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RECONSTRUCTING POPULATION HISTORY FROM PAST PEOPLES USING ANCIENT DNA AND HISTORIC RECORDS ANALYSIS: THE UPPER CANADIAN PIONEERS AND LAND RESOURCES

By

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ABSTRACT

It has been argued that the real anthropological potential of ancient DNA has yet to be realized (Stoneking 1995). Ancient DNA Research can only become truly anthropological when it is integrated holistically through a multidisciplinary approach within the bio-cultural framework. Reconstructions of past societies by definition necessitates the synthesis of other sources of culturally relevant information.

Attempts to interpret Upper Canadian pioneer population history from the ancient DNA recovered from two historic cemeteries (the nineteenth-century St Thomas' Anglican Church cemetery, Belleville, Ontario, and the Farewell Family Cemetery on Harmony Road, Oshawa, Ontario) revealed that there were a number of possible evolutionary explanations for the observed pattern in both nuclear and mitochondrial DNA data. The reconstruction of past sociocultural variables to facilitate further interpretation relied on the collection of scholarly historic research, primary records analysis, and archaeological theory and observations. Through this analysis it was shown that conclusions regarding past population history could not be drawn from any single source of information.

It was possible to observe the intragenerational and intergenerational kinship alliances influenced by a land resource stress through the establishment of a social context and an interment chronology. This finding provides strong empirical evidence in support of the Saxe (1970) and Goldstein (1976) theory which predicts the presence of a vital resource pressure when kinship structure is hypothesized in archaeological mortuary practice. While this theory may have use in broader archaeological contexts, it is maintained that its application can only be evaluated through a multidisciplinary approach involving ancient DNA and other relevant cultural evidence.

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To all of you, may our paths continue to wander together on occasion.

Sincerely,

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"Our real heritage is cut from a genetic cloth and it is this rich tapestry of interweaving threads of genetic descent that we must evaluate." (Jacquard and Ward 1976:139)

"From great-grandparents to great-grandchildren we are only knots in a string" (Naskapi-Cree saying, Speck 1935:245 as cited by Oberholtzer 1993)

Chapter 1: Introduction

Waldron (1991:156) has discussed the need for, "...some serious consideration to be given to determining how best we can use this [ancient DNA] knowledge to increase our understanding of human societies in the past." Stoneking (1995:1261) has similarly argued that,"...for the real anthropological potential of ancient DNA to be realized, we need to see more studies analyzing the sorts of questions that anthropologists are interested in". Inquiries of this type typically involve human population evolution as a result of: mutation, natural selection, genetic drift, and gene flow. Conclusions only become truly anthropological when these population histories are integrated holistically within a bio-cultural framework, a perspective that has been overlooked in ancient DNA (aDNA) research and under-utilized in anthropological genetics.

The testing of population history hypotheses from aDNA data requires the analysis of samples from archaeologically defined discrete peoples. Unfortunately, the vast majority of research with aDNA typically involves single remains (for example see Krings et al. 1997, Handt et al. 1994b, Pääbo 1985, Stone and Stoneking 1993) or only a handful of individuals, in many cases scattered across time and space

(for example see Hagelberg and Clegg 1993, Handt et al. 1996, Hauswirth et al. 1994, Horai et al. 1989, Kurosaki et al. 1993, Rogan and Salvo 1994, Tuross 1994). While these studies have advanced the sub-discipline, it is now time to break away from the methodological virtuosity of amplifying aDNA and integrate results into the broader scope of anthropology.

The major theme of this study involves the concept that human population history cannot be accurately or reliably tested using only the aDNA analyzed from small numbers of skeletons from archaeological collections. Reconstructions of past societies by definition necessitates the integration of other sources of culturally relevant information. Through a logical process of consilience, one is able to compare and contrast all available evidence in order to construct conclusions which have more confidence than those which utilize fewer sources of information (Rouse as reported in Siegel 1996). Only through this multidisciplinary approach will aDNA reach its true potential.

Hypotheses

The research undertaken in this dissertation is an effort to place aDNA within the bio-cultural model of evolution. This model has been described by Eckland (1971:90) as an, "...elaborate system of causal paths along which there is continuous feedback between the genetic and cultural tracks

from one generation to the next". The genetic constitution is known to restrict the range of human phenotypic evolution. Yet within these boundaries humans alter their physical surroundings and/or cultural arrangements, sometimes resulting in an alteration in the frequency or distribution of genetic variants in the next generation. New genetic patterns invariably emerge through this interaction of heredity and environment (Eckland 1971). The hypotheses presented address anthropological questions involving the identification of genetic drift influences in population history within the bio-cultural model, specifically that of the nineteenth century Upper Canadian pioneers. This has been accomplished by the aDNA and historic records analysis of two contemporaneous and geographically associated skeletal collections recovered from Southern Ontario (see Figure 1.1).

Skeletal tissue samples were analyzed from the St Thomas' Anglican Church parish cemetery from Belleville, Ontario (1821 to 1874), and the Farewell family cemetery on Harmony Road Oshawa, Ontario (1827 to 1900+, hereafter referred to as the Harmony Road cemetery). Nuclear DNA loci (short tandem repeats and amelogenin sex determination), and maternally inherited mitochondrial DNA (mtDNA) were amplified from the aDNA sample extracts. Given the known confines placed upon burial within the two cemeteries, what differences in genetic diversity can we expect to see between the limited interment

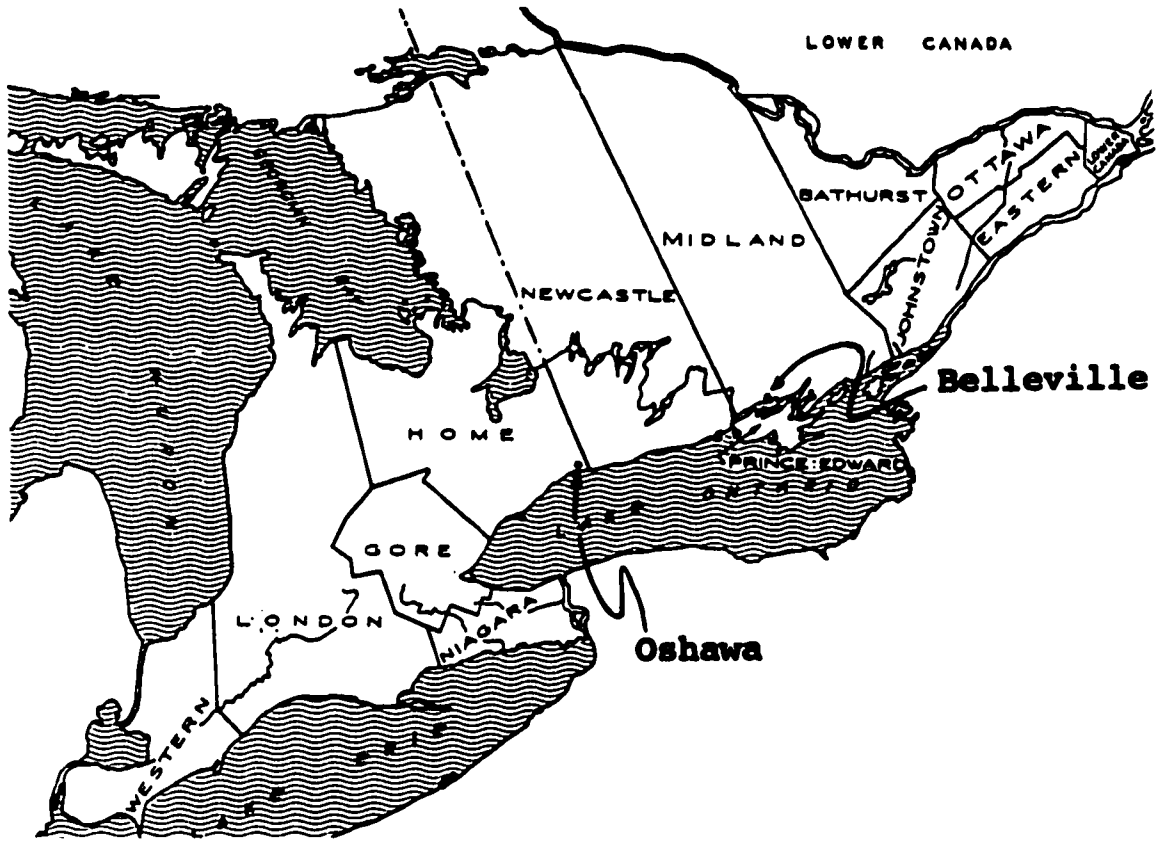


Figure 1.1: Location of the burials under study: The Harmony Road cemetery in Oshawa, and the St Thomas Anglican Church cemetery in Belleville. District divisions appearing in 1836 (adapted from Spragge 1967)

family cemetery of Harmony Road, and the wider range of people buried within the parish cemetery of St Thomas'?

This question has broad implications for applications of aDNA methodology to archaeological skeletal collections where sparse or no historical documentation exists. The determination of population relationships can be conducted on such collections with only aDNA data, yet the observed genetic differences may be explained through the action of a number of random and culturally driven events on an ancestral gene pool. Evolutionary catalysts may include any or all of the following: migration, random genetic drift, founder effect, population bottlenecks, and assortative mating. Investigators using only aDNA to reconstruct population histories must be conscious of the possibility of alternative explanations. It is necessary to define the limitations of aDNA population history research from an historic case example before our focus is turned to the more distant past.

Hypothesis #1:

The interment of biologically related individuals in the Harmony Road cemetery will be observed as a statistically significant difference in the aggregate STR allele frequency profile when compared to the St Thomas' parish STR profile and the allele profiles of various modern forensic populations derived from unrelated individuals. In addition, a significantly reduced number of mtDNA sequences are expected at Harmony Road when compared to St Thomas' and modern population databases.

Both cemeteries roughly encompass the historically documented increase in immigration to Upper Canada as well as the shift from subsistence agriculture to an increasing world market and industry driven economy. Changes in a population's perception of the social and biological environment can lead to stress responses that may have evolutionary consequences (Goodman et al. 1988). It is the evolutionary outcome of this culturally specific perspective that has often been overlooked in the studies of past peoples due to the myriad epistemological difficulties in recapturing that context. In what way can we ascertain such a bio-cultural stress in operation on a past population?

Much research has already been accomplished in establishing broad evolutionary trends and sociocultural responses of the pioneers of Upper Canada in general, but also for the parishioners of St Thomas'. Studies have been conducted on: colonial immigration, settlement (Houston and Smyth 1994, MacKay 1994, MacDonald 1939, Wood 1988) and economy (McCalla 1993, 1988); historic geography (Widdis 1991); Belleville community history (Boyce 1967, 1977, Mika and Mika 1975, 1977, 1978, 1986); St Thomas' parish demographic variables (Burke 1994); fertility patterns (Vanderlinden 1995); burial records and skeletal samples (Hoppa 1996, Saunders et al. 1995, Rogers 1991); cemetery paleopathology (Jimenez 1991, Saunders et al. 1997); and

infant growth, mortality, and weaning (Herring et al. 1998, Herring et al. 1991, Saunders et al. 1993). However, within the large corpus of work centred on the parish of St Thomas' Anglican Church in Belleville, assortative mating involving family surnames has been judged to be of no great significance (Sawchuk, personal communication).

Assortative mating, or the tendency for mate selection to yield partners with similarities or differences, has been described by Eckland (1971:86) as, "...one of the important links between the physical and cultural components of man's evolution." Divergence from random mating can split the population structure into kinship subgroups, such as religious isolates, socially isolated castes, and economic groups (Bamshad et al. 1998, Jacquard and Ward 1976). Kinship can be defined within an anthropological context as both genealogically structured (or consanguineous), and culturally specific non-genetic (or affinal) alliances of individuals with common interests.

> [kinship] is ascribed by birth and persists throughout life; is initiated by 'marriage'...is explained or justified in terms of a biological idiom...assigns the parties to an 'in' group or category, in opposition to persons not so assigned... entails the joint ownership, use, or serial inheritance of property and resources; serves as a medium for assigning hereditary social positions... (Barnard and Good 1984:188-189)

Gagan (1981) and Mitterauer and Sieder (1982) contend that pre-modern European and Upper Canadian pioneer marriages

were primarily determined by the social and economic needs of kinship, or family units.

> ...the choice of a marriage partner was very much controlled and influenced by the immediate family...[and] could affect the entire circle of relatives, as well as social, political and class interests.
> (Mitterauer and Sieder 1982:122)

> More commonly, daughters' choice of husbands had to be acceptable to their brother-parents. (Gagan 1981:55)

A search for assortative mating in the parish marriage records of St Thomas' may therefore help to illuminate the social and economic stress experienced by some of these kinship groups. The investigation of assortative mating through the analysis of isonymy, or same surname marriages, will also aid in the quantification of possible inbreeding effects on the gene pool of the congregation. The occurrence of nonrandom family unions would lend further strength to the view that cultural processes have effects on population structure that aDNA could not detect by itself.

Hypothesis #2:
The occurrence of positive assortative mating in the parish records of St Thomas', as detected by isonymous marriages, indicates that consanguineous unions were conducted by some members of the population experiencing a bio-social stress.

While the use of historic records analysis suits the major theme of this study, in what manner can we detect such a bio-cultural stress in cemeteries without such data sources? It has been argued that the evolution of biological descent

consciousness, or ancestor worship, may coincide with increasing pressure on vital resources (Saxe 1970). Biological and social pressures may have made restricted access to resources advantageous for the survival of the corporate, or kinship, groups that controlled them. This would lead to the establishment of inheritance of these resources from past ancestral rights and claims.

Saxe (1970) theorized that the emergence of formal cemeteries coincides with the evolution of ancestor worship. The alliances created with both living relatives and dead ancestors played an important role for kinship group control and inheritance of vital resources (an example being property transmission), and fostered the creation of burial areas specific to that kinship group as symbolic links to past claims/rights. What role did kin relationships play in the cultural milieu of the Upper Canadian pioneers, and how can we quantitatively or qualitatively detect this in the archaeological record?

Statistical methods were borrowed from paternity testing and forensic science and altered to suit an application to the identification of genetic relationships in the archaeological record. The calculation of a parentage index (PI) using short tandem repeat (STR) DNA fingerprinting is presented, as well as a method for the calculation of probability of kinship by chance (PrKBC) using mtDNA sequence data. Recommendations

were made for archaeological skeletal remains, such as the utilization of multiple STR loci, and the implementation of both cemetery and forensic databases in the calculation of the mtDNA kinship probabilities.

Hypothesis #3:
Spatially associated maternal mitochondrial haplotypes and short tandem repeat genotypes generated from the aDNA analysis reflect individual biological relationships, and thus allow the interpretation of genetic kinship.

The generation of statistical probabilities for kinship is generally not diagnostic unto itself in the archaeological record; in many cases the analysis of aDNA will only indicate with certainty who is not related (Waldron 1991). Therefore alternative data sources and methods must be used to further support any conclusions from the statistical analysis.

In support of Saxe's (1970) theory, Goldstein (1976) found that structured burial areas were more likely to be associated with agricultural societies that were based on a lineal descent kinship system. The use of mortuary structure indicators such as: spatial and temporal trends, archaeological features, and associated burial inclusions like coffin hardware items, may help reinforce the genetic relationships alluded to by the aDNA kinship probabilities.

Hypothesis #4:
Probable kin relationships identified by the aDNA analysis coincide with the spatial and temporal patterns, and the associated historic documentation of some of the burials. Furthermore, this mortuary analysis indicates an agricultural society with a lineal descent kinship system based on the theory of Saxe (1970) and Goldstein (1976).

Several independent lines of evidence have been brought together to reasonably define the cultural context and vital resource pressure perceived by the subsistence agriculturalists of Upper Canada. Historians have chronicled that land replaced money in the "extremely marginal economy" of this colony during the formative years of settlement and development (McCalla 1988:37), and was used like capital, accumulated and spent as needed (Johnson 1971). Those who owned agricultural land were also found to be significantly more likely to persist, while the landless majority were prone to migration (Gagan and Mays 1973, Gagan 1981, Wood 1988). In what way(s) will the aDNA based interpretation of population history change with the integration of the reconstructed cultural context?

If one integrates this ethnohistoric context with Saxe's (1970) theory of symbolic kinship mortuary practice, then the cemeteries under investigation must represent kinship burial grounds, or at least kinship clusters within a larger cemetery in the case of St Thomas'. By extension any cemetery from an agricultural society experiencing a vital resource pressure would also be unrepresentative of its population genetic diversity due to kinship mortuary practice. McCaa (1998) and Cadien and colleagues (1974) also submit that long chronological collections of skeletal remains cannot be used to represent populations. The characteristics that define a

biological population are not practically measurable in a skeletal series encompassing a long interment period.

Hypothesis #5

The integration of a multi-perspective ethnohistoric context with aDNA evidence and archaeological theory reveals that the Upper Canadian cemeteries are not representative of the population from which they once belonged.

