Memorial Hospital (July-November 2007).

With regard to dedicated facilities at UNM, ASU, and Mackay Memorial Hospital, their ancient DNA laboratories are all built in a separated building from where PCRs and post-PCR procedures are conducted. There are filters on air-in and air-out on the windows and doors, and UV lights are always on while no one is in the laboratory at UNM. There are class 10,000 Itepa filters and positive air pressure and UV light is always on at ASU. There is positive air pressure, while UV light is set up for certain time periods at Mackay Memorial Hospital. There are also three separate rooms for bone grinding, extraction, and pre-PCR at the Mackay Memorial Hospital.

A rotary tool was used to remove the outer surfaces of bone fragments from the skeletons (Stone et al. 1996). Teeth were soaked in hydrochloride for 30 seconds and then washed by double distilled H₂O for several seconds prior to removal of the root in order to reduce the possibility of modern contamination from previous handling. A bone mill or freezer mill (with liquid nitrogen) was used to grind the bone or tooth root into a fine powder. DNA then was extracted from approximately 0.25 g of bone or tooth root using the silica and guanidine thiocyanate extraction protocol (Hoss et al. 1993). Bone grinding and extractions for this study had been done in the ancient DNA laboratories at either UNM or ASU. None has been conducted at the Mackay Memorial Hospital.
<table>
<thead>
<tr>
<th>Population, Region</th>
<th>n</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ami, Taiwan</td>
<td>30</td>
<td>Sykes et al. 1995, Tajima et al. 2003</td>
</tr>
<tr>
<td>Atayal, Taiwan</td>
<td>21</td>
<td>Sykes et al. 1995, Tajima et al. 2003</td>
</tr>
<tr>
<td>Bunun, Taiwan</td>
<td>28</td>
<td>Sykes et al. 1995, Tajima et al. 2003</td>
</tr>
<tr>
<td>Cook Is., Polynesia</td>
<td>79</td>
<td>Skyes et al. 1995</td>
</tr>
<tr>
<td>Filippino, Philippines</td>
<td>37</td>
<td>Sykes et al. 1995</td>
</tr>
<tr>
<td>Han Chinese, Taiwan</td>
<td>59</td>
<td>Torroni et al. 1993, Horai et al. 1996</td>
</tr>
<tr>
<td>Indonesians, Indonesia</td>
<td>69</td>
<td>Lum et al. 1994, Redd et al. 1995, Sykes et al. 1995</td>
</tr>
<tr>
<td>Mongolian, China</td>
<td>103</td>
<td>Kolman et al. 1996</td>
</tr>
<tr>
<td>Papua New Guinea, Melanesia</td>
<td>54</td>
<td>Sykes et al. 1995, Redd et al. 1995</td>
</tr>
<tr>
<td>Puyuma, Taiwan</td>
<td>20</td>
<td>Tajima et al. 2003</td>
</tr>
<tr>
<td>Rukai, Taiwan</td>
<td>20</td>
<td>Tajima et al. 2003</td>
</tr>
<tr>
<td>Shan Dong, China</td>
<td>24</td>
<td>Oota et al. 1999</td>
</tr>
<tr>
<td>Samoa, Polynesia</td>
<td>51</td>
<td>Skyes et al. 1995</td>
</tr>
<tr>
<td>Tsuo, Taiwan</td>
<td>20</td>
<td>Tajima et al. 2003</td>
</tr>
<tr>
<td>Vanuata, Polynesia</td>
<td>51</td>
<td>Sykes et al. 1995</td>
</tr>
<tr>
<td>Yami, Taiwan</td>
<td>20</td>
<td>Tajima et al. 2003</td>
</tr>
</tbody>
</table>

Table 5.1 Comparative Populations of Modern Asian and Pacific Islander.

MtDNA loci examined included the Hae III restriction site at nucleotide 663+ (characteristic for haplogroup A), the 9-bp deletion (characteristic for haplogroup B), Hinc II restriction site at 13529- (characteristic for haplogroup C), Alu I restriction site at 5176- (characteristic for haplogroup D), Hinc II restriction site at 12406- (characteristic for haplogroup F), the Alu restriction site at I 7025- (characteristic for haplogroup H), the Dde I restriction site at 10394+/Alu I restriction site at 10397+ (characteristic for haplogroup M), and hyper-variable region I (HV1). In this regard, haplogroups A (00663G) and M (DdeI 10394/AluI 10397+) were also tested because they are believed to be Asian ancestral haplogroups (Ballinger et al. 1992, Merriwether et al. 1994, Macaulay et al. 1999). MtDNA HV1 (mutations at nucleotides 16217, 16247, and 16261) and 9-bp deletion (a 9-bp direct repeat sequence CCCCCTCTA) at COII/tRNA\(^{ts}\) were firstly targeted to identify the “Polynesian Motif” (Melton et al. 1995, Redd et al. 1995).
order to test if there was any ancient DNA for each SPC individual, haplogroups C and H were also tested. Although B, D, E, F, R9, M are all characteristic haplogroups in modern indigenous Taiwanese according to Trejaut and colleagues (Treajaut et al. 2005), haplogroups E and R9 were not tested in order to conserve ancient DNA (aDNA).