Summary

The hypotheses outlined for this dissertation are created to first test the application of aDNA methods on cemetery collections. Can these techniques reliably and accurately test past evolutionary events within skeletal samples? It is argued that aDNA cannot by itself fulfil the requirements necessary for the reconstruction of population histories. Many lines of independent evidence must be implemented in order to cull equally probable rival explanations.

The Literature Review that follows briefly outlines the theoretical manner in which evolution has shaped human societies, and how anthropologists have attempted to determine population history. This study integrates analyses of Upper Canadian aDNA, marriage registers, historic records, osteological analysis, and archaeological spatial relationships, with the theory that control of vital land resources was one socio-cultural factor influencing population history. The variety of data sources and practical approaches used in this synthesis are outlined in the Materials and

Methods Chapter. The outcomes and trends of the various investigations are summarized in the Results Chapter and the Appendices found at the end of this study. Integration and interpretation of the results can be found in the Discussion Chapter.

Through this analysis it was shown that conclusions regarding past population history in Upper Canada could not be drawn from any single source of information. Attempts to interpret pioneer population history from only the aDNA data revealed that there were a number of possible evolutionary explanations for the observed pattern. This may be a problem of viewing skeletal aggregates as 'snap-shots' of the populations from which they are derived, rather than as temporal collections of populations over several generations.

The reconstruction of past sociocultural variables to facilitate further interpretation relied on the collection of scholarly historic research, primary records analysis, and archaeological theory and observations. The hypothesized vital land resource pressure influencing kinship structure was found to be mutually supported by these independent lines of evidence. Only through the establishment of an interment chronology, by the use of time-dependent coffin hardware, was it possible to subdivide the burials and observe the intergenerational kinship mortuary practice. This conclusion concurs with the archaeological and ethnohistoric context.

Chapter 2: Literature Review

This chapter briefly outlines the theoretical manner in which evolutionary mechanisms have shaped the human gene pool. The biological paradigm of panselectionism based on Neo-Darwinian theory has been refuted by research at the nucleotide level of DNA. The neutral theory of molecular evolution has gained ascendency; random genetic events are required for the fixation of mutant alleles in the gene pool of a population. Only then can natural selection pressures operate upon the resulting phenotypes, and gene flow will spread advantageous genotypes to adjacent populations.

Researchers involved in the anthropological study of population structure and history have accepted the profound effects that genetic drift has had on relatively small and isolated human groups, a situation typical for most of our evolutionary history (Mange and Mange 1980). Yet these same investigators generally assume equal genetic drift effects with the populations studied, or make statistical attempts to control for these random events. The question may be asked, exactly how random are these events in human populations? While the influence of culture on human evolution is not in dispute its role in genetic drift is often overlooked, or these random events are dismissed as a confounding variable

in, the quantitative search for biological origins.

The perspective of this study views culture as playing a significant non-random role in evolutionary events through kinship marriages and kin migration as detected by the isonymy analysis, historic records research, and the molecular archaeology of skeletal remains. In order to implement aDNA in this study of culture through kinship influences on past populations, it is necessary to first evaluate the pitfalls inherent in aDNA research to identify possible difficulties in the determination of individual biological relationships from the archaeological record.

The Three Founders of Population Evolutionary Theory

Of the three founders of population genetics theory, Fisher, Haldane, and Wright, it was RA Fisher who had the strongest influence on the orthodox belief that the direction of evolution in large populations was almost entirely affected by natural selection. Fisher's (1930) mathematical investigations showed that stochastic, or random gene frequency fluctuations had little effect on total genetic variability, and therefore the process of evolution. He reasoned that random allele sampling from generation to generation, or genetic drift, would have a negligible effect on a population's gene pool with the size of most species.

Haldane (1932) investigated the quantitative process of natural selection and its effects on gene frequency variation.

By introducing the use of selection coefficients, Haldane showed that the number of generations required for a given change in gene frequency is inversely proportional to the intensity of selection. Since random events are not considered in his model, this theoretical approach is as deterministic, with respect to gene frequency changes, as Fisher's and contributed significantly to what Kimura (1983:8) calls the "panselectionism" bias observed in biology.

Opposed to the type of selectionism put forward by Haldane and Fisher, Wright's (1931) emphasis was on the importance of random genetic drift. His shifting balance theory examined the possibility that evolution has occurred in small, subdivided groups within a population with a limited amount of migration. Examples of this type of population abound in human prehistory and include hunting groups, tribes, or clans (Mange and Mange 1980). This limited isolation of subpopulations may allow for extensive gene frequency drift to occur due to random sampling accidents, resulting in the formation of unique nonadaptive, or neutral gene combinations. Natural selection may then occur on these phenotypes leading to rapid, adaptive gene frequency changes. A subpopulation undergoing this selection will possess a superior fitness compared to surrounding groups, and may expand via intergroup selection. These genotypes will spread by migration from different centres of increased fitness to overlap, and give

rise to a still superior centre. Thus a field of subpopulation gene pool interactions may be produced from a limited number of novel mutations. The rate of evolution in this model would be much faster than in a single large interbreeding population as pictured by Fisher. The introduction of this shifting balance theory sparked a debate that continues today regarding the role that random genetic drift plays in evolution and the generation of genetic variation. Wright's theory has often been misunderstood as proposing random drift as a direct alternative to natural selection (Kimura 1983), when in fact it employs all elements of evolution: mutation, drift, selection, and gene flow.

Kimura's (1983) neutral theory of molecular evolution asserts that the majority of evolutionary changes at the molecular level are caused by random genetic drift of neutral, or nearly neutral, mutant alleles (molecular variants of the same gene). Neutral alleles are defined as phenotypically silent molecular substitutions that have no influence on survival or reproduction. Thus the neutral theory rejects the selectionist dogma that the majority of polymorphic alleles are adaptive and maintained by natural selection. These alleles reach significant frequency levels by the action of random sampling in a manner similar to Wright's shifting balance theory. The neutral theory has been supported by molecular experimental evidence over the last two decades.

Majumder (1991:97) believes that there is "unequivocal evidence" in favour of the neutral theory. Natural distributions of allelic frequencies from a wide variety of protein loci closely approximate mathematical distributions of loci modelled on evolution by drift and mutation only (Weiss 1993). This suggests that most allelic variation segregating at any given time is effectively neutral, supporting the theorized function of genetic drift in evolution. As King and Jukes (1969:788) so eloquently wrote, "Natural selection is the editor, rather than the composer, of the genetic message."

Anthropological Study of Population Structure and History

The concept of a population is defined as, "...a geographically and culturally determined collection of individuals who share a common gene pool" (Molnar 1992:31). The gene pool can be described as all potential genetic contributions by the members of the population. Molnar (1992:31) believes that we should not think of populations as "...a reality of nature, but should recognize them for what they are - a construct, a means of organizing the diversity of data describing our species." These definitions and the perspective on their application will be in use throughout this study.

Relethford (1996) defines population structure studies as focusing on the balance between gene flow and genetic drift among a set of populations and deals with: migration, founder

effects, population distance, and the size and composition of breeding populations. Population history studies look at the degree of similarity between populations resulting from gene flow and/or common ancestry. Factors that affect population history are a subset of those affecting its structure, therefore the two terms, population history and structure, are often used interchangeably (Relethford 1996).

The analysis of genetic distances, or the measure of genetic dissimilarity between populations based on allele or haplotype frequencies, is central to studies of anthropological genetics (Relethford 1996). The key problem in interpreting genetic distances is the determination of the relative contribution of: common ancestry, gene flow from migration, and genetic drift. It is known that when population size varies between groups under study, the effects of drift can obscure the underlying population history (Relethford 1996). Genetic drift is therefore generally considered a confounding variable in these studies, and may encompass many past evolutionary drift events (Poirier et al. 1990). These can include: true random genetic drift when chance factors reduce some allele frequencies in subsequent generations, population bottlenecks due to drastic reduction in population size, founder effect of a new group due to an unrepresentative introduction of individuals from an ancestral population, and assortative mating or inbreeding reducing

heterozygosity or genetic variability.

Researchers have often dealt with genetic drift by assuming equal population size and long range migration rates, or the imposition of a generic mathematical model (Relethford and Harpending 1994). Others have developed sophisticated models which provide estimates of genetic distance under various demographic scenarios which can be compared to the observed data to test hypotheses (Rogers and Harpending 1986). Relethford (1996) has outlined a method by which the sizes of the populations under study are mathematically scaled with respect to each other to neutralize drift effects, however this approach still assumes a constant effective size over time and equal long range immigration into the groups.

Relethford (1996) discusses another alternative to cope with genetic drift through the qualitative interpretation of observed data with the known history and demography of the region. Roberts (1988:537) believes that this combination approach, "proves to be very powerful" for studies of past peoples. By integrating historical documents, indigenous oral histories, the archaeological record, as well as other forms of cultural information, one may be able to explain more of the observed trends in the population data.

The influence of culture can be best illustrated by considering the highly heterogeneous process of migration. The change in residence location of individual(s) due to

economic, social, political, or religious pressures results in the concomitant movement of alleles. Great changes to the gene pools of the host and recipient populations are possible if these relocations involve many people over a short period of time, as opposed to continued immigration of individuals over longer periods. Such migrations stemming from changes in attitudes and ideas can have considerable effects on population structure and can be considered one of the major mechanisms of evolution (Roberts 1988). The gene pools can be altered even more if migrations involve kinship units through the exaggerated changes in gene frequencies brought about by the relocation of families. This kin structured influence has been termed a 'booster effect'; its relative impact on gene pools is basically unknown since population genetic theory considers migration almost exclusively by random sample (Roberts 1988).

Clearly cultural forces have strong influences on some evolutionary events, such as kin structured migration and assortative mating involving kinship unions, making them non-random by any definition. The use of aDNA to study population structure/history is still in its infancy and should attempt to explore the possibility of uncovering culture and kinship effects on genetic diversity. Within aDNA lies the potential to definitively establish individual kin relationships from burials, something that has eluded past researchers using

archaeological material culture (Allen and Richardson 1971, Plog 1973), and non-metric skeletal trait analysis (Berry 1975, Corruccini et al. 1982, Rösing 1984, Saunders 1989). Analysis of aDNA yields observations of heritable genetic material, not presumed stylistic manifestations of kinship traditions handed down genealogically, nor variable phenotypic expressions of environmental interactions with unknown genes and genotypes. It is necessary to first review and evaluate the possible pitfalls inherent in aDNA research before we move on to present the study design and the statistical approaches modified for use in kinship determination from skeletal remains.

Ancient DNA Review

> ...the term "ancient DNA" (aDNA) covers any bulk or trace of DNA from a dead organism or parts of it, as well as extracorporeally encountered DNA of a living organism. Therefore, any DNA that has undergone autolytic or diagenetic processes or any kind of fixation is considered to be "aDNA". (Herrman and Hummel 1994:2)

Molecular biological advances in DNA sequence analysis methods have recently been included in the toolbox of the physical anthropologist studying evolution and variability of human populations (Cann 1988, Flint et al. 1989). This section discusses molecular anthropological research at the DNA sequence level since the introduction of the polymerase chain reaction technique and its application to past

populations. This is followed by an examination of the problems inherent in working with ancient DNA including damaged and altered template molecules, polymerase chain reaction inhibition, and fidelity of the Taq enzyme used in the amplification reaction. The effects of these obstacles on the determination of biological kinship from archaeological human skeletal remains is also addressed.

The Polymerase Chain Reaction

The polymerase chain reaction, or PCR, permits selective in vitro amplification of a particular DNA region by mimicking in vivo cellular mechanisms (Mullis et al. 1986). Specificity of the reaction to a certain region is conferred by the use of two oligonucleotides, short manufactured single stranded DNA sequences, that bind to unique areas flanking the region of interest and prime the extension of a new strand of DNA by the synthesizing action of the polymerase enzyme. The extension products produced from the 'primer pairs' are designed to overlap so that the reciprocal DNA strands are complementary, and can act as template molecules for further cycles of replication. In this way, geometric amplification of the original template is accomplished over several cycles. The reaction is so sensitive that potentially a single DNA molecule can be amplified (Higuchi 1989, Innis et al. 1990), thus permitting analysis of damaged and degraded samples. The application of this amplification technique to the recovery of

ancient human DNA has revolutionized the genetic study of past peoples (Herrmann and Hummel 1994).

Being able to directly observe DNA sequences from skeletal remains (Hagelberg and Clegg 1989, Horai et al. 1989) has allowed investigators to peer into the prehistory of Homo sapiens. Areas of anthropological research at the nucleotide level now include, human evolution (Relethford 1998, Krings et al. 1997, Stoneking et al. 1992, Vigilant et al. 1991), past migrations (Lorenz and Smith 1996, Stone and Stoneking 1996, Merriwether et al. 1994, Gibbons 1993, Hagelberg and Clegg 1993, Stone and Stoneking 1993), paleopathology (Yang 1997, Dixon et al. 1995), and biological kinship relationships (Soodyall et al. 1997, Kurosaki et al. 1993, Gill et al. 1994, Ginther et al. 1992, Jefferies et al. 1992).

Ancient DNA Damage

Successful PCR amplification from tissue samples derived from the living is straightforward when compared to the extensive damage and low extraction yields from ancient remains (Rogan and Salvo 1994). To some extent all ancient tissues have undergone a poorly understood decomposition process that begins with autolytic enzyme release and putrefaction, continued by microbial invasion and digestion (Hummel and Herrmann 1994, Rogan and Salvo 1990). The loss of enzyme regulation/repair and lactic acidosis following death also increases hydrolase class enzymes: endonucleases that act

on DNA to cleave it into small pieces, and exonucleases that remove nucleotides from the ends of these fragments (Rogan and Salvo 1990). Oxidative damage to nucleotides, especially the pyrimidines, and hydrolytic damage to the DNA sugar backbone appear to be the major form of taphonomic modification, followed by baseless sites and DNA strand crosslinks (Handt et al. 1994, Pääbo 1989). Reductions in quality can be observed by the short sequences recovered, 300-400 base pairs at an optimum, but typically less than that (Krings et al. 1997, Handt et al. 1994, Jeffries et al. 1992, Hagelberg and Clegg 1991, Pääbo et al. 1989).

Necrotic processes can reduce the quantity of DNA yield by more than 100 fold, leading to estimates that less than one percent of aDNA molecules will be undamaged (Pääbo and colleagues 1989). Tuross (1994) has determined that fresh, surgically removed bone yields 3 to 5.5 µg of DNA per gram of wet tissue, reflecting both cellular and vascular content, while DNA bound to hydroxyapatite ranged from 1.5 to 3 µg/gram of bone. It is unclear how representative this last figure is for DNA solely bound to the mineral portion of bone as both results were achieved by using the same fresh bones. Researchers should probably expect to extract yields of less than 10 ng, or 0.01 µg from archaeological bones using Tuross' lowest estimate and Pääbo and colleagues' (1989) one percent expectation for aDNA preservation.

Despite these potential aDNA preservation problems, there are several characteristics of the polymerase chain reaction that favour evolutionary analyses (Hagelberg 1994). Selecting a short sequence as a template for PCR will increase the probability of a successful reaction. Overlapping primer pair sets can be used to achieve longer sequences in a 'step-wise' manner, a procedure used by Krings and colleagues (1997) to successfully reconstruct a Neandertal sequence. Any preserved tissue from a human body can be submitted to extraction and yield target DNA for PCR amplification. An example is the successful analysis of aDNA from mummified muscle and connective tissue, as well as bone, of the Tyrolean Ice Man (Handt et al. 1994b). The Taq polymerase enzyme will preferentially replicate undamaged template molecules; baseless sites and cross linked DNA strands will slow down or stop polymerase advancement, placing damaged molecules at a replicative disadvantage (Pääbo et al. 1989). This results in the 'dilution' of incomplete, damaged reaction products, and the geometric increase of complete templates by the chain reaction principle (Pääbo et al. 1990).

"The Ever-present Spectre of Contamination"
(Handt et al. 1994a:526)

The fact that PCR will preferentially amplify undamaged template means that the smallest amount of modern DNA sequence or post PCR product may form the majority, or at least part of

the final product. Richards and associates (1995) have speculated that even minute amounts of modern contamination are sufficient to outcompete aDNA for polymerase activity. Contamination will interfere with the accurate and reliable assessment of the DNA extract, potentially leading an investigator to falsely conclude a positive result (Handt et al. 1994b). The magnitude of this problem should not be underestimated, especially by the uninitiated, nor should the discovery of contamination necessarily dismiss all related experiments.

Krings and colleagues (1997:25) encountered a, "...larger amount of contemporary human DNA, probably stemming from laboratory contamination...", with their third independent extract from the Neandertal specimen. Fifteen clones created from a PCR product identified the source as collaborator A. Stone's mtDNA sequence, and 2 others of unknown contemporary origin. Lindahl (1997:2) similarly reports that, "Occasionally, a DNA sequence could be amplified from amber, apparently independent of the presence or absence of a fossil insect...[and] were unrelated to the insects investigated." The fact that even seasoned aDNA researchers can still experience contamination difficulties indicates that some element of random chance is involved.

Contamination may be present on the specimen from previous handling, or introduced at any stage during the

extraction or PCR setup through reagents or airborne aerosols. Handt and associates (1996) identify two types of contamination, the first will affect all amplifications systematically and is caused by the presence of exogenous DNA in extract and amplification reagents. The second type, or 'spot' contamination, affects individual extracts and amplifications and is caused by prior handling of the sample or from laboratory aerosols containing modern DNA or PCR product. Procedures have been established to identify and correct widespread systematic contamination and reduce spot contamination to acceptable levels in the presence of ubiquitous modern DNA.

Systematic contamination of extract and PCR reagents is relatively easy to identify if the proper controls are established, such as reagent blanks for mock extracts and PCR blanks that contain no DNA (Handt et al. 1996). While a positive result in these 'negative controls' may also be due to spot contamination, the more parsimonious explanation is that the extraction or PCR reagents have become fouled. The presence of an identical PCR product (for example the same mtDNA sequence) in each specimen will confirm systematic contamination. The solution is straightforward: discard all potentially fouled reagents, create/purchase new reagents, and re-amplify all samples associated with the old PCR reagents, and if necessary re-extract all tissue samples.

Spot contamination on the other hand is of greater concern because of its insidious nature (Handt et al. 1996). It is not usually detectable from negative controls. Only the final analysis of the PCR product may reveal outside sources as the origin because of previous handling of the specimen, or 'investigator introduced' through airborne routes such as sloughed skin particles and cough or sneeze aerosols. Some deductive reasoning is necessary here, if one is working on Native American aDNA then mtDNA sequences typical of Europeans should be viewed suspiciously. Steps to identify spot contamination involve the comparison of DNA sequences under study to that of all personal sequences of researchers with access to the laboratory. Solutions are again relatively straightforward: the implementation of more stringent cleanliness protocols and reamplification and if necessary, re-extraction of affected samples. The easiest way to identify some forms of contamination is to establish reagent extraction blanks that contain no sample material but are run concurrently with a small batch of specimens, usually less than ten. PCR blanks are similarly created for each amplification reaction.

Cleanliness protocols have been extensively outlined to control for both types of contamination (Handt et al. 1994a, Kwok 1990). Of primary importance is the physical separation of the extraction and PCR setup areas from post PCR analysis.

Dedicated laboratory clothing such as lab coats, hair nets, and paper masks, as well as pipettors, centrifuges, and reagents should also be specific to these areas. Daily UV irradiation and wiping down of bench tops, pipettors and centrifuges with commercial bleach solution will considerably reduce the chance of airborne DNA contamination. The establishment of a separate ventilation system is also recommended, but is of relatively less importance when compared to the effects achieved through the lower cost precautions discussed above.

Characterization of aDNA: Reliability and Accuracy

A number of methods have been devised to test results, and strengthen the confidence that aDNA has been amplified. DNA results should be considered suspicious if an inverse relationship between the size of the amplified fragment and amplification efficiency is not observed (Hagelberg and Clegg 1991). If false positive or ambiguous sequences are suspected/observed from the direct sequencing of PCR products, then molecular cloning of the product and subsequent sequencing of clones will reveal different 'PCR lineages' of molecules (Handt et al. 1994a). These different lineages may be caused by contamination (as discussed above), or PCR artefacts due to base pair misincorporations, or 'jumping PCR' (see Pääbo et al. 1990). Jumping PCR occurs when the polymerase enzyme is stopped, because the aDNA template is too

fragmented, and then jumps to another piece of template to create a chimeric molecule. If one is working with mitochondrial DNA, additional rare explanations of ambiguous sequences may include a nuclear insertion of a piece of mtDNA sequence, and heteroplasmy, the presence of more than one mtDNA lineage in the cell lines of an individual due to a mutation event. Both would mimic the appearance of more than one mtDNA lineage in a single individual (Gill et al. 1994).

Very often, aDNA is so badly damaged that base modifications occur or baseless sites are present (Pääbo 1989). If the PCR is started from a single molecule or a few damaged molecules, then the final product will contain the same error(s) or a mix of lineages of molecules with a variety of artefacts yielding an ambiguous genotype if directly sequenced (Handt et al. 1996). If the PCR is started from several molecules however, then chances are that undamaged template exists and will be preferentially amplified to produce the majority of the final product.

A related subject is the occurrence of 'allelic drop-out', defined as a preferential amplification of one allele in a two allele nuclear system, thus leading to the conclusion of homozygosity when in fact the sample genotype was heterozygous (Zierdt et al. 1996, Ramos et al. 1995). Allelic drop-out is a serious hurdle to the accurate and reliable determination of short tandem repeat genotypes for aDNA, and may be caused by

a stochastic problem of amplifying low copy number and degraded aDNA (Gill et al. 1994). Therefore the quantification of the initial number of molecules is very important to rule out PCR-induced heterogeneous artefact sequences for mtDNA and false homozygotes for nuclear STR analysis.

Direct quantification via standard photometric methods is not applicable to aDNA due to the major presence of soil micro-organisms such as bacteria and fungi (Tuross 1994). It has also been argued that the amount of human aDNA recovered is often too small to be directly detectable by radioactive probes, and that a PCR quantitative method must be used (see Handt et al. 1994a). PCR quantitation is based on the principle of polymerase competition between a series of PCR reactions containing a constant amount of aDNA extract and a serial dilution of a template constructed to be slightly longer. The DNA concentration of the longer template that approximately matches that of the appearance of aDNA amplification is thus used to estimate the number of ancient molecules. Krings and associates (1997) speculate that PCR started from fewer than 1000 ancient mtDNA molecules tend to yield results that vary between experiments. Gill and colleagues (1994) place their cut off for STR analysis at 10 molecules (50 pg).

While quantitative PCR is currently the only method to

quantify aDNA, it has some significant drawbacks and it is argued here that it provides a false sense of accuracy. The necessary assumption of equal competition for polymerase activity between aDNA molecules and the constructed template is likely false given Richards and associates (1995:291) experience that "...even minute quantities of modern contaminating sequences are sufficient to out-compete ancient template during amplification by the polymerase chain reaction (PCR)." In addition to this, the method also assumes that the same quantity and quality of aDNA molecules are present in each reaction, and ignores the possible effects of pipettor error involved in the preparation of the serial dilution and the PCR reaction. This leads to a further false assumption of identical reaction conditions for every vessel. While many of these points can never be fully substantiated, it can be surmised that the number of aDNA molecules predicted by this method will at best provide the lowest range of a very conservative estimate. It is therefore concluded that this method may prevent researchers from attempting experiments that have as much likelihood of succeeding as failing based on quantification error.

One final source of possible error involved in achieving an analyzable aDNA product is the fidelity, or error rate, of base pair incorporation of the Taq polymerase molecule itself. Taq has been shown to decrease in activity and fidelity with

repeated exposure to the high temperatures necessary to separate target DNA during the denaturing phase of PCR (Gelfand 1989). Of primary concern is a possible decrease in fidelity of the amplified product as the polymerase enzyme is used over the 40 or more cycles sometimes required for aDNA detection. Some researchers have sanctioned the removal and reamplification of the PCR product itself. Yang (1997) used nested PCR for 80 cycles, Zierdt et al. (1996) used 90 cycles, Stone (1996) used 60 cycles, Kurosaki et al. (1993) used 60 cycles, Holland et al. (1993) for 54 cycles. This is not generally recommended as it places all template on a level footing in the second PCR, including artefacts and even the slightest amount of contamination from the previous reaction.

Ancient DNA Kinship Determination

The determination of definitive biological kinship from skeletal material has involved forensic cases (Hagelberg et al. 1991, Ginther et al. 1992, Jefferies et al. 1992), repatriation of Vietnam soldiers (Holland et al. 1993), the recent historic burials of the Romanov family (Gill et al. 1994), and archaeological remains (Kurosaki et al. 1993). The resolution of aDNA genetic relationships involves all of the problems of contamination and artefacts due to template quantity, quality, and Taq error that were discussed previously. Determining biological kinship from aDNA is thus a daunting task when one considers that the data generated

must represent precise genotypes in order to compare individual profiles and definitively identify possible parents and siblings. Ancient DNA research involving close genetic relationships has used mitochondrial DNA and repetitive nuclear sequences; both approaches have positive and negative characteristics associated with them. Accuracy and reliability are of primary importance in these studies, leaving little flexibility in the interpretation of PCR artefacts and contamination.

Mitochondrial DNA (mtDNA)

There are several advantages to using mtDNA extracted from archaeological skeletal remains. The entire molecule itself is very well characterized (Anderson et al. 1981, Stoneking et al. 1991), and is composed of 16,569 base pairs with numerous polymorphisms throughout the one kilobase non-coding control region, owing to a rate of evolution approximately 10 times faster than nuclear DNA (Holland et al. 1993). Mitochondria themselves are present in large quantities in human cells (averaging 1000/cell) and are passed on to the zygote through the egg, therefore mtDNA does not follow Mendelian inheritance but a simple maternal lineage pattern (Hagelberg 1993). This limited inheritance system can be used to great advantage in evolutionary and population level kinship analyses of burials across time and space (Pääbo et al. 1988, Krings et al. 1997). These characteristics,

especially the high copy number, make mtDNA an ideal source of template for most aDNA studies (Rogan and Salvo 1990).

The use of mitochondrial DNA for genealogically based kinship identification from archaeological burials is perhaps less than satisfactory for determining individual biological relationships. Stone (1996) has discussed the need to use a more definitive marker of biological relationship than mtDNA, such as nuclear short tandem repeats. This is especially pronounced within small homogeneous archaeological communities and/or maternal clan burial grounds, typical of North American Indian societies (Maracle 1997). Soodyall and co-workers (1997) have found that mtDNA is transmitted with high fidelity with no mutations detected in over 100 independent transmissions from mother to offspring. Their finding could in fact hinder the resolution of refined biological kinship analysis in relatively small, stable, or isolated 'island' populations over time and space. The problem may arise due to the maintenance and/or decrease of a limited number of maternal lineages in one region because of a population bottleneck event. This would result in the potentially false assumption of a closely knit kin burial ground if other sources of archaeological evidence were not available.

In some situations mtDNA does possess unique characteristics, such as the heteroplasmy observed in the putative Romanov family burials which was also seen in the

living maternal relatives of this famous Russian lineage (Gill et al. 1994). Stoneking and associates (1991) have used sequence-specific oligonucleotide probes (SSO) across nine particularly polymorphic areas of the mtDNA sequence to determine that any one SSO type would exclude 88.3 to 94.6% of modern forensic populations for which data sets exist. While this technique has great potential for perhaps more heterogeneous contemporary populations, it would require large quantities of good quality aDNA, which is not the case in the vast majority of ancient sample extracts (Pääbo 1989).

Ancient mitochodrial DNA kinship analysis is also hampered by the difficulty of calculating likelihood ratios from haplotypes (Hagelberg 1996). The use of any DNA locus for the calculation of these statistics requires the comprehensive understanding of the population variability and possible substructure at that locus (Melton et al. 1997). While this work is currently under completion for contemporary populations (Melton et al. in press, Melton et al. 1997, Melton and Stoneking 1996), it will likely continue to be an unknown variable for most, if not all, ancient populations due to the fact that any aggregate of skeletal collections should not be considered a population (Cadien et al. 1974, Stoneking 1995). Mitochondrial sequence information should therefore be used only to exclude maternal ancestry or cautiously conclude it with additional evidence, such as unique mtDNA

heteroplasmy, nuclear markers, or relevant material culture.

Short Tandem Repeats (STRs)

Identification of individuals, or DNA fingerprinting, for forensic use was developed using non-coding regions of nuclear DNA that demonstrated a variable number of tandem repeats in their sequences (Jeffries et al. 1985). STR's can have di-, tri-, tetra-, pentanucleotide and larger repeated core elements with alleles representing different lengths due to repeat number. They are short (generally less than 400 base pairs) and thus potentially provide a good DNA template for PCR when tissues have undergone taphonomic alterations (Hagelberg et al. 1991). Ancient DNA analysis of STRs have used dinucleotide repeats (Ramos et al. 1995, Hauswirth et al. 1994, Kurosaki et al. 1993), and tetranucleotide repeats (Gill et al. 1994, Zierdt et al. 1993) in attempts to establish a basis for aDNA fingerprinting of individual relationships.

Polymerase chain reaction amplification of short tandem repeat loci from ancient DNA extracts have yielded frustratingly inconsistent results in some cases with repeated reactions achieving only 3% reproducibility (see Ramos et al. 1995, and Zierdt et al. 1996). The problem of reliability may stem from a lack of sufficient copies of ancient template molecule in the initial cycles of the amplification causing allelic dropout (Zierdt et al. 1996, Gill et al. 1994), and jumping PCR in the early cycles of PCR contributing to

artefactual alleles by the end of the reaction. These problems of template preservation are doubled because each Short Tandem Repeat locus has potentially two different alleles present, a maternally and a paternally inherited allele. Since the Taq enzyme will preferentially amplify intact sequences, the PCR reaction may geometrically increase only one allele during one PCR, and in subsequent verification experiments amplify only the other allele, leading to confusion and incorrect conclusions about the genotype.

In addition to these problems, dinucleotide repeats are known to yield a, "...relatively complex pattern of PCR products per allele..." due to Taq enzyme slippage and non-template insertion of an extra nucleotide (Jeffries et al. 1992:72-73). This 'complex pattern' can be composed of up to three or four extra bands migrating both directly above and below the true allele in positions where other alleles would be (see figures in Kurosaki et al. 1993, Jeffries et al. 1992 for examples of these artefacts). These artefactual bands are of an intensity to confound any interpretation of genotype, especially when working with contamination and damage prone aDNA. Interestingly, Hauswirth and associates (1994) did not observe these artefactual bands for some unexplained reason. They used a ^{32}P end labelled primer and ran PCR products on a high resolution sequencing gel to separate alleles by size. By underexposing the resulting gels to radiograph film they

may have been able to increase the contrast, masking the minor products so that only the 'hot' major product was observed. It still must be concluded that dinucleotide STRs are not appropriate for aDNA analysis of biological kinship due to their potential production of PCR artefacts.

Ancient DNA results from tetranucleotide repeat STR loci, such as those found by Zierdt et al. (1996), have led to the need for expensive 'consensus allele' approaches where numerous PCR reactions (up to six reactions reported) are required to derive tentative genotypes. Zierdt and colleagues (1996) were able to generate the same alleles for only three out of seven reported samples submitted to multiple PCR amplifications. Results such as these originally led Gill and colleagues (1994) to recommend that all amplification reactions started below 50 pg should be analyzed a minimum of four times, with any apparent homozygotes amplified an additional two times for a total of six PCR reactions!

It is argued here that the consensus allele approach can never provide a confident genotype in an ancient DNA extract with a low template number due to allelic dropout, PCR artefacts, and the increasing chance of spot contamination with each reaction. Not only would precious aDNA extract be used up amplifying the same locus, but the expense of PCR reagents and post-PCR analysis would drain project budgets to the point where little data is retrieved. It is thus further

advanced in this dissertation that a methodological solution to the reliability issue of ancient STR analysis can be found in acquiring more template at the extraction step. By using aDNA purification methods utilizing silica suspensions developed by Hoss and Pääbo (1993), and technically improved by Yang and colleagues (1997, 1998), a more concentrated aDNA extract is achieved to allow accurate and reliable STR analysis for biological kinship determination.

Summary

The importance of genetic drift events in biological evolution has been substantiated by modern molecular research. In human populations founder effect and population bottlenecks may be influenced by cultural shifts in economic, social, political, and religious pressures. It has been shown that migrations stemming from changes in attitudes and ideas can be considered one of the major mechanisms of our evolution (Roberts 1988). This cultural effect on population gene pools can be boosted by the relocation of entire families. With the advent of aDNA research, it has become possible to analyze heritable genetic material from skeletal remains and therefore test for possible kin relationships in the archaeological record. Many methodological difficulties must be overcome before achieving confident PCR results that can be used in the statistical analyses of kinship.

Chapter Three: Materials and Methods

This chapter summarizes the materials used and the methods required to test the hypotheses outlined in the introduction. Background is provided on the St Thomas' (Belleville) and Harmony Road (Oshawa) pioneer cemeteries used in the extraction and amplification of aDNA. This molecular analysis of skeletal tissue samples consists of: mitochondrial DNA (mtDNA) sequence determination, short tandem repeat (STR) DNA fingerprinting, and molecular sex determination using the amelogenin gene. Statistical protocols were borrowed from paternity testing (Parental Index, or PI), forensic mtDNA identity testing (Probability of Kinship by Chance, or PrKBC) and altered to suit the requirements of archaeological applications.

A strategy is presented to integrate the aDNA results with historical documents, osteological, and archaeological evidence in order to strengthen conclusions about potential genetic relationships within burial grounds. Background is also provided on the marriage records from the parishes of St Thomas' and Christ Church Anglican used in isonymy analyses to demonstrate intragenerational consanguineous and affinal kinship alliances. In this way, many potential sources of data are tapped to provide an integrated approach to the

reconstruction of population history.