<table>
<thead>
<tr>
<th>Population, Geographic origin</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balochi, Pakistan</td>
<td>24</td>
</tr>
<tr>
<td>Bedouin, Israel (Negev)</td>
<td>46</td>
</tr>
<tr>
<td>Biaka Pygmies, Central African Republic</td>
<td>28</td>
</tr>
<tr>
<td>Brahui, Pakistan</td>
<td>25</td>
</tr>
<tr>
<td>Burusho, Pakistan</td>
<td>25</td>
</tr>
<tr>
<td>Druze, Israel (Carmel)</td>
<td>42</td>
</tr>
<tr>
<td>French, France</td>
<td>27</td>
</tr>
<tr>
<td>French Basque, France</td>
<td>24</td>
</tr>
<tr>
<td>Han, China</td>
<td>44</td>
</tr>
<tr>
<td>Hazara, Pakistan</td>
<td>22</td>
</tr>
<tr>
<td>Japanese, Japan</td>
<td>29</td>
</tr>
<tr>
<td>Kalash, Pakistan</td>
<td>24</td>
</tr>
<tr>
<td>Makrani, Pakistan</td>
<td>25</td>
</tr>
<tr>
<td>Mandenka, Senegal</td>
<td>21</td>
</tr>
<tr>
<td>Maya, Mexico</td>
<td>21</td>
</tr>
<tr>
<td>Mozabite, Algeria</td>
<td>29</td>
</tr>
<tr>
<td>Palestinian, Central Israel</td>
<td>46</td>
</tr>
<tr>
<td>Pathan, Pakistan</td>
<td>24</td>
</tr>
<tr>
<td>Russian, Russia</td>
<td>25</td>
</tr>
<tr>
<td>Sardinian, Italy</td>
<td>28</td>
</tr>
<tr>
<td>Sindhi, Pakistan</td>
<td>25</td>
</tr>
<tr>
<td>Yakut, Siberia</td>
<td>24</td>
</tr>
<tr>
<td>Yoruba, Negeria</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 5.2 Comparative populations (Hunley and Healy, unpublished data).

Each aDNA mtDNA PCR protocol (Table 5.3 lists all primers used in the present study; Table 5.4 for protocol content) consisted of a standard PCR with the addition of bovine serum albumin, and forty cycles were used to amplify DNA. Amplification
products were then visualized by electrophoresis with ethidium bromide in 2.8% NuSieve agarose gels (Stone et al. 1996). Additionally, if amplification was successful with a

<table>
<thead>
<tr>
<th>RFLP</th>
<th>Primers</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hae III 663+</td>
<td>L635</td>
<td>5'-TGAAATGTCTAGCAGGCTCACATC</td>
<td>Handt et al. 1996</td>
</tr>
<tr>
<td></td>
<td>H708</td>
<td>5'-TAGAGGGTGAACCTACTGGAAC</td>
<td>Handt et al. 1996</td>
</tr>
<tr>
<td>COII/trn A</td>
<td>L8215</td>
<td>5'-ACAGTTTCATGCCATCGTC</td>
<td>Wrischink et al. 1987</td>
</tr>
<tr>
<td>Lys 9-bp deletion</td>
<td>H8297</td>
<td>5'-ATGCTAAGTTAGCTTTACAG</td>
<td>Wrischink et al. 1987</td>
</tr>
<tr>
<td>Hinc II 13259</td>
<td>L13527</td>
<td>5'-AATCGTAGCCTCTCCACTTCA</td>
<td>Handt et al. 1996</td>
</tr>
<tr>
<td></td>
<td>H13393</td>
<td>5'-TCCTATTTTTCCAATATCTGTTC</td>
<td>Ward et al. 1991</td>
</tr>
<tr>
<td>Alu 5176-</td>
<td>L5127</td>
<td>5'-ACTACCAGACCCATACTACTCA</td>
<td>Handt et al. 1996</td>
</tr>
<tr>
<td></td>
<td>H5189</td>
<td>5'-GGTTGGATGAATTAGGGGTG</td>
<td>Handt et al. 1996</td>
</tr>
<tr>
<td>Hinc II 12406-</td>
<td>L12368</td>
<td>5'-CCCTGACTCTCCCTAACATCC</td>
<td>Mooeder et al. 2006</td>
</tr>
<tr>
<td></td>
<td>H12473</td>
<td>5'-TGTTGTTGGGAGAGACTGA</td>
<td>Mooeder et al. 2006</td>
</tr>
<tr>
<td>Alu 7025-</td>
<td>L6991</td>
<td>5'-TAGACATCGTACTACACGAC</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>H7058</td>
<td>5'-AGAAGCGTCCGTACCCGCAC</td>
<td>This study</td>
</tr>
<tr>
<td>DdeI 10394+</td>
<td>L10361</td>
<td>5'-TCTGGCCTATGAGTGACTACA</td>
<td>Mooeder et al. 2006</td>
</tr>
<tr>
<td>Alu 10397+</td>
<td>H10458</td>
<td>5'-TGAGGGGCATTTGGTAATATG</td>
<td>Mooeder et al. 2006</td>
</tr>
<tr>
<td>DdeI 10394+</td>
<td>L10342</td>
<td>5'-TCATCATCCTAGCCCTAAGT</td>
<td>This study</td>
</tr>
<tr>
<td>Alu 10397+</td>
<td>H10471</td>
<td>5'-ATTATGTAATAGGGGCA</td>
<td>This study</td>
</tr>
<tr>
<td>HV1 - 1</td>
<td>L16055</td>
<td>5'-GAAGCGATTTGGGTACC</td>
<td>Handt et al. 1996</td>
</tr>
<tr>
<td></td>
<td>H16142</td>
<td>5'-ATGTACCTAGGTGGGTCAAG</td>
<td>Stone et al. 1998</td>
</tr>
<tr>
<td>HV1 - 2</td>
<td>L16131</td>
<td>5'-CACCAGGATATTGTACGCT</td>
<td>Handt et al. 1996</td>
</tr>
<tr>
<td></td>
<td>H16218</td>
<td>5'-TGTGTGATAGTGAGGGGTG</td>
<td>Handt et al. 1996</td>
</tr>
<tr>
<td>HV1 - 3</td>
<td>L16209</td>
<td>5'-CCCCATGCTTACAAGCAAGT</td>
<td>Handt et al. 1996</td>
</tr>
<tr>
<td></td>
<td>H16303</td>
<td>5'-TGGCTTTATGTACTATGT</td>
<td>Handt et al. 1996</td>
</tr>
<tr>
<td>HV1 - 4</td>
<td>L16287</td>
<td>5'-CACTTAGGATACAAACAACC</td>
<td>Handt et al. 1996</td>
</tr>
<tr>
<td></td>
<td>H16356</td>
<td>5'-GTCATCCATGGGGGAGGAA</td>
<td>Handt et al. 1996</td>
</tr>
<tr>
<td>HV1 - 5</td>
<td>L16347</td>
<td>5'-CGTACATAGGACATTACGT</td>
<td>Handt et al. 1996</td>
</tr>
<tr>
<td></td>
<td>H16410</td>
<td>5'-GCGGGATATTGATTTACAGG</td>
<td>Handt et al. 1996</td>
</tr>
</tbody>
</table>