3.1: The St Thomas' Cemetery

St Thomas' Anglican Church cemetery in Belleville, Ontario, was in use from 1821 through to 1874, after which all interments took place in the municipal grave yard (Mika and Mika 1977). Three other religious cemeteries were established during the period of activity at St Thomas': a Roman Catholic, a Presbyterian, and a Methodist cemetery. Members of the St Thomas' congregation and their relatives, as well as Anglicans from surrounding rural parishes, were buried in the cemetery (Saunders et al. 1993).

The cemetery was excavated in the summer of 1989 under the license of Northeastern Archaeology Associates, with subsequent osteological analysis and tissue sampling directed at McMaster University during the following year. A total of 579 grave features were excavated from a 1.75 acre portion of the cemetery yielding 576 intact skeletons with associated grave goods (Saunders et al. 1993). Tissue preservation is acknowledged to be excellent overall (Jimenez 1991). The skeletal collection represents 37 per cent of the total number of burials (N=1564) that occurred at the cemetery. Chi square tests comparing the skeletal and parish records revealed no statistically significant difference in the age cohorts (Herring et al. 1991, Hoppa 1993). Unfortunately, the lack of

a surviving historic cemetery plan and the occurrence of two church fires causing the partial destruction of some grave markers, meant that only 80 individuals were personally identifiable from coffin plates (Saunders et al. 1993). In addition to these problems, Ontario Government legislation denies the acquisition or observation of the original report submitted by H. McKillop, hampering all scholarly research related to the site. A partial reconstruction of the site plan involving some burials with identifying feature numbers up to and including the 200 range has allowed limited spatial analysis of the aDNA data (see Figure 4.3.16 in Results Chapter, section 4.3).

3.2: The Harmony Road Cemetery

The Regional Municipality of Durham contracted Archaeological Services Inc. of Toronto, Ontario, in the spring of 1992 to assess the impact of a proposed expansion of Harmony Road into the pioneer cemetery of the Farewell family, or Harmony Road cemetery (Austin 1992). This cemetery was established on Lot 4 concession 1 of East Whitby Township by one of the region's first pioneers, Ackeus Moody Farewell, and was used from approximately 1827 to 1937 (Hood 1978, Austin and Dudar 1993). The cemetery had succumbed to the neglect of several decades so that most grave markers were damaged and no longer retained their original positions. In 1979 the Oshawa Parks Commission constructed a large cairn on the site

composed of all of the remaining complete and fragmentary head stones (Goodwin 1993). It is from this cairn that a cemetery inventory was created.

Initial surveying and subsequent excavation established that approximately 40 grave shafts were encroaching on the road allowance (see Figure 3.1). The long axis of two features were oriented in a north-south direction (features 35 and 36) and were later determined to be television cable trenches. Other burials (features 1, 2, 3, and 4) were apparently disturbed by the trench used to place the fire hydrant and water main (see Figure 3.1 northwest corner). A small unused portion within the legal cemetery limits was identified for the relocation of the suspected remains that would be uncovered. The exhumation and relocation process began in July of 1993 and was completed in September when the last two burials were removed from beneath the mature maple tree bordering the west sidewalk.

A very brief period of osteological analysis followed during which the remains were not allowed to leave the grounds of the cemetery because of legislative confines. This situation mirrored that experienced by Pfeiffer and colleagues (1989) at another pioneer cemetery in Ontario, and thus provided the salvage procedures that were implemented for the determination of basic skeletal biology. Age at death, sex, stature, race, and evidence of gross level pathology were

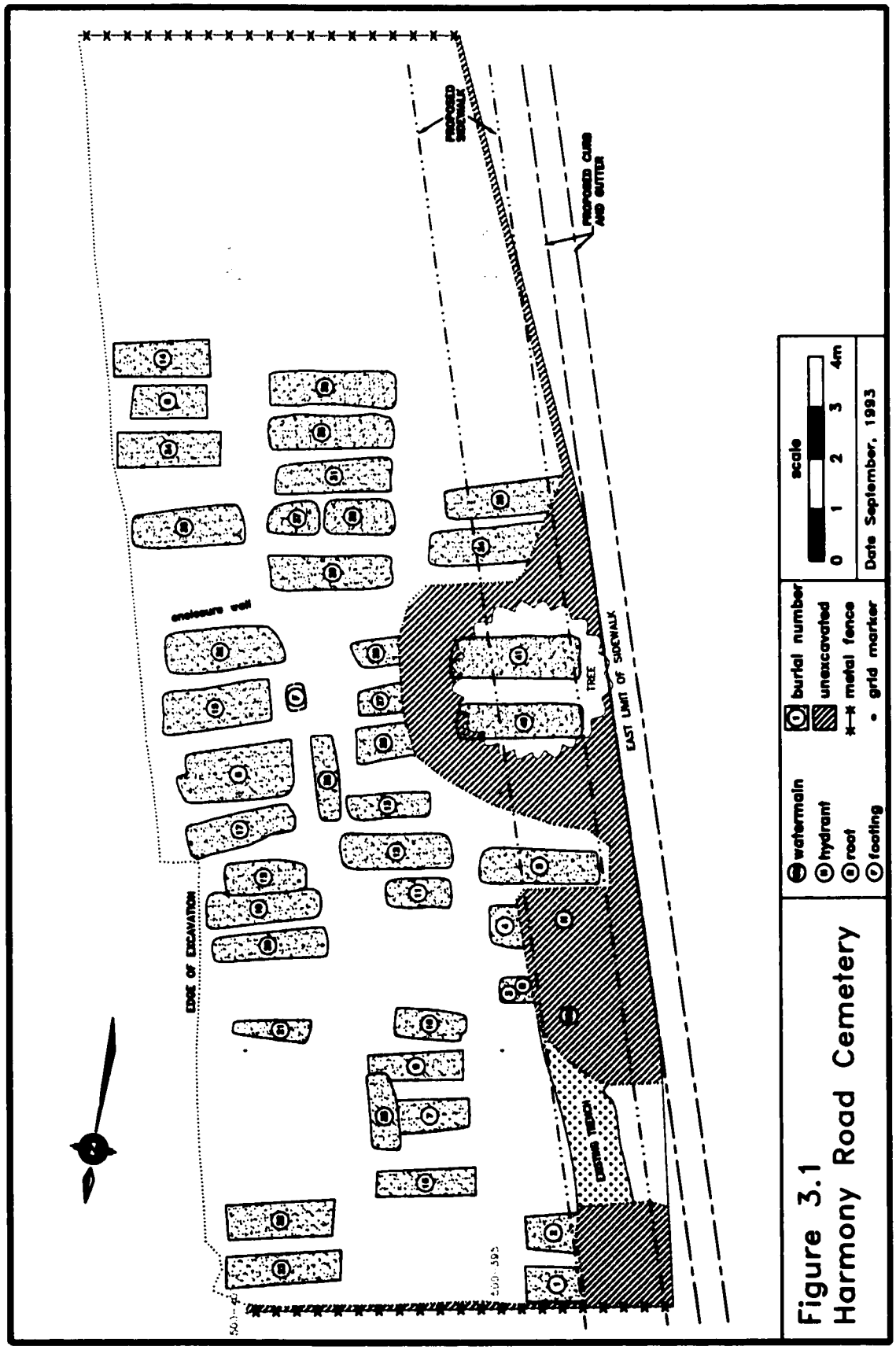


Figure 3.1
Harmony Road Cemetery

determined for each burial. Small samples of bone and teeth were retained by the principal investigator of the site, this author.

The excavated portion of the cemetery produced 38 individuals: 27 young adults, adults, and elderly (15 males and 12 females), 6 juveniles of estimated age 18 years or less, and 5 infants who died before their first year. The dates of interment for the majority of these burials (N=35) has been estimated by the associated coffin hardware (using Woodley 1992) to fall within the nineteenth century, ranging from 1827 to 1850 (N=7), 1850 to 1875 (N=16), and 1875-1900 (N=12). Only three post-1900 burials were uncovered, and of these two were positively identified from coffin plaques. A Chi square test of association conducted between the estimated age at death profile of the recovered skeletons and the reconstituted cemetery inventory found no significant difference in the age cohorts (Austin and Dudar, 1993). The sample is therefore considered representative of the cemetery as a whole.

The cemetery inventory lists 38 surnames from gravemarkers, representing approximately 117 individuals. Of these surnames, 11 represent multigenerational families of between three to 26 individuals totalling 77, or 66% of the cemetery. Eleven surnames consist of 18 interments after the year 1900 (and would therefore be easily distinguished from

earlier burials under study by their coffin hardware), leaving 16 surnames of husband/wife couples or single interments from the 19th century. At least three of the multigenerational surnames are linked by marriages: the Coryell, Farewell, and Pickell families, as ascertained from grave marker inscriptions. Certain archaeological features, such as the low enclosure wall surrounding burials 6, 15, and 32, as well as other apparently spatially discrete clusters of burials (see Figure 3.1), led to the hypothesis that these remains may not be a random sample from the cemetery; rather, this suggests that an orderly program of burial was undertaken with clusters of burials representing certain biological kin units.

Since 19th century coffin hardware has been determined to change with time and across regional and cultural spheres in Upper Canada (Woodley 1992, Saunders and Lazenby 1990, and this author's personal observations involving three historic cemeteries of Upper Canadian pioneers), the interment artifacts were deemed inappropriate for use in status determination from archaeological mortuary analysis. Woodley (1992:62) suggests that,"...if a model based on a unilateral correlation between the presence and absence of grave inclusions does not work for historic cemeteries, then analyzing status from prehistoric cemeteries based on a similar assumption may be somewhat simplistic." Analysis of interment data to resolve possible kinship patterns was

therefore limited to using the spatial co-ordinates of each burial and the genetic data from the aDNA analysis. The cluster analysis and multidimensional scaling options from the statistical package SPSS (SPSS Inc. Chicago) were attempted but the data set was not large enough to meet the entry criteria. The identification of possible kin groups was limited to visual observations of spatial relationships and aDNA data in a manner similar to Stone's cemetery study (1996:135) where, "simple observation" was used to identify patterns between relative interment location and aDNA data.

3.3: Estimating Interment Dates from Coffin Hardware

In order to test the hypothesis of a symbolically based intergenerational kinship mortuary program, as well as ensure the sampling of burials over the entire time span of the St. Thomas' and Harmony Road cemeteries, it was necessary to establish the interment dates for every skeleton used in the DNA analysis. But due to the relatively small number of remains with associated documentation it was necessary to establish an approximate time of interment for the burials whose interment dates are unknown. Since a working chronology of coffin hardware has been established for nineteenth century burials elsewhere in Upper Canada (Woodley 1992) and at St. Thomas' (Springate 1994), and good artifact records are available for each burial, this approach was used to achieve approximate interment dates for the undocumented burials at

St. Thomas' and Harmony Road. Woodley's (1992) chronology was helpful with the Harmony Road burials, however the majority of his observations deal with changes in hardware after 1878, and therefore was not helpful in dating the St Thomas' burials. It was necessary to refine Springate's (1994) inspection of this hardware to suit the needs of this study.

The method of using hardware to date interments presupposes that new coffin construction technology and decoration would be used as it came into style locally, and that large inventories of older items would not be maintained for extended periods of time. Springate hypothesizes that:

> Coffin hardware is unique archaeologically, in that deposition is an integral part of its intended function. There should be, therefore, little manufacture to deposition lag, and the artifacts should represent the fashions and trends of the time. (Springate 1994:1.9)

Springate (1994) compared St Thomas' documented burials (N=72) with their associated grave goods, to historic research involving period hardware catalogues from North America (Russell and Erwin Manufacturing Company 1865, as cited in Springate 1994). A good example of one chronologically linked hardware item is found in the use of copper tack initials (CTI) and nameplates on coffins. Both of these hardware styles were used to provide a means of personally identifying and decorating the coffin, and were used exclusively before 1849 and after 1846 respectively (Springate 1994). Woodley's (1992) Stirrup Court hardware chronology also supports the

date of use of coffin plaques and similar decoration seen at St. Thomas' to after 1850. By using chronologically linked coffin hardwares, two sub-samples of burials were created for St Thomas' separated at mid-century (see Table 3.1).

Table 3.1: Chronology of coffin hardware used to date unknown burials at St. Thomas' (modified from Springate 1994).

Coffin Hardware	Date in Use	Number of burials with hardware item
A) Pre 1850:		
Copper Tack Initials	1825-1849	11
Copper Tacks	1825-1847	6
Carpenter's hinge	1825-1847	6
Handle, Lion's Head	1830	2
Handle, Kidney shape	1834	1
Handle, Wire	1825	1
Total identified burials from parish records		N=14
B) Post 1850:		
Nameplates	1846-1874	61
Caps & Studs	1865-1874	12
Coffin hinge	1862-1874	11
Coffin Tacks		
-with filigree	1857-1874	6
-fancy or plain	1862-1874	7
-white metal	1856-1974	22
Viewing Glass	1849-1874	11
Thumb screw/Escutcheon /Fancy Trim	1860-1870	3
Total identified burials from parish records		N=62

In order for an unidentified burial to be classed within the first half of the century for DNA analysis, the presence of at least copper tack initials, copper tacks, or a carpenter's hinge had to be present. These particular coffin hardware items were deemed more accurate and reliable indicators of interment date, rather than the more fashionable handles, because the tacks and hinges occurred with greater frequency and are probably related to the local manufacturing

technology then available in Upper Canada. The sample sizes for the pre-1850 coffin handle styles was also rather small, and would not necessarily eliminate the possibility of an 'out of style' handle appearing on a later interment. Besides buttons, handles were the only items to be found throughout the years the cemetery was in use; over 1500 handles of twelve different types are included in the hardware assemblage.

The presence of any item from Part B of Table 3.1 automatically categorized the burial in the latter half of the 19th century. Anomalous unidentified burials, in which items from both Parts A and B were present, totalled only six out of over 500 interments. Of these six, three features included carpenter's hinges and filigree screws, one with a mix of carpenter's hinges and coffin hinges, and two with copper tacks and filigree screws. The four burials having carpenter's hinges and Part B hardware may possibly be explained by the occasional practice of placing coffins inside coffin shipping boxes during physical burial (Austin and Dudar 1993). The hardware used on the coffin shipping box would have been more functional and less expensive and ornate than the coffin itself (ie plain carpenter's hinges). Upon subsequent deterioration of the wood, these items would have been incorporated into the general feature perhaps appearing to be part of the true coffin hardware. The two interments with both copper tacks and filigree screws may represent the

stylistic transition period from simple items to the more fashionable mass produced hardware from catalogues.

An alternative explanation may be found in the gender and class associations seen in mortuary styles. Cannon (1997, 1995, 1989) found that the rate of adoption of new gravestone styles in Victorian England was biased towards the upper classes, and male interments in particular. He demonstrated that 19th century English mortuary behaviour was grounded in the socio-economic dynamics of fashion, and that women were less socially prominent in Victorian England. Cannon (1997, 1995, 1989) concluded that this trend may be used in a more generalized way to infer relative social status in archaeological contexts. This may be the explanation for the problematic occurrence of hardware types from both periods.

The possibility also exists that lower social status burials in St. Thomas' cemetery may contain earlier hardware types despite a true (yet unknown) post-1850 burial date, thus leading to the incorrect assignment of these unidentified burials to the pre-1850 period. However, Cannon's findings do not refute the hardware chronology established by Woodley (1992) or Springate (1994) because both used male and female burials with documented interment dates. The fact that there appears to be a 'no overlap' time period of approximately ten years between Part A Hardware and the appearance of Part B Hardware lends strength to the assignment of interment phases.

Submitting the hardware data from the unidentified burials at St Thomas' to Springate's (1994) modified system of categorization resulted in a pre-1850 sample size for aDNA extraction of N=45 burials, and a post-1850 sample size of N=55 burials. Similarly, the Harmony Road burials were submitted to Woodley's (1992) hardware chronology to create 4 interment periods roughly corresponding to pre-1850 (N=11), 1850 to 1875 (N=12), 1875 to 1900 (N=13), and post-1900 (N=3). These 139 burials represented the maximum number of skeletal tissue samples that could be extracted and analyzed within the budget limitations of the Ontario Heritage Foundation Grant held by this author. Unfortunately, due to the early detection of systemic contamination problems, the actual number of extractions is less than the target number and does not involve the re-analysis of independent extractions.

3.4: Ancient DNA Extractions

Extraction of aDNA from the Harmony Road and St Thomas' cemetery samples was performed using the laboratory handling and cleaning protocols (with some minor modifications) of the Armed Forces DNA Identification Laboratory (1995), and the extraction method created by Yang and coworkers (1997, 1998). The samples used for aDNA extractions from Harmony Road included the first metatarsal (N=7), and maxillary molars (N=26), for a total of 33 extracts. The sample locations for St Thomas' included midshaft femur (N=77), clavicles (N=5),

and one radius with copper staining, totalling of 83 extracts.