Table 5.3 Primers used in this study.
positive PCR result, the bands were cut and placed in 100-150 ul of ddH2O for re-amplifying.

<table>
<thead>
<tr>
<th>Master Mix</th>
<th>Vol/Rxn (ul)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10x buffer (gold)</td>
<td>5.0</td>
</tr>
<tr>
<td>10 mM dNTPs</td>
<td>2.0</td>
</tr>
<tr>
<td>20 mg/ul BSA</td>
<td>2.0</td>
</tr>
<tr>
<td>25 mM MgCl2</td>
<td>4.0</td>
</tr>
<tr>
<td>20 uM primers</td>
<td>0.5 each</td>
</tr>
<tr>
<td>Taq (gold)</td>
<td>0.5</td>
</tr>
<tr>
<td>H2O</td>
<td>30.5</td>
</tr>
<tr>
<td>Total MM</td>
<td>45.0</td>
</tr>
<tr>
<td>DNA</td>
<td>5.0</td>
</tr>
<tr>
<td>Total rxn</td>
<td>50.0</td>
</tr>
</tbody>
</table>

Table 5.4 Ancient DNA mtDNA PCR protocol in this study.

Five short, overlapping segments of HV1 were amplified and then sequenced. If PCR products of aDNA mtDNA HV1 were observed, then these products were subject to purification (SAP/EXO protocol), cloning (TOPO TA Chemical Cloning Kit) (Invitrogen Life Technologies version R, 4 April 2008), and sequencing, after a re-amplification using 25 cycles at a higher annealing temperature (56°C). The purpose of cloning was to test whether there was any contamination, indicated by the presence of multiple different sequences. Markers for haplogroups A, B, C, D, F, H, and M were digested with the appropriate restriction enzyme and visualized using a 2.0% agarose gel.

With respect to independent laboratory analyses, Independent extractions of 14 individual samples were conducted by Dr. Cecil Lewis Jr. at the University of Oklahoma. These individuals were G11 II B1, G16 II B3, G18 II B2, H16 II B8, H17 II B3, H17 II B5, J16 II B3, J17 II B3, J17 II B4, J17 II B5, K16 II B1, K16 II B2, K17 II B1, and K17 II B2. Additionally, these samples were all long bones such as metacarpals instead of
teeth. The salting out extraction protocol (Miller et al. 1988) was applied using one to two grams of ground sample. DNA and blank extractions were purified and concentrated using Wizard prep SV columns (Promega). For all samples, the 9-bp deletion was used to assess human DNA preservation except for two samples (the 9-bp deletion as well as Hae III, Hinc II, and Alu I primers were used for G18 II B2 and K17 II B1). Table 5.5 shows the primers that were designed for quantitative PCR, rather than traditional gel electrophoresis.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>mt-8261F</td>
<td>ACCCTATAGCACCCCCTCTA</td>
</tr>
<tr>
<td>mt-8355R</td>
<td>AGAGGTGTTGGTCTCTTTAAT</td>
</tr>
</tbody>
</table>

Table 5.5 Primers for quantitative PCR used in the independent extraction by Dr. Lewis.

**Analytical Methods**

Sequenced HV1 data were presented in ABI files, and both directions were checked using Seqman (Lasergene, © 2007 DNASTAR). Population genetic distances were estimated using dnadist.exe and use to construct a neighbor-joining (NJ) tree with neighbor.exe in Phylip (Felsenstein 2006). Neither the estimation of neither population structure nor comparison for the frequencies of haplogroups A, B, C, D, F, H, and M was applied because of the small sample sizes of the SPC people.

**Results**

A total of forty-one individuals (108 human skeletal samples) from the SPC site were tested (Table 5.6) and six out of these forty-one (14.63%) individuals (F11 II B2,
G11 II B1, G18 II B1, H17 II B4, and I14 II B2) provided neither mtDNA HV1 nor positive haplogroup marker PCR results. The success rate for DNA extraction using teeth was much higher than using bones. In the total of 48 successful reactions (23 from 4 segments of the HV1 and 25 from restriction enzymes for haplogroups A, B, C, D, F, H, and M), 35 (72.92%) of them are from tooth powder and only 13 (27.08%) are from bone powder.

<table>
<thead>
<tr>
<th>Loci Analyzed</th>
<th>Sample Size/ND</th>
<th>Amplified (+, -, DNW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hae III+ (A)</td>
<td>41/10</td>
<td>18 (2, 16, 13)</td>
</tr>
<tr>
<td>9-bp deletion (B)</td>
<td>41/6</td>
<td>12 (0, 13, 22)</td>
</tr>
<tr>
<td>Hinc II 13259- (C)</td>
<td>34/20</td>
<td>4 (2, 2, 10)</td>
</tr>
<tr>
<td>Alu I 5176- (D)</td>
<td>30/15</td>
<td>2 (0, 2, 13)</td>
</tr>
<tr>
<td>Hinc II/Hpal I 12406- (F)</td>
<td>23/19</td>
<td>0 (0, 0, 4)</td>
</tr>
<tr>
<td>Alu I 7025- (H)</td>
<td>29/25</td>
<td>4 (1, 3, 0)</td>
</tr>
<tr>
<td>Dde I 10394+/Alu I 10397+ (M)</td>
<td>36/16</td>
<td>2 (1, 1, 18)</td>
</tr>
<tr>
<td>HV1 16055-16142</td>
<td>38/21</td>
<td>0 (0, 0, 17)</td>
</tr>
<tr>
<td>HV1 16131-16218, G11 II B4: cloned</td>
<td>38/18</td>
<td>5 (5, 0, 15)</td>
</tr>
<tr>
<td>HV1 16209-16303, G17 II B1: cloned</td>
<td>38/2</td>
<td>11 (11, 0, 25)</td>
</tr>
<tr>
<td>HV1 16287-16356, H16 II B6: cloned</td>
<td>38/4</td>
<td>10 (10, 0, 24)</td>
</tr>
<tr>
<td>HV1 16347-16410, H17 II B1: cloned</td>
<td>37/14</td>
<td>2 (2, 0, 21)</td>
</tr>
</tbody>
</table>

Table 5.6 List of Amplification Results of the SPC Samples. (ND meaning “not done” if that marker was not tested in that sample.) “+” or “-” indicating that the reaction for that specific sample did work or did not cut at that restriction site. DNW meaning “did not work.”).

Nucleotides 16209-16303 in the HV1 region have the highest success rate for amplification and sequencing (28.95% of n = 38), then nucleotides 16287-16356 (26.32% of n = 38), nucleotides 16131-16218 (13.16% of n = 38), and nucleotides 16347-16410 (5.40% of n = 37) (Table 5.7). No samples were successfully amplified for nucleotides 16055-16142 (0 of n = 38). K17 II B1 is the only one that could not be amplified for any segment of HV1; however, the amplification of the haplogroup C maker (Hinc II 13259-)

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for this individual was successful. Furthermore, 62.86% (of n= 35) of the collected SPC samples were amplified and sequenced at least one segment of the HV1, but no SPC sample had sufficient DNA preservation allowing the amplification and sequencing of the entire mtDNA HV1 region.

<table>
<thead>
<tr>
<th>assigned haplogroup</th>
<th>HV1 fragment</th>
<th>From</th>
<th>To</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>G11 II B4</td>
<td>M</td>
<td>2</td>
<td>16112 - 16237</td>
<td>125</td>
</tr>
<tr>
<td>G18 II B2</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G18 II B4</td>
<td>H</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G16 II B3</td>
<td>A</td>
<td>3</td>
<td>16189 - 16279</td>
<td>90</td>
</tr>
<tr>
<td>I15 II B1</td>
<td>A</td>
<td>4</td>
<td>16268 - 16363</td>
<td>95</td>
</tr>
<tr>
<td>K17 II B1</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5.7 Individual Samples with assigned haplogroup markers by restriction analysis only.

Most of the recovered sequence data are short fragments (2 with 50 bps, 9 with 65-95 bps, and 5 with 102-127 bps) for HV1, although in 22 out of 35 individuals (62.86%) one or two segments of HV1 were successfully amplified (Appendix IV). However, these 22 sequences represent only a small portion of HV1 (e.g., Chang 2005, Hill et al. 2006, Kivisild et al. 2002, Pierson et al. 2006, Mooder et al. 2006). Therefore, it is difficult either to assign one haplogroup from HV1 sequence data or to test the overall population structure. Only 6 sequences/individuals (G17 II B1 – 138 bps, H15 II B8 – 133 bps, H16 II B6 – 178 bps, H16 II B8 – 176 bps, H17 II B3 – 211 bps, and J17 II B4 – 154 bps) (Table 5.8) were selected for distance analysis because of the length of HV1 sequences. A neighbor-joining tree (Figure 5.2), which was generated using these six individual sequences by Phylip (Felsenstein 2006), suggests that the San-Pau-Chu people and Japanese are closely related to each other and separated from other populations.
Finally, there are several mutational site differences between the reference sequence and the researcher’s personal sequence for HV1 sequencing data, but none of these were found in any of the SPC sample sequences. Sample G11 II B4 is the only one with the same macro-haplogroup as the researcher’s (M7b). On the other hand, haplogroup H for Sample G18 II B4 is unexpected. Could this be contamination because the laboratory works on G18 II B4 was done during the stay at the Arizona State University?

Unfortunately, not enough of the sample remains for duplicated extractions of each individual in the current study because extraction(s) from the power of one tooth/bone were not sufficient to get HV1 sequence as well as haplogroup data most of the time.

<table>
<thead>
<tr>
<th>Sample Name (fragment/s of HV1)</th>
<th>From</th>
<th>To</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>F11 II B3 (3)</td>
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</tr>
<tr>
<td>G11 II B4 (2)</td>
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<td>125</td>
</tr>
<tr>
<td>G12 II B4 (4)</td>
<td>16273</td>
<td>16375</td>
<td>102</td>
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<tr>
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<td>16375</td>
<td>106</td>
</tr>
<tr>
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<td>G17 II B10 (3)</td>
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<td>176</td>
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<tr>
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<td>16267</td>
<td>16317</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 5.8 SPC individuals with successful amplifications of HV1 fragments.
Using a restriction site or 9-bp deletion alone, only six (G16 II B3 & I15 II B1 to A, G18 II B2 & K17 II B1 to C, G18 II B4 to H, and G11 II B4 to M) out of 41 individuals (15% of n = 41) were able to be assigned to a specific haplogroup. Additionally, haplogroup A (Hae III+) has the highest success rate (43.90% of n = 41), and haplogroup D has the lowest success rate (6.67% of n = 30). For the other haplogroups, the success rates were as follows: B (29.27 of n = 41), F (17.39% of n = 23), C (11.76% of n = 34), M (8.33% of n = 36), and H (6.90% of n = 29), respectively.