Modifications to the handling and cleaning protocols for aDNA extraction include the elimination of the bone surface bleaching and bone pulverization steps. The use of bleach on the deteriorating periosteal surfaces of archaeological bone to destroy contaminating DNA may have also resulted in bleach access to deeper cortical tissues and endogenous aDNA. Bone pulverization would have required the purchase of expensive liquid nitrogen freezer mill equipment beyond the budget of this study. These procedures were thus replaced by the removal of an outer 2-3 mm of bone (for femora, clavicles, radii, metatarsals) with unused aluminum-oxide 80 grit sandpaper, and the use of titanium steel drill bits that had been soaked and scrubbed with 10% commercial bleach solution after every application. Disposable rubber gloves were changed, and bleach used to clean bench tops and exposed drill surfaces after every step. Approximately 1 to 1.5 grams of bone powder was obtained for the extraction protocol.

For extraction of aDNA from molars, the teeth (possessing protective enamel and cementum surfaces) were scrubbed in a 10% commercial bleach solution for approximately one minute, followed by several washes in double distilled water to remove traces of bleach. The teeth were then exposed to 20 minutes of UV light crosslinking on each side. Following surface decontamination, the teeth were frozen in liquid nitrogen and

submitted to the mechanical force of an industrial machine press. This provided sufficient force to powder even the largest of molars. The use of disposable rubber gloves and bleach on bench tops and exposed press surfaces was also completed after every sample. In this way one gram of powder was obtained containing enamel, cementum, and DNA rich dentin.

Each bone or tooth powder was placed in a new, sterile screw top 15 ml tube, and decalcified in 10 ml of 0.5 M EDTA pH 8.0 with agitation at 40 °C for 2 days. At this time, blank extracts were established containing no sample tissue, only the extraction reagents, and processed concurrently with each batch of 5 to 7 extracts. Each tube was then centrifuged and the resulting bone/tooth pellet retained. Three millilitres of Lysis buffer (0.5 M EDTA, 0.5% sodium dodecyl sulfate, pH 8.0) was added to the tube and the pellet resuspended. One 25 ml aliquot of 100 mg/ml proteinase K was added to each tube approximately every 12 hours for 48 hours during a period of agitation in a shaking water bath set at 40 °C.

Each tube was centrifuged to separate undigested tissue and the supernatant retained for concentration in a Centricon 30™ Microconcentrator. This was accomplished by centrifugation at 2000 g over several hours, with time dependent on the amount of extract volume. Approximately 250 µl of retentate was transferred to a clean sterile labelled tube and 1.25 ml of commercial PB buffer (Qiagen Inc.) was added (see QIAquick

spin Handbook 1997). This salt buffer/aDNA solution was then loaded in a clean and labelled QIAquick™ silica spin column and centrifuged for 1 minute. This last step had to be repeated twice as the silica column only holds 0.75 ml of solution. Unwanted oils, detergents, and other organic molecules were washed through the column by centrifugation of 0.75 ml of commercial PE buffer (Qiagen Inc.). Ancient DNA was then eluted with 30 to 40 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) by first incubating the column with the elution reagent for 1 minute, followed by centrifugation.

The extraction method using silica spin columns published by Yang and colleagues (1997, 1998) was chosen over the more established protocols of Hagelberg and Clegg (1991) when it was shown experimentally that ancient DNA extracted with the traditional phenol/chloroform and Centricon™ filtration technique did not amplify well, if at all. When re-extracted with the newer method, these failed extracts yielded a clean and concentrated template, without inhibitors, resulting in a successful PCR reaction (see Yang et al. 1997). The advantages and limitations of this method have not been totally explored, and are assessed in this study using a large number of individuals and a wide range of tissue sample types.

Disposable aerosol barrier pipette tips and screw top sample tubes were used in preference to the 'pop-top' style reaction tube. These cut down considerably on spot

contamination problems due to the reduction in aerosol formation. All extraction steps were conducted in a location separate from the area where PCR reactions and post PCR analyses were conducted. The use of designated laboratory clothing, reagents, and equipment solely for use in extraction of aDNA helped to eliminate early systemic contamination.

3.5: PCR Amplification of Mitochondrial DNA

The entire mitochondrial DNA (mtDNA) molecule is well characterized. It is composed of 16,569 base pairs arranged in a circle, with numerous polymorphisms throughout the 1.1 kilobase non-coding control region due to a rate of evolution approximately 5 to 10 times faster than nuclear DNA (Holland et al. 1993). The control region contains both the origins of transcription and replication, as well as the D-loop area (Anderson et al. 1981). Variability within the control region is not distributed randomly but is condensed within two hypervariable segments of approximately 400 base pairs each (Stoneking et al. 1991, Vigilant et al. 1991). Because of the polymorphisms and high copy number, mitochondrial DNA has been intensively studied in both living and past populations.

Stoneking (1995) has also discussed the inductive use of mtDNA as an indicator of contamination in ancient DNA studies. A good example can be seen with the dinosaur DNA sequence "discovered" by Woodward and colleagues (1994), but later shown to be most likely of human origin (Hedges and Schweitzer

1995, Zischler et al. 1995). In ancient human DNA studies this application would consist of determining the mtDNA haplotype of all those involved in handling the tissue samples, as well as those working in the lab. The mtDNA sequences amplified should also make contextual sense in both time and space; observing European lineages in precontact North America should warn researchers of contamination.

Holland and co-workers (1993) were able to identify the skeletal remains of an American Vietnam War aviator by comparing the mtDNA sequence of one of the hypervariable regions to that of the soldier's surviving mother and siblings. The existence of two polymorphisms within this maternal lineage at locations 16240 and 16291 was not found amongst a forensic database of 650 sequences. On this evidence alone the Armed Forces DNA Identification Laboratory was able to legally repatriate these skeletal remains to this family (Holland et al. 1993). Based on these findings, it was assumed that one hypervariable region would provide sufficient information to infer maternal lineage relationships in the cemeteries under study here. The established protocol of the hypervariable region of the D-loop investigated by Holland and associates (1993) and Yang (1997) was therefore implemented:

Primers:
MT1 5' CACCCTATTAACCACTCACG 3' Tm=55 °C
MT2 5' TGTGTGGAAAGTGGCTGTGC 3' Tm=53 °C
MT3 5' TGGAAAGTGGCTGTGCAGAC 3' Tm=57 °C (for sequencing)

For a 50 µl PCR reaction:

Buffer	50 mM KCl and 10 mM Tris-HCl
MgCl	2.0 mM
dNTP	0.2 mM
primer MT1	100 pmole
primer MT2	100 pmole
AmpliTaq	0.25 U
aDNA	5 µl of extract

PCR Cycling Protocol: 40 cycles
Initial denaturing at 94 °C for 5 minutes
Denature at 94 °C for 40 seconds
Anneal at 56 °C for 30 seconds
Extend at 72 °C for 40 seconds
Final extension at 72 °C for 7 minutes

A 'master mix' containing all PCR reagents sufficient for ten reactions was created and aliquoted into individual reaction vessels before any ancient DNA or positive control DNA was added to individual reactions. Blank PCR reactions containing only water were established for every eight reactions containing ancient DNA. These PCR Blanks were used to monitor spot contamination due to modern DNA aerosols or post PCR product fouling of the reaction reagents. The set up of PCR reactions in an area physically removed from the PCR machine and post-PCR analysis greatly reduced the chance of the latter source of spot contamination. No PCR blanks demonstrated spot contamination during the collection of the data presented here. PCR was carried out in a GeneAmp™ Thermocycler Model 2400 (Perkin-Elmer) set at 40-45 cycles.

3.6: Sequencing DNA

The mtDNA primers listed above amplify a 261 base pair sequence between positions 15 and 275 which contains a minimum

of 30 known polymorphisms (Piercy et al. 1993, Stoneking et al. 1991). Sequence determination of this aDNA PCR product followed the chain termination technique of Sanger and associates (1977). This sequencing technique depends on the use of the polymerase enzyme and inhibitors that terminate the newly synthesized DNA strand at each of the base pairs of the original template molecule. The inhibitors, or terminators, randomly take the place of normal deoxyribonucleotide triphosphates (collectively known as dNTP's) in the sequence. The best terminators for this method of sequencing are:

2',3'-dideoxyguanidine 5'-triphosphate (ddGTP),
analogue to guanidylic acid (dGTP), or 'G'

2',3'-dideoxyadenine 5'-triphosphate (ddATP),
analogue to adenylic acid (dATP), or 'A'

2',3'-dideoxythymidine 5'-triphosphate (ddTTP),
analogue to thymidylic acid (dTTP), or 'T'

2',3'-dideoxycytosine 5'-triphosphate (ddCTP),
analogue to cytosylic acid (dCTP), or 'C'

Since the terminators contain no 3'-hydroxyl group, the DNA strand cannot be extended further by polymerase action from the point at which the individual ddNTP's were incorporated. Termination therefore occurs specifically at positions analogous to their dNTP counterparts. If a primer and template molecule are incubated with polymerase in the presence of ddGTP and dGTP, as well as dTTP, dCTP, and radiolabelled [α-^{32}P]dATP, then a melange of fragments of different lengths all having the same 5' end (due to the

primer) and terminating at the various positions where dGTP normally is in the sequence, will be created. By obtaining products using the analogous terminators for the other dNTP's in separate incubations, loading them into adjacent wells of a denaturing polyacrylamide gel and applying current to the gel on an electrophoretic apparatus and subsequent autoradiography, a step-like pattern of bands is visualized on the film from which a sequence can be determined.

The sequencing of the 261 base pair mtDNA PCR product under study was carried out on each sample extract and reagent blank that yielded an observable product band under ethidium bromide fluorescence (see Figure 4.3.3 in Results chapter for an example). An AmpliCycle™ sequencing kit was used (Perkin Elmer) which provided the majority of the reagents, however the exact reaction conditions were altered to suit the individual level of amplification of each PCR product. This entailed altering the amount of starting template for the sequencing cycle, and increasing cycle number. In many cases, a very faint band could be observed under fluorescence but protocol manipulations did not yield a legible autoradiograph.

Successful PCR products were treated with 1 unit of exonuclease and 2 units of shrimp alkaline phosphatase for each 5 µL of PCR product, and incubated at 37 °C for 15 minutes followed by 80 °C for 15 minutes to heat denature these proteins. These steps were required to digest existing

primers and nucleotides that would interfere in sequencing.

Four separate labelled colour-coded tubes were organized for each mtDNA PCR product, corresponding to G, A, T, and C, and 2µL of each ddNTP terminator loaded into the respective analogue tube. A master mix for each sequencing reaction (the 4 tubes above) was created that contained all of the necessary reagents in these proportions to a total volume of 30 µL:

4 µL of AmpliCycle™ cycling mix (buffer and Taq enzyme)
1 µL of primer MT3 (100 ng/µL)
21 µL to 14 µL of double distilled water
3 µL to 10µL of PCR template digest
1 µL of [α-^{32}P]dATP (3000 Ci/mmol)

Manipulation of the master mix allowed the incorporation of more PCR template when ethidium bromide fluorescence indicated a weak amplification. Six microlitres of this mix was aliquoted into each of the coloured tubes containing the ddNTP's and mixed thoroughly. Inadequate mixing of the ddNTP in the master mix produces a failure of the reaction to terminate all possible positions in the DNA strand, resulting in many blank positions (if not a blank lane) where a base pair should be legible. Sequence cycling took place in a GeneAmp™ Thermocycler (Perkin Elmer):

Sequencing Cycle: 25 to 40 cycles
Denature at 94 °C for 40 seconds
Anneal at 58 °C for 40 seconds
Extend at 72 °C for 60 seconds

Following cycling, the reaction was incubated in 1 µL of terminal deoxynucleotidyl transferase, or TdT (2 units/µL) for

30 minutes at 37 °C followed by 3 minutes at 95 °C. The TdT was prepared by adding 1 µL of TdT (15 units/µL) to dNTPs and TE Buffer (10 mM Tris-HCl, 1mM EDTA, pH 7.5). This was done in order to avoid premature termination artefacts caused by DNA strands that did not successfully incorporate a ddNTP at the 3' end. These strands are grossly extended by this step and run much slower than the properly terminated DNA strands.

Four µL of commercial denaturing stop solution (Promega™) was added to the sequencing reactions and then denatured for 3 minutes at 94 °C to separate the double helix. They were immediately placed on ice before loading on 6% denaturing polyacrylamide gel. Three microlitres from each tube, corresponding to G, A, T, C were added to adjacent wells and run under current until the turquoise dye reached the bottom. A second loading was not required as the sequence is already well characterized by Anderson et al. (1981) and Stoneking et al. (1991), and separation of the bands was good over the area containing polymorphisms. The gel was dried and exposed overnight on Kodak™ XR-1 autoradiograph film.

3.7: Using mtDNA Sequences to Determine Individual Kinship

Allen and colleagues (1998) have described statistical approaches for using mitochondrial DNA sequences in human identification from forensic evidence. They suggest that the probability of identity by chance (PrIBC) can be calculated by determining the number of matches between a mtDNA population

database, the suspect's sequence, and the forensic evidence. By estimating this matching frequency, a 95% confidence interval (95% CI) can then be created. The application of this forensic method was altered slightly for use in this study by determining the probability of genetic kinship by chance (PrKBC) for those burials sharing a mtDNA lineage and associated within an identified cluster. This involved the use of the same equations outlined by Allen et al. (1998), however a more conservative estimate of this probability was calculated by the inclusion of both of the suspected kinship burial lineages in the database of the cemeteries.

PrKBC = x_i/n, where x_i is the number of database matches and n is the number of sequences in the database

95% CI = PrKBC +/- 1.96 x $[(PrKBC \times (1-PrKBC))/n]^{0.5}$

3.8: PCR Amplification of an aDNA Short Tandem Repeat Locus

The tetranucleotide tandem repeat polymorphism of the human tyrosine hydroxylase gene (HUMTH01 $[AATG]_n$) has been extensively researched and validated in a number of forensic populations (Gill and Evett 1993). This locus was used to demonstrate the efficacy of the extraction protocol to provide sufficient clean template aDNA for use in individual identification. Short tandem repeats are non-coding regions of nuclear DNA that demonstrate a variable number of tandem repeats (VNTR's) in their sequences (Jeffries et al. 1985). VNTR's can have di-, tri-, tetra-, pentanucleotide and larger

repeated core elements with alleles representing different lengths due to repeat number. They are short (generally less than 400 base pairs) and thus potentially provide a good DNA template for PCR when tissues have undergone taphonomic alterations (Hagelberg et al. 1991). The primer sequences presented by Kimpton and associates (1993) were utilized, and the PCR protocol optimized for use on ancient DNA as follows:

Primers:
TH01/1 5' GTGGGCTGAAAAGCTCCCGATTAT 3' Tm=72 °C
TH01/2 5' GTGATTCCCATTGGCCTGTTCCTC 3' Tm=74 °C

For a 50 µl PCR reaction:

Buffer	50 mM KCl and 10 mM Tris-HCl
MgCl	2.5 mM
dNTP	0.2 mM
primers	100 pmoles each
AmpliTaq	0.25 U
aDNA	5 µl of extract

PCR Cycling Protocol: 40 or 45 cycles
Initial denaturing at 94 °C for 5 minutes
Denature at 94 °C for 30 seconds
Anneal at 65 °C for 30 seconds
Extend at 72 °C for 60 seconds
Final extension at 72 °C for 7 minutes

A 'master mix' containing all PCR reagents was created and aliquoted into individual reaction vessels before any ancient DNA or positive control DNA was added. Blank PCR reactions containing only water were used to monitor spot contamination. No PCR blanks demonstrated spot contamination during the collection of the data presented here. PCR amplification was carried out in a GeneAmp™ Thermocycler Model 2400 (Perkin-Elmer) set for 40 or 45 cycles. Post PCR

genotype determination involved the separation of PCR products on a 6% denaturing polyacrylamide gel and the use of a Promega Silver Stain Plus™ kit (Promega 1996) to delineate individual allele bands. The individual PCR products were loaded beside an 'allelic ladder' representing each tandem repeat allele in order to accurately determine genotype (see Figure 3.2).

3.9: Interpretation of aDNA Short Tandem Repeat Genotypes

The interpretation and assignment of short tandem repeat alleles to aDNA amplifications can be difficult depending on the template quality and quantity. Figure 3.2 depicts a typical St Thomas' cemetery HUMTH01 STR analysis on a denaturing polyacrylamide gel with silver staining. PCR amplification of contemporary DNA is known to generate artifacts that appear as faint bands below true alleles, or "shadow bands" (Zierdt et al. 1996:190), presumably caused by slippage of the Taq enzyme at secondary DNA structures as it replicates the template molecule (Jeffries et al. 1992, Schlotterer and Tautz 1992). An example of this artefact can be seen in Lane B (contemporary DNA positive control: DY's DNA, genotype 9/9) of Figure 3.2 as a weaker PCR product migrating as an allele 8, below that of the true allele at position 9. Lanes E, N, P, Q, U, and V of Figure 3.2 are aDNA STR amplifications that also show this artefact but to a much greater extent.