Only the bone samples of G18 II B2 and K17 II B1 (out of 14 individuals) were amplified with a positive PCR result (DNA was not retrieved from any other bone sample) for the Hinc II site, and these results indicate poor aDNA preservational conditions for these bone samples, which is consistent with this study. Specifically, whereas I was able to amplify DNA for the Hinc II 13259- from sample G18 II B2, Dr. Lewis was not. His result showed the presence of this Hinc II site. However, the results for K17 II B1 from both Dr. Lewis and this study show the absence of the Hinc II site. None of other loci (other than Hinc II) was successfully amplified for either these two or other samples.

**Discussions and Conclusions**

Although six SPC individuals (out of n = 41) were assigned to specific haplogroups (G16 II B3 & I15 II B1 to A, G18 II B2 & K17 II B1 to C, G18 II B4 to H, and G11 II B4 to M) using solely haplogroup markers, these results only indicate that the SPC people are related to other Asians (83.33% of n = 6), which is also supported by the neighbor-Joining tree constructed using Phylip. However, the relationships between the SPC, modern Taiwan indigenous people, and other peoples in the Pacific region remain
unanswered. Additionally, none of the twelve successfully amplified individuals has the 9-bp deletion, which is one of the characteristic genetic traits for “Polynesian Motif” (Melton et al. 1995; Redd et al. 1995) and generally present in modern Taiwanese aborigines (Tajima et al. 2003).

The most frequent substitution for the SPC people is the C to T at nucleotide 16261 which is hypervariable (found in various, such as haplogroups D5 and E1b)(Trejaut et al. 2005, Friedlaender et al. 2007): 31.82% (of n = 22) have this transition, 22.73% have no mutation, and 50% have no sequence data for this site. Interestingly, this substitution is the second specific change for the “Polynesian Motif.” It was observed in four SPC individuals (H15 II B8, H16 II B8, H17 II B3, and J17 II B3). Additionally, these four individuals also have mutations (C to T) at nucleotides 16266, 16278, and 16294, which are not typical mutations in Polynesians.

The SPC site is an open site and its overall ancient DNA amplification with positive PCR results is low: the success rate for the entire HV1 region was 0% (out of n = 41) and the success rate for haplogroup determination was likely 14.63% (6 out of n = 41) (in the condition for a lack of multiple extractions). In this regard, Yan (2006) only use 3 dental roots from the Hui-Lai-Lee site in the central-west Taiwan and her results indicate only one sample (33.33%) with a positive PCR amplification. Zheng’s study (2004), on the other hand, only stated that either tooth roots or bones were used from the Wu-Shan-Tou site in the southwest Taiwan and did not specify which samples are bone samples and which samples are from tooth roots (aDNA study of R. exulans for PCR amplification success rate on open sites in the Pacific region see Robin et al. 2001). In brief, these
results may indicate the ancient DNA preservation in western Taiwan is poor, even while bone preservation itself is in excellent/good condition such as that of SPC.

Finally, the limitations of this study are as following, 1) the preservation of ancient DNA at SPC is poor in general as the results show in the current study and from attempts to extract and amplify DNA independently by Dr. Cecil Lewis at the University of Oklahoma. Poor preservation of ancient mtDNA, potential contamination, or a combination of factors resulted in a different result from G18 II B2. For this sample, Dr. Lewis’ independent analysis suggested it did not belong to haplogroup C as indicated by the current study. 2) The sequence data are too short and the data are not sufficient to check whether HV1 sequences and haplogroup markers match each other. 3) The sample size is small and the ancient DNA preservational conditions at the SPC site are insufficient to determine the biological affinities of this people by using ancient DNA alone. Future studies using a larger sample size and independent laboratory analyses will be required to answer questions of genetic diversity and maternal ancestry in the SPC people.
Figure 5.2 A Neighbor-Joining tree by HV1 for worldwide population.
Chapter 6: Summary of the Evidence for the Biological Affinities of the San-Pau-Chu People

Human diversity can be influenced by various factors, such as migrations, admixture, language, and culture. This variation is evident in the Pacific islanders, even though a large number of them are classified linguistically as Austronesian speakers. This diversity is also evident in Taiwan and indigenous Taiwanese, who are also Austronesian-speaking populations. There was no written record for this island until few hundred years ago; it is generally assumed that prehistoric sites in Taiwan were left behind by ancestors of modern indigenous Taiwanese (Chang and Ward 1996).

The Tainan Science-Based Industrial Park is located at the Tainan Prefecture and archaeological sites discovered in this Park represent a microcosm of Taiwan’s prehistory. Up to November 2007, thirty-one archaeology sites, which include 17 Taiwanese prehistoric cultural complexes, have been excavated in this park (Tsang et al. 2007). Skeletal analyses and artifact studies of these sites may unveil secrets of this island’s prehistory back to 4,800 years ago. Three archaeological sites were chosen in this study because they contain comparatively large and well-preserved samples of human skeletal remains: the San-Pau-Chu (SPC), Wu-Chen-Tzu South (WCTS), and Nan-Kong Li East (NKLE) sites. The focus is on the SPC people because this site has the largest available sample size, are in the best condition, and, out of the three sites, its skeletons are least likely to be contaminated by modern DNA.

This research is the first study in Taiwan that combines both morphological and genetic evidence to understand the biological nature of one prehistoric population. The underlying significance of this research is that it demonstrates the Asian origins and
affinities of the SPC people as well as suggesting a possible, but enigmatic, European affinity for at least one individual based on ancient DNA. A study combining analysis of dental data and ancient DNA also provides direct biological evidence and avoids the uncertainty that arises from using more recent Taiwanese samples that have been affected by recent population admixture between aborigines and Han Chinese.

Methods

To access the biological relationships between the ancient Taiwanese samples (SPC, NKLE, and WCTS) as well as between those samples and modern populations from the surrounding Pacific area, this research examined molecular and dental morphological data from the prehistoric archaeological sites in Taiwan. Morphological data comprised measures of tooth crown width and nonmetric traits, listed in the Arizona State University Dental System. Molecular data included assays for the markers for maternally inherited mitochondrial DNA (mtDNA) haplogroups A (HaeIII 663+/00663G), B (the 9 base pair deletion), C (Hic II 13259-), D (Alu I 5176-), F (Hinc II /Hpa I 12406-), H (Alu I 7025-), and M (DdeI 10394/AluI 10397+) as well as sequences of the hypervariable region I (HV1) of maternally inherited mitochondrial DNA.

Materials

After inter-group tests between crown width of the SPC, NKLE, and WCTS samples, SPC and NKLE samples were grouped together because their teeth were not significantly different in size. WCTS forms a group by itself. Therefore, 30-60 individuals were included (the numbers vary by trait), excluding the 3rd molars, for comparison for the
SPC-NKLE group, while approximately 10-15 persons were available for the WCTS group.

For the SPC dental sample, approximately 10-15 individuals per tooth, excluding M3, were sufficiently well preserved to be scored per nonmetric trait. However, by dental field (i.e. maxillary incisors, maxillary canines, maxillary premolars, maxillary molars, mandibular incisors, mandibular canines, mandibular premolars, and mandibular molars), approximately 21-30 teeth per trait could be scored for the SPC sample.

For the ancient DNA analysis, a total of 108 SPC samples, including 23 long bones and 85 teeth, were collected from 41 individuals. Sequence data obtained from these remains were compared to published and unpublished data. Markers included the maternally inherited mitochondrial DNA (mtDNA) haplogroups A (HaeIII 663+/00663G), B (the 9 base pair deletion), C (Hic II 13259-), D (Alu I 5176-), F (Hinc II /Hpa I 12406-), H (Alu I 7025-), and M (DdeI 10394/AluI 10397+) as well as sequences of the hypervariable region I (HV1). Partial sequence results were obtained from 22 individuals and positive haplogroup results were obtained from six individuals.

**Results**

In terms of dental morphological traits, the SPC sample showed an affinity to northeastern Asian (sinodont) dentitions, although not all features diagnostic of Sinodonty as defined by Turner were included in this study. Over 90% (of n=30) shoveling and double-shoveling (of n=29) were observed in the SPC samples. Additionally, substantial frequency of enamel extension on molars, moderate numbers of hypocone (62.93% of n = 27) on maxillary molars and of cusp 5 (58.82% of n=17) on
mandibular molars, marginally elevated numbers of 2-rooted 1st premolars (15% of n=24) and of Carabelli’s cusp (10.71% of n = 28) on maxillary 1st molar, low frequencies of metaconule (4.76% of n=21) on maxillary 1st molar, of cusp 6 (4.55% of n=22) on mandibular molars, and of cusp 7 (4.74% of n=24) on mandibular molars, and absence of protostylid on mandibular molar were also observed.

In brief, the frequencies of incisor shoveling and 1-root 1st premolar agree with Sinodonty, which has characteristic traits including winged (inward-rotated) maxillary central incisors, shovel-shaped central incisors, 1-rooted maxillary 3rd premolars, and 3-rooted mandibular molars. Genetic matrixes (Neighbor-Joining trees, SAHN trees, and Consens subroutines in NTSYS-pc) also suggest closer relationship between the SPC-NKLE cluster, Japanese, and Native Americans. Additionally, Mean Measures of Divergence (MMD) from maxillary central incisors, Carabelli’s cusp on maxillary 1st molar as well as Cusps 6 and 7 on mandibular 1st molars seems to indicate an agreement with the Sinodont dental pattern. To sum up, the analytical results of the SPC discrete dental traits differ from previously published studies of indigenous Taiwanese dentitions, which are regarded as having a more generalized, Australoid morphology and having a longer ancestry than populations of the derived, Sinodont morphology.

Interestingly, although the mandibular crown widths suggest the SPC-NKLE cluster is closer to the Japanese (a Sinodont population) using multidimensional scaling, the maxillary crown width of the SPC-NKLE cluster is most closely related to populations from Tonga and modern Niah Cave, which seems to suggest a more generalized, or at least larger, dentition. Therefore, this phenomenon suggests that perhaps gene flow over a
longer period existed between indigenous Taiwanese, Island Southeast Asian, and Oceanians.

For the study of ancient DNA, only six individuals (G17 II B1 – 138 bps, H15 II B8 – 133 bps, H16 II B6 – 178 bps, H16 II B8 – 176 bps, H17 II B3 – 211 bps, and J17 II B4 – 154 bps) were used for distance analysis because of the longer length of the preserved portions of their HV1 sequences and it reveals a close relationship between the SPC sample and Japanese. However, this result should be taken with extreme cautions given the tiny sample size and sequence length, and the fact the ancient DNA results for SPC could not be independently confirmed. None (0 of n=12) of the SPC sample has the 9-bp deletion, a typical haplogroup for Polynesians. Although most of the positive PCR re-amplifications show negative reactions for restriction analyses of A, B, C, D, F, H, and M, six individuals were assigned to A (n=2), C (n=2), H (n=1), and M (n=1).