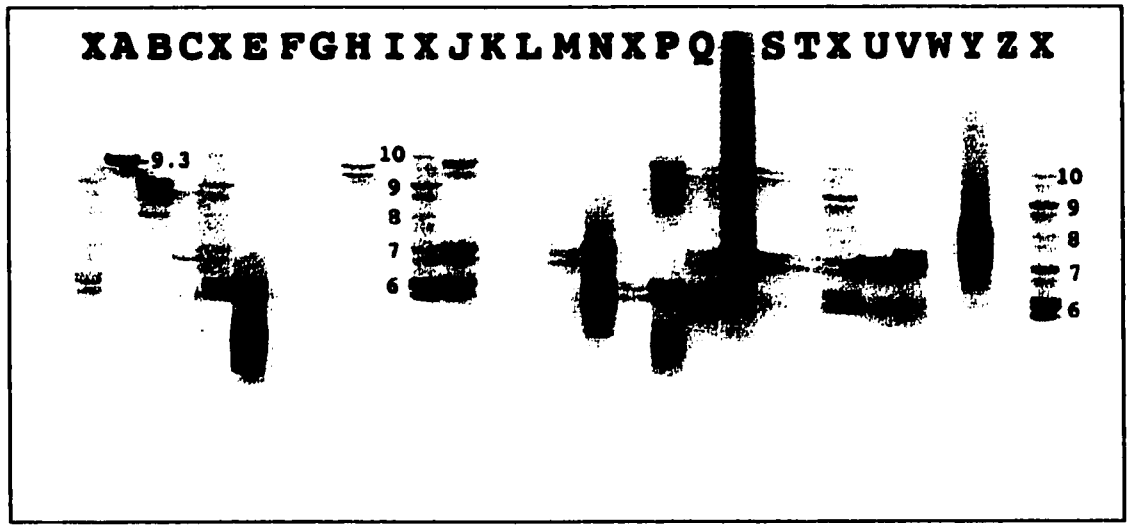


Figure 3.2: aDNA amplification of the HUMTH01 STR locus demonstrating PCR artefacts from St Thomas' extracts.

Lane X) Allelic Ladder; **A)** K562 control DNA; **B)** DY's control DNA; **C)** JCD's control DNA; **E)** burial 2; **F)** b 28; **G)** b 58; **H)** b 67; **I)** b 69; **J)** b 87; **K)** b 97; **L)** b 103; **M)** b 111; **N)** b 137; **P)** b 211a; **Q)** b 268; **R)** b 272; **S)** b 336; **T)** b 351a; **U)** b 361; **V)** b 362; **W)** b 397; **Y)** b 398; **Z)** b 423

In some aDNA amplifications the shadow band may be wider and darker than that seen with modern DNA because intact ancient template may be at a low copy number, prompting greater artefact replication. In some cases the shadow band does not present a characteristic allele band definition, but more of a "smudgy" thick band appearance. Ancient DNA STR analysis may be prone to this type of artefact because of template damage, such as baseless sites and strand breaks, and perhaps non-template Taq insertion of an extra nucleotide, more typical of di-nucleotide STRs (Jeffries et al. 1992). These problems of template preservation are doubled because each STR loci has potentially two different alleles present, a maternally and a paternally inherited allele.

'Thick shadow bands' and 'non-allele bands' running off the diagnostic allelic ladder positions (usually just above or below), can lead to confusion with regard to genotype scoring. The 'thick band' artefact is typified by Lane Y of Figure 3.2, and the 'non-allele band' artefact can be seen in Lane V also from Figure 3.2. Non-allele bands usually give some indication of a true allele position because they are generally thicker towards one ladder position and can therefore be easily rounded up or down. In the case of Lane V, Figure 3.2, the band was scored as an allele 7 because it overlapped with the ladder at that position. "Thick band' artefacts are more difficult to interpret, and may be

dismissed as a shadow band initially. Careful observation of the original gel with the thick band (no gel facsimiles should be used) over a strong light source can reveal a more defined band migrating at an allelic ladder position, something not seen with an ancient DNA shadow band artefact (Lanes E, N, P, Q, U, and V of Figure 3.2). In all of these cases, repeated PCR analysis is required to confirm the initial genotype.

Another problem leading to interpretive difficulties is the occurrence of allelic dropout. This occurs when insufficient undamaged template exists for one or both alleles of a two allele system (Zierdt et al. 1996, Gill et al. 1994). Since the Taq enzyme will preferentially amplify intact sequences, the PCR reaction may geometrically increase only one allele during one PCR, and in subsequent verification experiments amplify only the other allele. When shadow, thick, and non-allele bands are combined with allelic dropout, one can easily appreciate the difficulties inherent with determining an STR genotype for ancient skeletal extracts.

It became necessary in this study to set some criteria for genotype determination in order to deal with the problems discussed above, and thus be able to arrive at some tentative conclusions for the aDNA amplifications. 'Questionable genotypes' were defined as those PCR analyses which did not demonstrate the identical allelic bands in subsequent reactions, yet provided data to infer a genotype based on the

assumption that shadow bands and dropout are a common phenomena with aDNA and need to be understood as such in these circumstances. Further evidence that the sample extract contained endogenous aDNA was also required, such as a successful mtDNA sequence or an amelogenin result in agreement with previous documentation or morphological sex estimation.

An example of a questionable genotype is St Thomas' burial 2 (see Appendix 4.3.1) in which the first PCR yielded a strong 7 allele and a weak 6 allele, followed by the second PCR which yielded a strong 6 allele, a weak 7 allele, and a large shadow band at about allelic position 5 (see Lane E Figure 3.2). The questionable genotype was scored as a 6/7 taking into account that allelic dropout must have alternated between allele 6 in the first PCR, and allele 7 in the second PCR. The shadow band at allele position '5' is dismissed as a PCR artefact. Burial 2 also demonstrated an unambiguous mitochondrial DNA sequence, but failed to produce a PCR product for sex estimation.

'Confident genotypes' were defined as those that demonstrated the same alleles in repeated short tandem repeat PCR amplifications, and possessed unambiguous mtDNA sequences and/or successful amelogenin sex estimations. An example is St Thomas' burial 211a (see Appendix 4.3.1) in which the first STR analysis yielded strong bands at alleles 6 and 9.3, with subsequent analysis demonstrating strong bands at alleles 6

and 9.3, and weaker diffuse bands at allele positions 5 and 8 (see Lane P Figure 3.2). Since burial 211a also had an unambiguous mtDNA sequence (confirmed with an independent PCR amplification and sequencing reaction) and a successful sex estimation (albeit a weak X band), this data overwhelmingly supports the conclusion of endogenous aDNA in the extract, and allows the weaker diffuse secondary bands in the last STR analysis to be dismissed as PCR shadow band artefacts.

3.10: Using STR Genotypes to Determine Parent/Child Kinship

One of the problems with using mitochondrial DNA to study archaeological genetic relationships is that it can only identify possible maternal kinship ties. Stone (1996) has discussed the need for a more discriminating analysis, such as short tandem repeats, in order to discern precise genetic affinities between individuals who share mtDNA types. Even if researchers were able to reliably determine ancient STR genotypes, we would still need a method to quantify the probability of kinship since only one parent (likely the mother by mtDNA analysis) can be assumed with any degree of confidence. A method is presented here to calculate the probability of parentage amongst archaeological burials that is based on Brenner's (1993) paternity computation equations for cases where the genotype of the mother is unknown.

Paternity cases involve two scenarios: **Hypothesis 1)** The Alleged Father (AF) is the true father, and **Hypothesis 2)** The

father is an unknown Random Man (RM). DNA typing is then performed on the alleged father, the child, and the Mother (M) if available, and the ratio X/Y is calculated where:

X = the probability of observing the child and adult(AF and M) DNA genotypes assuming Hypothesis 1 from above,

Y = the probability of observing the child and adult(RM and M) DNA genotypes assuming Hypothesis 2 from above,

The quantity X/Y is known as the Paternity Index (PI) which reports how many times more likely the observed results are explained by true genetic kinship rather than by coincidence. The PI can be calculated for any number of statistically independent nuclear loci, and the simple product of these ratios used to derive a more discriminating overall, or total PI (Brenner 1993). The calculation of the individual X and Y probabilities can be best explained by a rudimentary example where the child's genotype is PQ at one locus.

X = Probability(AF passes P) x Probability(M passes Q)
+ Probabilty(AF passes Q) x Probability(M passes P)

Y = Probability(RM passes P) x Probability(M passes Q)
+ Probability(RM passes Q) x Probability(M passes P)

These general equations will work whether the mother's DNA is available or not, with changes needed only in the calculation of maternal contributions, such as the Probability(M passes Q). When the mother's genotype is known, these maternal probabilities can only be equal to 1 (when the mother is homozygous for that allele), 0.5 (when the mother is heterozygous at the loci), and 0 (when the mother does not

share that particular allele with her child). In cases where the mother's genotype is unknown, the maternal probabilities must take the point of view that the maternal allele is a random representative, ie. a Probability(M passes Q) with probability q, with q equal to the allele frequency from the relevant forensic population (Brenner 1993). Equations are created for each homozygous/heterozygous combination.

The archaeological situation for determining parentage is identical to the modern one except that instead of an alleged father (AF), this study uses the more general term 'alleged parent' (AP), to denote the ability to test either paternity or maternity (Parentage Index, or PI) depending on the archaeological and aDNA evidence. The equations for the various genotype combinations using this nomenclature are:

1)Heterozygous AP and Child:

AP		Q	R
Child	P	Q	

PI = X/Y = 1/4q where q is the frequency of the allele Q

2)Homozygous AP and Heterozygous Child:

AP		Q/Q
Child	P	Q

PI = X/Y = 1/2q where q is the frequency of the allele Q

3)Heterozygous AP and Child with the same genotype:

AP	P	Q
Child	P	Q

PI = X/Y = 1/4p + 1/4q where p and q are frequencies of the shared alleles P and Q

4)Homozygous AP and Child:

AP	Q
Child	Q

PI = X/Y = 1/q where q is the frequency of the allele Q

5)Heterozygous AP and Homozygous Child:

AP	Q	P
Child	Q/Q	

PI = X/Y = 1/2q where q is the frequency of the allele Q

The number values generated by the paternity index calculations have been assigned a verbal predicate by Hummel and associates (1971, as cited by Bryant 1988) and used widely in the legal milieu for parentage disputes. Table 3.2 presents the scale of PI values and the verbal predicates used by laboratories involved in paternity disputes (Bryant 1988).

Table 3.2: Verbal predicates associated with Paternity Index values (Hummel et al. 1971, as cited by Bryant 1988)

Paternity Index Range	Verbal Predicate
+399	Practically Proven
+95	Extremely Likely
+19	Very Likely
+9	Likely
+4	Hint of paternity
<4	Not Useful

The Parentage Index equations were tested on four contemporary paternity cases (provided by Dr J. Waye) with both the mother and father used as alleged parents, mimicking a variety of situations that may occur within a burial site environment (see Appendix 3.2). A variety of Parentage Indices were generated in each case for an increasing number of STR loci in order to demonstrate the range of PI values possible, and to make recommendations for future use in archaeological applications.

3.11: PCR Amplification for Sex Determination

The determination of sex from archaeological skeletal remains is important for reconstructing the demographic structure of past populations (Acsadi and Nemeskeri 1970) and establishing possible kinship structure (Howell and Kintigh 1996). In this study, aDNA sex determination was used to aid in the verification of individual identification through comparisons to historical documention of burials and via traditional osteological determinations of sex. Estimation of sex from skeletal remains has traditionally utilized morphological differences between the pelvis and cranium of male and female skeletons. Sexually dimorphic traits and measurements can establish sex with 80 to 90% accuracy (St Hoyme and Işcan 1989). But problems arise if remains are fragmentary and poorly preserved, if they come from a pre-pubertal individual, or if the observed variable falls in the range of overlap of male and female distributions.

Molecular genetic methods use the differences present on the X and Y chromosome to determine sex from skeletal remains, offering a means to analyze problem burials. Hummel and Hermann (1991) were the first to present a method for the determination of sex from human aDNA. Their technique involves the 60 PCR cycle amplification (40 plus 20) of a Y-specific sequence that contains an Eco RI restriction enzyme site. The 154 bp product can then be cleaved into fragments

of 102 and 52 bp in order to accurately ensure that the Y sequence was amplified. Their technique unfortunately relies on the absence of an amplification product in order to conclude female. This is unacceptable when working with aDNA because of the possibility of false positive results.

Other researchers have approached this problem by amplifying a sequence from the intron 1 region of the amelogenin gene, a locus found on both the X and Y chromosome (Sullivan et al. 1993). This specific region contains a 6 base pair deletion on the X chromosome that is not found on the Y. Male samples should thus produce two PCR products of unequal length (because they have both X and Y), and females only one product (only X). This technique was used with success on the Romanov family burials (Gill et al. 1993). Some difficulties have arisen producing mixed results possibly due to sensitivity problems (Stone et al. 1996) or unequal amplification of the X and Y sequences (Yang et al. 1998b).

Stone and coworkers (1996) have developed a technique that amplifies the amelogenin gene, but instead of relying on fragment size differences, they use sex-specific oligonucleotide probes (SSOP's) that only hybridize to X or Y. With 55 cycles of PCR (40 plus 15) and the use of this dot blot procedure, the amplification products produce two 'spots' for a male, and only one 'spot' for a female. They have found this technique to be accurate (95 to 100%) and sensitive,

attaining resolution with only 5 pg (one genome equivalent).

While this method solves earlier technical problems, it introduces the possibility of further spot contamination through the re-amplification of an existing PCR product. In order to access only the PCR product of interest, their method requires that the primary PCR product be run on an agarose gel, the band then excised, suspended in TE buffer, melted at 65 C, and then re-amplified for a further 15 cycles before the use of the lengthy dot blot procedure. All extra manipulations of a sample, or in this case a potentially powerful contaminating PCR product, before PCR amplification increase the risk of exogenous DNA entering the reaction and producing false results. In addition, Stone and associates' (1996) method is more expensive and time consuming to use because of the extra PCR and dot blot procedures.

Yang and associates (1998b) have improved upon the deletion-based amelogenin sex estimation method by incorporating a 3 primer system designed for a simultaneous amplification reaction that again produces two unequal fragments for males and only one for females. The primers consist of a common primer 'COM' to both the X and Y chromosome versions of amelogenin, an XA primer for the X chromosome allele, and a YA primer for the male chromosome amelogenin allele. The PCR conditions are optimized such that the efficiency of the COM-YA sequence is increased and the

efficiency of the COM-XA sequence is lowered, thus the COM-YA sequence will be amplified if present in sufficient amounts and quality. If COM-YA cannot be amplified from an aDNA extract, then COM-XA will not be produced because of its lower efficiency, virtually guaranteeing no false female results.

The efficiencies of the primer pair combinations are altered by the ingenious manipulation of their melting points (see below), and the purposeful lowering of the annealing temperature during the PCR cycle to suit the COM common primer. With the higher theoretical melting point of male YA (62 °C) compared to female XA (54 °C), and the annealing temperature of 50 or 51 °C, the male sequence will have an advantage in competition for the polymerase enzyme. This method has been shown to be very sensitive in tests with modern DNA serial dilutions with COM-YA product detected at the 5 pg level, with COM-XA 'dropping out' of competition at the 50 pg range (Yang et al. 1998b). This technique was implemented in the sex determination of the Harmony Road and St Thomas' skeletal remains because it requires only one PCR reaction, and its ease of analysis entails only polyacrylamide 'minigels' and ethidium bromide staining to achieve visualization of diagnostic bands:

Primers:

COM	5'-TCATTAACCACTR(A or G)CTCAG-3'	Tm=50 or 51 °C
XA	5'-TGTGACTATCTTAGAATCAG-3'	Tm=54 °C
YA	5'-TGCCCAAAGTTAGTAATTTTACC-3'	Tm=62 °C

For a 50 µl PCR reaction:

Buffer	50 mM KCl and 10 mM Tris-HCl
MgCl	2.0 mM
dNTP	0.2 mM
primer	100 pmole
primer	100 pmole
primer	100 pmole
AmpliTaq	0.25 U
aDNA	5 µl of extract

PCR Cycling Protocol: 45 cycles
Initial denaturing at 94 °C for 9 minutes
Denature at 94 °C for 40 seconds
Anneal at 50 °C for 30 seconds
Extend at 72 °C for 30 seconds
Final extension at 72 °C for 7 minutes

A 'master mix' containing all PCR reagents sufficient for ten reactions was created and aliquoted into individual reaction vessels before any aDNA or positive control DNA was added to individual reactions. Blank PCR reactions containing only water were established to monitor spot contamination. No PCR blanks demonstrated spot contamination during the collection of the data. PCR amplification was carried out in a GeneAmp™ Thermocycler Model 2400 (Perkin-Elmer) set for 45 cycles. Visualization of PCR products for sex determination was conducted on polyacrylamide gels stained with ethidium bromide. Both male and female positive controls were run with aDNA product to assist in the determination of sex.