Additionally, the rate of successful DNA extraction using teeth was much higher than that by using bones, although they are mostly fragments in terms of successful amplification of the entire HV1 sequence.

In conclusion, the dental evidence in this project seems to suggest a Northern Asian affinity for the SPC people, which is unexpected and varies from previously proposed models of Austronesian dispersals. The ancient DNA evidence is, unfortunately, too poor to clearly support or refute the result from dental analysis. The dental results differ from any of original hypotheses of this project for the role of Taiwan in Austronesian migrations. Interestingly, however, the dental results accord with result from the Hui-Lei-Lee site that the M9a haplotype recovered from the site is most likely of Northern Asian origin (Yan 2006). This does not exclude the possibility that the SPC people are related to
Austronesian speakers in the South Pacific. It is evident that there may have been some level of gene flow between the SPC people, mainland Asian, and Oceania according to the sizes of maxillary crown width (which explains the similarity to Tonga) and perhaps the presence of mitochondrial haplogroups A and M (ancestral Asian haplogroups).

Finally, contamination is always a major concern for an ancient DNA study. However, the researcher’s personal sequence and assigned haplogroup do not match any individual from the SPC sample and dedicated aDNA facilities were used for this study. Unfortunately, results from 14 bone samples sent to another laboratory (Dr. Lewis at the University of Oklahoma) were not successfully replicated either. A problem for multi-discipline research such as this study is that there are inconsistencies between populations analyzed. Because available archaeological sites and well-preserved human skeletons from these sites are unpredictable and limited, population samples used by skeletal biologists and molecular anthropologists are different. Increased sample sizes within and among sites and compatible modern populations are two important factors for future multi-disciplinary studies.

**Discussion and Conclusion**

Although the SPC and WCTS people were from the same time period (around 2,500 BP), the crown width of the SPC people could be grouped with the NKLE people (around 4,800-4,200 BP), who occupied a location that is geographically closer to the SPC site, instead of with the WCTS people. This observation, and the finding of a largely Sinodont pattern of dental morphology in the SPC people, raises many additional questions. Will this pattern occur throughout in this Park or in additional prehistoric sites in Taiwan?
Does it mean that spatial distances are less affected by population affinities than by time differences? Will further pooling data and increased sample sizes tell us a different story than the SPC-Japanese affinity? Can traits, such as Carabelli’s cusp, which are less characteristic of East Asian populations, be observed at elevated frequency in elsewhere of Taiwan? Are observed traits in the SPC people unique?

Because the dental morphological study and ancient DNA analyses seem to suggest a Northern Asian affinity for the SPC people, it is proposed here that approximately 2,500 BP, some prehistoric Taiwanese came from Northern Asia. However, the WCTS people, contemporaries of SPC, show a closer relatedness with the Namu from the Hawai‘i. Therefore, a simple model of “Out of Taiwan” or “Indigenous Melanesian Origin” cannot explain the whole picture of prehistoric Taiwan. In this circumstance, it seems to indicate that Taiwan in the past may have harbored diverse populations. If so, one cannot simply assume that contemporary aboriginal Taiwanese are a reasonable representation of the people who were involved in the initial Austronesian expansion. Likewise, it does not rule out that Taiwan was the origin of Austronesian speakers. It is also possible that the San-Pau-Chu people did not belong to the Austronesian speakers who migrated into Oceania or that there were extensive population admixtures before the Austronesian speakers of Taiwan, including the San-Pau-Chu people, moved to Oceania. If Taiwan is indeed an origin, a multi-faceted model must be considered.

**Avenues for Future Research**

The results of this project illustrate the need for additional, future studies of skeletal materials from Taiwanese archaeological site. First, with respect to ancient DNA
studies, tooth samples (72.92% of n = 35) tend to have a much better preservational condition than that of bones (27.08% of n = 13) from the SPC site (in the sense of getting positive amplification results rather than getting the entire sequences of HV1). It is highly recommended to use tooth samples instead of bone samples for future ancient DNA work in Taiwan. Additionally, since the general preservation of ancient mitochondrial DNA seems to be poor (the entire HV1 segment could not be amplified in any of the individuals and the haplotype could be determined in only 14.63% of the individuals who preserved some DNA), it may be better to conduct tests only with restriction fragment length polymorphisms (haplogroup data). However, sequencing along with restriction enzymes’ tests will further confirm the haplogroup determination and may help to address the issue concerning the short fragment of the total sequence for each individual as shown in this project. Tests on diagnostic loci for haplogroups B, D, E, F, M, and R9 are highly recommend because these are now known to be common in indigenous Taiwanese (Tiejaut et al. 2005). Only assays for diagnostic restriction fragment length polymorphisms on haplogroups A, B, C, D, F, H, and M were conducted in this project because less was known about the genetics of indigenous Taiwanese when this research was designed. Tests for haplogroup A, which is an ancestral Asian haplogroup, remain worthwhile.

In terms of dental morphological studies, studies with larger samples sizes and from a wider range of archaeological sites will help to shed light on the issue regarding affinities between ancient Taiwanese and other (prehistoric and contemporary) Asians, especially populations from Northern Asia and South Pacific. Other skeletal measurements, such as those used in Peitrusewaky’s studies (2005) of crania, might be
also informative. No artificial and cultural cranial deformation was seen in the SPC, WCTS, and NKLE sites. However, at least some taphonomic distortion of the crania is expected, but metric studies of these crania would be useful. Lastly, study of historic crania of indigenous Taiwanese housed at National Taiwan University may help to clarify the place of Taiwan in Austronesian dispersals. These crania are generally well preserved and derive from indigenous people who were killed as a result of conflict between the mountain tribes and the Japanese government during the period of Japanese colonization in the early 19th century.
Appendix I: Standard of Nonmetric Traits (1 as presence, 0 as absence)

Shoveling/incisors
0. None: lingual surface is essentially flat.
1. Presence: very slight elevation to strong development of mesial and distal aspects of lingual surface.

Double Shoveling/incisors
0. None: labial surface is smooth.
1. Presence: mesial and distal ridging can be easily seen to very prominent.

Hypocone/upper molars
0. None: without distolingual cusp or cusp 4. Site is smooth.
1. Presence: faint ridging to very large cusp present.

Carabelli’s Cusp/upper 1st molar
0. None: mesiolingual aspect of cusp 1 is smooth.
1. Presence: groove to a large free cusp is present on the lingual surface of the mesiolingual cusp (cusp 1)

Cusp 5/upper molars
0. None: without metaconule or cusp 5 in the distal fovea between the metacone and hypocone. Site is smooth.
1. Presence: faint cuspule to medium-sized cusp present

Protostylid/lower molars
0. None: buccal surface is smooth.
1. Presence: a pit to a cusp with a free apex is present.

Cusp 5/lower molars
0. None: the distal occlusal aspect is smooth. Molar has only 4 cusps. Only be recorded in the absence of cusp 6.
1. Presence: very small to very large cusp 5 is present.

Cusp 6/lower molars
0. None: absent.
1. Presence: a small to much large cusp in the distal fovea lingual to cusp 5.

Cusp 7/lower molars
0. None: absent.
1. Presence: faint cusp to large cusp in the lingual groove between cusps 2 and 4.

Enamel Extensions
0. None: enamel border is straight or rarely curved toward the crown.
1. Presence: a faint, approximately 1.00 mm long extension projecting to a lengthy
extension, generally larger than 4.00 mm extension projecting toward and along the root is present.
**Appendix II: Crown length and height of the SPC samples**

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<td></td>
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<tr>
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### Appendix IV: Crown width (mixture of males and females)


#### Part I: Maxillary tooth

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## Appendix V: Non-metric (discrete) Traits

### The SPC Site

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### Groove Pattern (right maxillary molars)

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Appendix VI: Metric (continent) Traits

The SPC Site

*Mesiodistal diameter for (left/right) maxillary teeth*

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224
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APPENDIX VIII: Permission Letters

To Whom It May Concern:

This is to testify that, I, the director of National Museum of Prehistory and of the Project for rescuing the archaeological sites in Tainan Science-based Industrial Park, would like to give permission to Miss Hsin-Man Lin to use selected samples of human teeth and bones excavated from the San-Pan-Cha and the Wu-Chen-Tsa South at Hsin-shih, Tainan county, Taiwan, for conducting amino acid racemization test and ancient DNA analyses.

Truly Yours,

Cheng-Hwa Tsang, Ph.D.

Director of National Museum of Prehistory

December 31, 2003
This is to certify that the bone samples carried by Miss Lin Hsiu-man were archaeological samples excavated by the Rescue Archaeology Project from the archaeological sites in the Tainan Science-based Industrious Park and will be brought to the laboratory of the Department of Anthropology, University of New Mexico, USA for ancient DNA analysis.

Chang-hwa Tsang, Ph.D.
Project Director
APPENDIX VIII: Copyrights
@ for photos cited in this study are available in Chinese only. The following pages are
permissions from Dr. Cheng-Hwa Tsang (the author of "The Archaeology of Taiwan"),
Council for Cultural Affairs, Academia Sinica, the National Museum of Prehistory,
Administrative Bureau of the Kenting National Park, and National Taiwan University.

Institute of History and Philology
Academia Sinica
128 Yuanchuyuan Road, Sec. 2
Taipei
Taiwan
Tel.: +886-2-27829555
Fax: +886-2-27365834

17 January 2008

To Whom It May Concern:

This is to testify that I, as the authors of "The Archaeology of Taiwan" and
"The Jomon of Prehistoric Peoples" would like to give permission to Ms.
Hsia-Man Lin to use selected figures, pictures, maps, and/or photos for citations
of her dissertation.

Truly Yours,

Cheng-Hwa Tsang
Research Fellow
Division of Archaeology
台北市文献委员会函

地址：台北市大同区承德路2段
33號7樓
承辦人：張先生
電話：02-2556-4966轉30
傳真：02-2550-1064
電子信箱：cc-
hou@mail.taipei.gov.tw

受文者：國立臺灣史前文化博物館

發文日期：中華民國96年12月11日
發文字號：北市獻編字第09630353100號

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副本：

96.12.12

臺史前館字第 3889 號

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國立台灣大學人類學系 教授兼主任

中華民國九十七年六月二十五日
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Robert Bollt, Tony Djubiantono, Bion Griffin, Michiko Intoh, Emile Keane, Patrick Kirch, Kuang-Ti Li, Michael Morwood, Lolita M. Pedriña, Philip J. Piper, Ryan J. Rabett, Peter Shooter, Gert Van den Bergh, Eric West, Stephen Wickler, Jing Yuan, Alan Cooper, and Keith Dobney (2007) Phylogeny and ancient DNA of Sus provides insights into neolithic expansion in Island Southeast Asia and Oceania. PNAS 104: 4834-4839


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Chapter 3
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http://www.dmtip.gov.tw/ (“Digital Museum of Taiwan Indigenous Peoples” is a digital database, which has been set up by the National Museum of Prehistory)

Chapter 5
http://infinity.gen.emory.edu/mitomap.html for Mitomap
http://db.eva.mp.d/Hvrbase/ for HvrBase
http://www.megasoftware.net for Mega 2.0
http://anthro.unige.ch/arlequin/ for Arlequin 2.0
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http://cmpg.unibe.ch/software/simcoal/ for SimCoal