3.12: aDNA Quantification

Ancient DNA quantification was attempted using the protocol of Waye and coworkers (1989) and the related protocol of the Quantiblot™ kit from Perkin Elmer (see also Walsh et

al. 1992). Three attempts at quantifying aDNA using these probe based methods were conducted without success. The reason for the failure of these established protocols is unknown. It has been argued that the amounts of human aDNA recovered from archaeological bones is often too small to be directly detectable by methods using radioactive probes and that a PCR quantitative method should be used (Handt et al. 1994a). While this is a possible explanation, it has been demonstrated that as low as 80 pg (16 cell equivalents) of contemporary nuclear DNA can be detected with the ^{32}P labelled p17H8 probe after only a 12 hour exposure on autoradiographic plates (Waye et al. 1989). Extending the exposure time (such as the two day exposures attempted here) should theoretically increase the sensitivity of this procedure (Waye et al. 1989).

Ancient DNA quantification involving the competitive PCR method of Handt and colleagues (1994a) could not be attempted because this technique requires that multiple aDNA amplification reactions be conducted simultaneously on every extract. This is required in order to compare the strength of the aDNA product, and the point at which aDNA is no longer amplified, to that of a known dilution series of modern DNA. Insufficient extract volume was available to conduct this protocol because the sample extracts were used up in amplification reactions involving STR reliability, mtDNA sequencing, sex determination, and quantification involving

the other protocols previously mentioned. Funding limitations precluded the re-extraction of the samples from the bone or tooth tissue. Accomplishing aDNA quantification from a second extract would not yield an accurate template estimate in the first extract from which the majority of data was collected.

3.13: A Model for Using aDNA Data to Infer Biological Kinship

The use of aDNA data to infer biological kinship amongst archaeological burials must involve multiple lines of evidence. Many genetic indicators of relationship need to be used in conjunction with supporting archaeological and osteological analysis, and if possible, historic documentation. The following section outlines the inferential strategy used to test for biological relationships within the pioneer cemeteries in this study. This recommended strategy is based on the principle of consilience, that two or more independent yet supporting lines of evidence are used to draw conclusions that possess more confidence than those using fewer sources (Rouse as reported in Siegel 1996).

Mitochondrial DNA haplotype data can be used to provide initial points of kinship investigation such as nonrandom clusters of lineages observed within burial grounds. In the absence of mtDNA groupings, unique mortuary arrangements and spatial distributions, cultural manifestions of burial practice, or osteological nonmetric traits can be used as foci for further analyses. The establishment of a burial

chronology through stratigraphy or other meaningful material culture evidence, such as coffin hardware, will greatly aid in the reconstruction of kinship from the archaeological record.

With the points of interest thus located, the use of standard osteological analyses, such as age at death and sex estimation, will help in the resolution of hypothesized relationships. It is very unlikely that a young adult female sharing a mtDNA lineage with an elderly male of the same burial phase is that man's mother (depending on the accuracy of the estimated interment dates). It is also unlikely that the elderly male is the father of the young female unless consanguinity is suspected between the father and mother. This suspicion must be placed within the context of that particular mtDNA lineage frequency. Other maternal links are more likely such as a sibling, or a maternal cousin or uncle, but these types of genetic ties are considerably more difficult to test statistically. With any suggestive intergenerational genetic relationship, the use of the mtDNA probability of kinship by chance, and STR based parentage index can be computed and tentative inferences drawn.

Results from these statistical procedures are only meaningful if placed in some context. Returning to the example above, depending on the context of the community and the frequency of the mtDNA lineage, the possibility exists that there is no close genetic relationship between the young

woman and the older man. Over one-fifth to one-third of all Britons possesses the mtDNA reference sequence (Piercy et al. 1993, Richards et al. 1996), yet have no known recent genetic link. In this study the probability of kinship calculations were evaluated based on the presence of archaeological spatial and historical document evidence of biological relationships. Only after thorough consideration of all lines of data were inferences made on the putative kinship relationship, the nature of the spatial relationship to family property, and how formal family burial areas support the hypothesis of land as a critical resource in pioneer Upper Canada.

3.14: Isonymous Marriage Records Analysis

Assortative mating strategies have been described by Eckland (1971:86) as, "...one of the important links between the physical and cultural components of man's [sic] evolution." Divergence from random mating, due to a common cultural characteristic amongst partners, can split the population structure into kinship groups, such as religious isolates, socially isolated castes, and economic groups (Bamshad et al. 1998, Eckland 1971). A vast number of population structure studies on past and contemporary societies have shown a significant departure from random mating (see Crow 1996, Lasker 1985). Mitterauer and Sieder (1982) and Gagan (1981) contend that pre-modern European and Upper Canadian pioneer marriages were primarily determined by

the social and economic needs of the family.

Marital isonymy refers to marriages where the bride's maiden name is the same as the bridegroom's, and therefore indicates some form of assortative consanguineous or affinal union based on an inherited surname. A search for these types of non-random matings in the St Thomas' marriage records would help to reconstruct intragenerational, or 'sibling' kinship alliances (Keesing 1975, Marshall 1979). Basic isonymy analyses following Crow and Mange (1965) were carried out on the marriage records from St Thomas' and Christ Church Anglican parishes, both located in Belleville. The original St Thomas' congregation split with a significant portion leaving St Thomas' to form Christ Church Anglican in 1865 (De Vito 1994a). Isonymy analyses conducted on documents after 1865 incorporates both sets of marriage records.

The genetic relationship between isonymous couples is taken to be the probability that they share an autosomal gene by reason of descent from a common ancestor. Their kinship is estimated as twice the degree of their genetic relationship, but the degree of inbreeding in any individual is only half the degree of relationship of the parents to each other (Lasker 1985). When a proportion of isonymy is used to express a genetic coefficient of relationship it is divided by 2, but when used to express an inbreeding coefficient by isonymy, it is divided by 4. Since the majority of the

surname analysis literature is geared towards coefficients of inbreeding by isonymy, this calculation was chosen to simplify comparisons. Crow and Mange (1965) developed the following equations for estimating the total inbreeding coefficient F, the random component F_r, and the non-random component F_n.

$$F=I/4 \qquad F_r=\Sigma(p_iq_i)/4 \qquad F_n=F-F_r$$

where I = the proportion of marital isonymy (number of isonymous marriages divided by the total)
p_i = the frequency of the ith surname in males
q_i = the frequency of the ith surname in females

The random component refers to events in past generations, and is a measure of gene frequency change during that time. It is the cumulative result of all processes, random and non-random, that have produced changes in surname frequencies and hence gene frequencies (Crow 1983). The non-random component is a measure of departure from random mating in the current generation. Effects measured by F_n would disappear if the population were to mate at random, whereas effects of F_r are permanent (Crow 1983).

In order to complete a study of isonymy on the marriage records of St Thomas' and Christ Church, certain assumptions must be made about the population of parishioners in order to satisfy the theoretical foundations of the statistical analysis:

1) Surnames are monophyletic, each surname has one origin in a common ancestor and follows biological inheritance. Name changing, adoption, illegitimacy, changes in spelling, and errors of transcription may lead to errors in interpretations.

2) Non-random mating must not discriminate between males and females. Cousins related through brothers must mate with the same frequency as cousins related through sisters.

3) The population should be a monogamous society due to differential numbers of children possible between sexes. eg. polygyny or polyandry

4) Both sexes are equally represented among migrants

Keeping in mind the crude nature of isonymy analysis, decisions about study samples violating these four assumptions must be put in perspective. If a population could theoretically meet all of the assumptions, the isonymy method still does not take into account, nor is there any way that it could, the cumulative effects of inbreeding, ie. a distant common ancestor that was inbred. Isonymy will thus underestimate the total inbreeding coefficient (Crow 1983). The assumptions should, in this author's view, be used as guidelines to remind investigators of the "...quick, easy, cheap and crude..." nature of isonymy studies (Crow 1983:383). With the assumptions put in perspective, in what way(s) are they violated at St Thomas' and Christ Church?

The fact that name changing, adoption, and illegitimacy are generally not part of aggregate historic records means that the first assumption of monophyletic origin of surnames may never be fully established. To fully meet this assumption would require the detailed genealogical research of several families across multiple countries. Herring and coworkers (1998) have found a low ratio of illegitimate to legitimate

babies in the baptism records of St Thomas', however Drake (1974) has observed such ratios to vary dramatically.

Transcription errors are similarly an unmeasurable commodity, especially primary errors in the original records. Saunders and colleagues (1993) state that the transcription of the St. Thomas' and Christ Church records into subsequent database files were checked twice by different individuals for such errors. Spelling changes of surnames over time may be negligible due to the relatively brief duration and recent source of the marriage records. The fact that the majority of immigrants into Upper Canada and Belleville had English as their first language (Saunders et al. 1993) probably meant that their surname was presumably well established by the time they emigrated in the nineteenth century.

The calculation of inbreeding coefficients and surname frequencies did involve the use of both the raw marriage records and standardized spelling for surnames involved in isonymous marriages (see Appendix 3.2 for a list of alternative spellings). The Family Search computer data base at the Mormon Family Library provides a list of alternative spellings for each surname based on verbal similarity unaffected by minor spelling variation, as well as common country/ethnic group origins.

It must be assumed that the marriages on record at St. Thomas' and Christ Church were in fact monogamous marriages,

both bride and groom having only one spouse at one time. This assumption is in accordance with the societal beliefs in Upper Canada and Canon law in practice in the Anglican Church (Goody 1983). Any rare deviations would have a negligible impact on this study, and would be very difficult to uncover if present.

The assumption of non-random mating and discrimination between males and females is similarly unquantifiable and beyond the scope of this study. It would again require the accurate reconstruction of several genealogies involving multiple families in each genealogy in order to determine whether cousins related through brothers had mated with the same frequency as cousins related through sisters. This assumption must therefore be accepted with some caution.

Are both sexes equally represented among the migrants into Upper Canada? This assumption for isonymy studies is in fact violated due to the observed bias in the male to female ratio in Upper Canada (Gee 1982, McCalla 1993), and most certainly at Belleville (Burke 1994). Males probably represented the majority of the inhabitants for most of the early history of Upper Canada, however no reliable or accurate data sources exist prior to 1851 (Gee 1982). This sex ratio may have been due to the early bias towards male immigration because of the physically demanding pioneer lifestyle. The male to female ratio did not approach 1:1 until approximately 1861 (Gee 1982) or 1871 (McCalla 1993), after which the ratio

was in favour of females. This reversal may be explained by the re-emigration of thousands of males to the expanding frontiers of the United States (Darroch and Soltow 1994) or Western Canada, and/or the influx of predominantly female immigrants at this time (Saunders et al. 1995). As we will see later, this may have had an influence on the marriage patterns of the St Thomas' parishioners.

3.15: The St. Thomas' and Christ Church Marriage Records

Marriage records from the years 1821-1899 from the St. Thomas Anglican Church and the years 1865-1899 from Christ Church Anglican (see Figure 3.3) serve as the samples for isonymy studies in this dissertation. Both Rogers (1991) and De Vito (1994a,b) have submitted the marriage, baptism, and burial records of St. Thomas' and Christ Church to Drake's (1974) analysis to insure that the parish records do not misrepresent the population. Roger's (1991) conclusions were that the St. Thomas' records are not perfect in every respect. De Vito (1994b) has also identified several time periods in the St. Thomas' records where they deviate from established trends, such as drops that occurred in marriage records from 1831-1841, and 1865-69. Her study was limited to the time period of 1821-1874, and thus did not notice the conspicuous drop that also occurred from 1875 onwards to the end of the century at St. Thomas' and Christ Church (see Figure 3.3).

The anomalous drop in marriages that occurred during

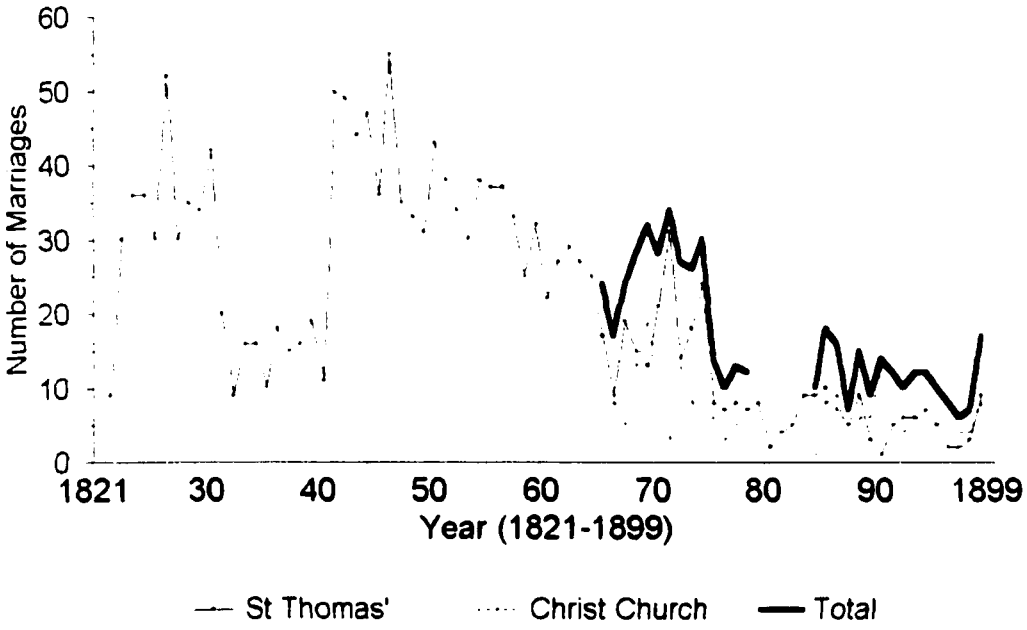
1831-41 at St. Thomas' can be partially explained by the cholera epidemics of 1832 and 1834. During this time the town council posted armed guards on the roads entering Belleville (Mika and Mika 1986). This may have prevented many rural St. Thomas' parishioners from contributing to the records. A more cogent theory can be found in the passing of the Marriage Act of Upper Canada in March 1831, which gave clergy of all denominations the right to perform ceremonies (De Vito 1994a).

> The Rev. Thomas Campbell, rector of St. Thomas' in Belleville, moaned to his bishop: 'Previous to the passing of the marriage act, my marriage fees averaged about £40 a year. One year they exceeded £55. In the year 1832, they were so few [nine, he reports elsewhere] that I kept no regular acct.[sic] of them. They could not have exceeded four or five pounds. (Lamb 1990:72)

Also during this time period, several churches of various denominations opened their doors for services in Belleville, theoretically drawing from St. Thomas' practical monopoly on marriages. A small wooden Roman Catholic Church, St. Michael's, was built in 1829 to service the growing Catholic community (Mika and Mika 1977). St. Andrew's Presbyterian Church was erected in 1831 (Mika and Mika 1977).

The 1865-69 drop in marriages at St. Thomas' can be attributed to the opening of Christ Church Anglican parish. The St Thomas' congregation was established after a split occurred between Reverend Jones and Reverend Grier over the renting of pews and other conservative practices such as vestment colour and table leg design (Bellstedt 1968). The

Figure 3.3: St Thomas' and Christ Church Marriage Records 1821-1899



more liberal Christ Church was thus established as the first church in the Anglican Diocese to abandon renting pews (Mika and Mika 1986). Figure 3.3 demonstrates that if the St. Thomas' and Christ Church marriage records are totalled, the resultant line shows no visible drop in the trend of marriages until 1875. It appears that the events recorded following the schism of the two parishes may in fact represent a single Anglican community divided over fundamental differences in opinion on access to community religion, and other conservative dogma.

The Christ Church records have unfortunately been deemed "inadequate for use in any large scale analysis" by themselves (De Vito 1994a:7). This conclusion was reached due to the small number of total entries (less than 50 entries for 26 of the 36 years analyzed) for baptisms, burials, and marriages; too many gaps appear in the records that cannot be accounted for over such a short period of time. In addition, the fact that 31 different individuals are responsible for the entries may lead one to conclude that the records were not systematically or competently maintained.

The final decline of the total marriage records of St. Thomas' and Christ Church from 1875 to 1899 (see Figure 3.3) may be partially explained by the addition of a third Anglican parish, St. John's, to Belleville in 1874 (The Archivists of the Ecclesiastical Province of Ontario 1990, as cited by De

Vito 1994b). This coupled with the gutting of the St. Thomas' church by fire in 1876, and the fire at Christ Church in 1881, provides additional evidence for the decline in the number of marriages seen at St. Thomas' and Christ Church.

As the rural regions surrounding Belleville developed and grew in population, more Anglican churches were established in Hastings County. The Archivists of the Ecclesiastical Province of Ontario (1990, as cited by De Vito 1994b) list seven additions over a 50 year period:

1829	Tyendinaga, Christ Church
no date	Marmora, St. John's
1840	Shannonville
1850	Stirling, St. John's
1853	Rawdon
1863	Madoc, St. John's
1873	Marmora, St. Paul's.

The addition of these churches to the small communities that surround Belleville may also have contributed to the declining marriage numbers seen in the St. Thomas' and Christ Church records. Instead of prospective brides and grooms and families travelling to Belleville for their marriage ceremony, it would have been more convenient to be wed closer to home.

The St. Thomas' marriage records thus represent a segment of the Anglican parishioners of the Belleville region. The conspicuous declines of marriage totals can be explained by the Marriage Act of Upper Canada in 1831 and the addition of several new Anglican parishes both within Belleville and in the developing rural areas of Hastings County. Unfortunately,

the parish records of Christ Church do not meet the requirements as set out by Drake (1974). These marriage records were still submitted to the isonymy analysis of Crow and Mange (1965) in order to detect any possible trend in inbreeding at St. Thomas' that was continued at Christ Church after the schism. The two parish records were also pooled into a single sample for analysis. Any conclusions drawn from the analysis of the Christ Church records must necessarily be interpreted carefully.

3.16: Summary of Materials and Methods

A number of lines of evidence are used in this study to test the association between symbolic kinship mortuary behaviour, kin marriage alliances, and control and management of vital land resources in Upper Canada. The combined implimentation of archaeological, osteological, historic records, and aDNA has created a powerful test for these hypothesized relationships. By incorporating a variety of data sources within an ethnohistoric context, more confident interpretations are made and a much richer understanding of past events is achieved.

Chapter Four: Results

The influence of a vital resource pressure on a corporate kinship structure will be demonstrated through: the symbolic meaning of mortuary programs as observed in patterns of aDNA, and the alliance strategy detected from analysis of related marriage records. It is however necessary to first document the historiographic social context that acted as this vital resource pressure. Research involving human aDNA runs the risk of becoming an end unto itself unless it is linked in a systematic way to the archaeological record and ethnohistoric context of the individuals and communities under study.

It is argued that the two perspectives on kinship under study here, inter- and intragenerational, are significantly interwoven with the social pressure of access to good quality land in pioneer Upper Canada. The ethnographic identification and support of the premise of land as a vital resource is integral to this research. The quantitative and qualitative results of the historiographic research will therefore be discussed first in some depth, followed by the marriage records analysis and finally the aDNA. This presentation order will provide a solid socio-cultural contextual base for interpreting the more technical molecular methods used in this study.

4.1: Historiography of Land, Policy, and Immigration in Upper Canada

> The holding of exclusive rights, then, gives rise to the general problem of possession, the tension between the haves and the have-nots. (Goody 1962:283)

The first substantial wave of Europeans to arrive in Upper Canada took up land and formed communities and centres of trade near the waterways. This provided not only ease of transportation, but also hydraulic power for grist and saw mills. Land location was vital to the success of any pioneer endeavour in a wilderness devoid of roads and the support of neighbours and kin. These first settlers were responsible for establishing the access to the best locations through the manipulation of formal land policy, for much of the good arable land was alienated at an early date, despite the very small population. This section presents both quantitative historic research, and qualitative ethnohistoric accounts supporting the argument that land was perceived as a vital resource. While a biased account may be presented by some of the primary and secondary sources used here, it is believed that the opinions themselves represent a valuable indication of popular belief at the time (Cox 1995). This perception, perhaps more than reality, would therefore have worked to shape symbolic intergenerational mortuary practice and intragenerational kinship marriage alliances amongst the pioneers under study.

The Family Compact in Upper Canada

It has been argued by 19th century contemporaries, and the historians studying them, that a nebulously defined 'Family Compact' caused a shortage of land to develop across Upper Canada through the political manipulation and patronage appointments within the bureaucracy of colonial Upper Canada (Gourlay 1822, Mackensie 1837 as cited by Johnson 1971, Canniff 1872, Haight 1885, Wallace 1915, Saunders 1957, Hamil 1967, Gates 1968, Landon 1974, Baskerville 1981, Gagan 1981, Johnston 1977, 1990). In this way unimaginable amounts of land were presumed to be held by these inbred aristocratic absentee land owners. Recently, Moorman (1997) has criticized this position believing that various forms of favouritism were not the important factors involved, but that frontier and technological limitations such as: problems with communication, office and survey equipment, poor records, and working conditions, invited confusion and delays. He still concludes however that poor policy inadvertently encouraged land speculation which contributed to pioneer hardships.

That a division of wealth and class developed and propagated itself in Upper Canada through land acquisition is not unique amongst other developing colonies. Weaver (1996:982) states that, "Diverse peoples in the London-based world economy of the early nineteenth century --from frontier settlers to urban capitalists-- contrived low-cost methods of

grabbing land." This most certainly was the case for the first settlers of Upper Canada who had early access to choice land as township surveys were completed. These families, mostly United Empire Loyalists, made the effort to persist on their founding farms in well established communities, often selling free ancestor land grants in wilderness locations to pay for farms closer to home and kin.

Early Immigration, Land Policy, and Settlement

After liberty was granted to the new United States of America many refugees, and the militia and British soldiers who fought in the war, were settled in Upper Canada with the hope of stopping American expansion into Crown land. Farms and communities were established on choice land ranging from the headwaters of the St. Lawrence River, across the north shore of Lakes Ontario and Erie, over to the Niagara and Detroit rivers. This pattern of settlement along waterways not only provided the best means of transportation, but also established a defensive border against the United States (see Figure 4.1.1). Darroch and Soltow (1994) estimate the population of Upper Canada at 20,000 people at the turn of the 18th century, composed mostly of wealthy land owners who had occupied high positions in the old British colonies (Canniff 1872, Haight 1885).

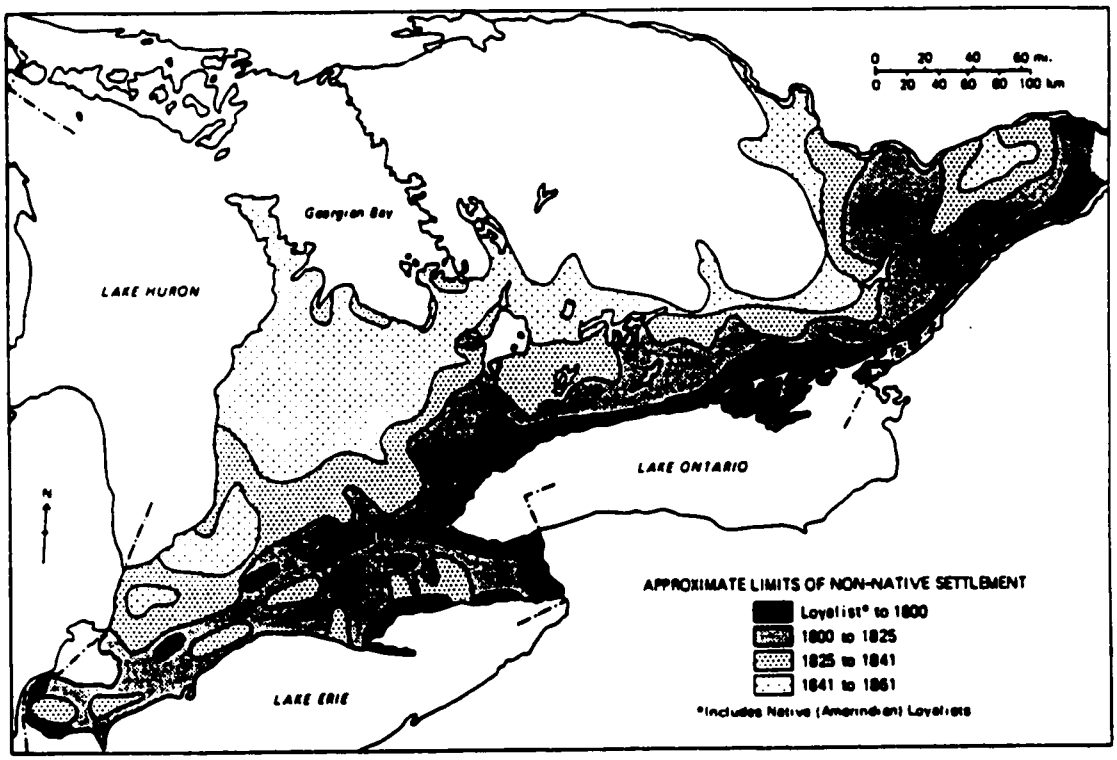


Figure 4.1.1: Limits of settlement in Upper Canada to 1861 (adapted from Keane and Read 1990)

The privilege of freehold land tenure was bestowed on all soldiers and refugee families by General Frederick Haldimand, later know as Lord Dorchester, when he realized that the 'Loyalists' were unlikely to remain loyal without some material compensation (Moorman 1997). Gagan (1981:6) believes that at this time "... owning [land] or speculating in it was the proven road to security" in the embryonic economy of Upper Canada. This opinion has broad support from recent scholars (Darroch and Soltow 1994, McCalla 1988, Moorman 1997). The early settlement process was often haphazard with no clear method other than to entice Loyalist pioneers with the offer of free land. Non-loyalist immigrants from the British Isles were required to pay settlement fees and perform duties on granted lands.

The Loyalist land grants consisted of 5000 acres for a field officer, 3000 acres to a captain, 2000 acres to a subaltern, and 200 acres to a private soldier or refugee citizen (Gates 1968). This land grant policy caused a renewed influx of families from the United States, or 'Late Loyalists', who were seen as opportunists by the original settlers (Canniff 1872). In order to keep the peace, Lord Dorchester granted a further 200 acres to settlers who had already improved their land. This popular provision, coined "Lord Dorchester's Bounty", was meant to be only temporary (Gates 1968). In addition to this, the United Empire Loyalist

(UEL) regulation was passed in 1789 allowing for the immediate descendants (at first only the sons, but later the daughters, and then their grandchildren...) entitlement to grants of 200 acres each, free from all expenses. However, like many of the colonial land laws created in this era, both varieties of loyalist took full advantage of the system (Gates 1968:18).

Dorchester's Bounty and UEL descendent rights "took on a life of their own" causing many complications in future land policy decisions (Moorman 1997:19). Applications for Lord Dorchester's Bounty continued to be received to 1797, and UE Loyalist grandchildren fought for, and secured, their 200 acre grants up to 1837 (Johnson 1971). The final cost to the Crown would be 3,300,000 acres and £75,000 in land revenue (Gates 1968). Instead of a healthy economy, the land grant and alienation process encouraged elements of lax regulation and petty bureaucratic corruption (Moorman 1997), but surely created an unbridled land speculation business that inhibited growth and caused undue hardship to common immigrant pioneer families (Gourlay 1822).

The creation of Crown and Clergy reserves were initially proposed as an alternative to taxation. These lands would be leased or rented to provide for the financing of the administration. Reserves were laid out in a chequered pattern of 200 acre blocks, totalling one seventh of all the land in townships surveyed (see Figure 4.1.2). However the option of

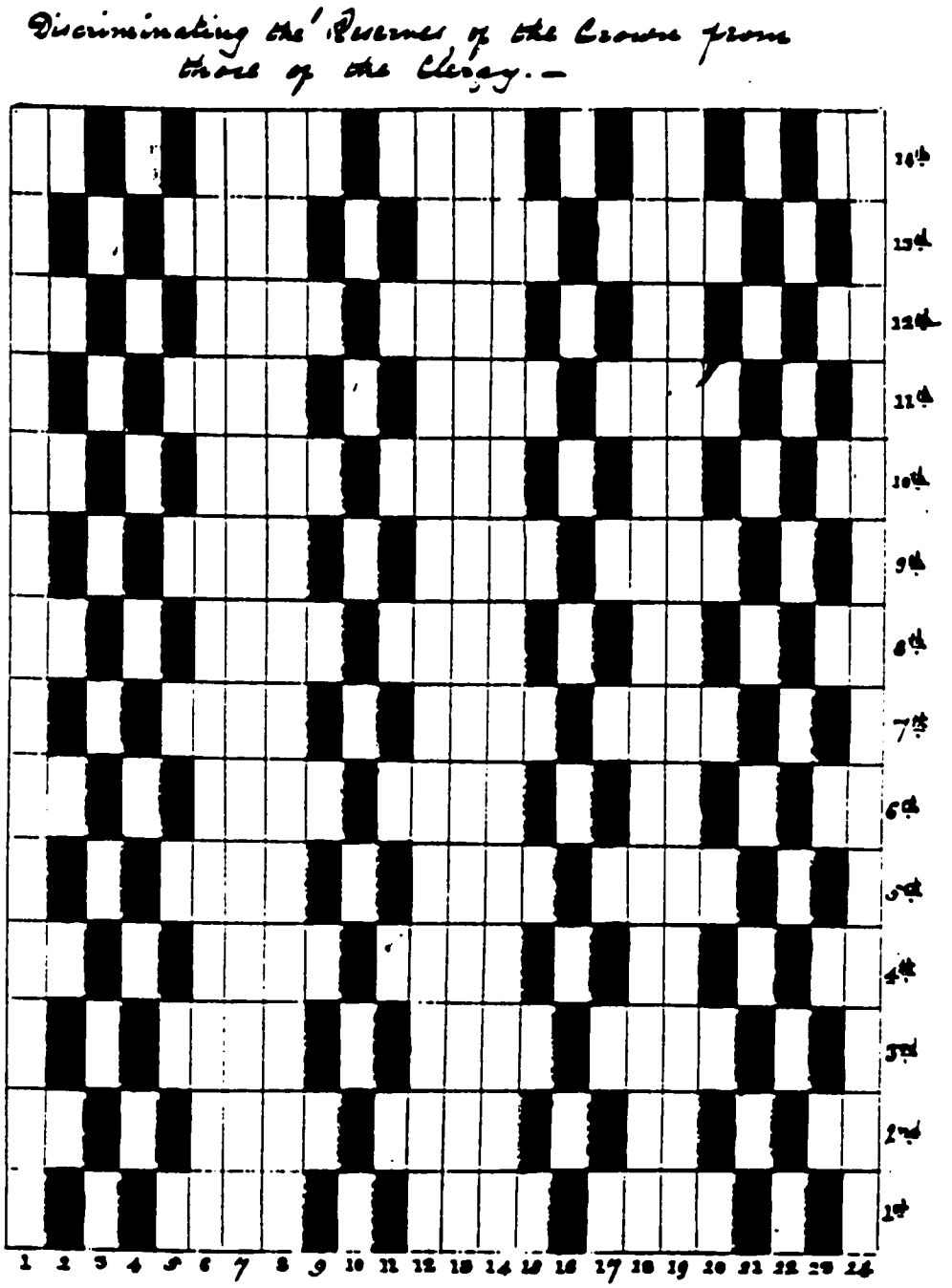


Figure 4.1.2: The chequered plan of Crown and Clergy Reserves totalling one seventh of the area of every township (adapted from Moorman 1997)

owning land outright was readily available to all immigrants, making the action of improving rented land a rather foolhardy undertaking. Approximately three or four acres of forest could be cleared in a year, with the soil fit for a plow in approximately ten years after the stumps had rotted and could be leavered out (Houston and Smyth 1990). Not only was the leasing of these reserve lots a "dismal failure", but it left nearly one third of all concession roads unfinished (Moorman 1997:309). Since by law settlers had to perform the duty of clearing the thickly forested front of their property for these roads, many were left with the added task of clearing the reserve portions as well, or be left with a road that went nowhere.

The lack of a levy on unused land combined with the inability, or lack of motivation of the administration to enforce settlement duties, meant that many of the early pioneers took up more land than they could occupy. This, in concert with the blocks of Crown and Clergy reserves, prevented the orderly and compact formation of townships and support groups of farms (Gourlay 1822). Later pioneers would therefore have to take up land locations further into the bush with no neighbours, bordered by Crown and Clergy reserves and speculated property owners with no intention of clearing their road allotment. More burdens were placed on these struggling families who arrived after the initial wave of Loyalists.

Wood (1988) has detected demographic differences within the population of Upper Canada during this early settlement period. He identifies three types of pioneer: transients who stayed less than two years, sojourners of five to seven years who either farmed, rented land or sold their grant, and the persisters, the founding families of the townships, who accounted for one-third to half of the population (Wood 1988). Gagan and Mays (1973) support this generalization, finding that approximately three-fifths of all Peel County households were landless, with a 75% turnover of these families. In contrast, the remaining two-fifths of landed households experienced only a 25% turnover. These persisting families must have quickly established themselves, expanded their holdings, and perhaps increased their influence in the colonial capital, York.

> These leading men throughout the Province were in most cases closely united by consanguinity and marriage; and soon became even more closely identified in interest - forming a strong political body, which derived its life-blood from the Executive. ...choice bits of land were granted to members of this strong family, compacted together, to help one another, and the land was left uncultivated, unimproved, until the energies of the pioneers around had made it more valuable.
>
> (Canniff 1872:584)

The Methods of the Land Speculator

Most land speculators prospered without the official favours of the Executive and Legislative Councils (Moorman 1997). These individuals acquired thousands of acres by

securing the land rights from indebted settlers who later failed in their struggle against the bush (Gates 1968, Howison 1821). These purchases propagated a vicious cycle of hardship for new immigrants. Most were without capital, and could not pay the speculator's price for land near established communities (McCalla 1988). This forced settlers to take unpatented land further and further into the backwoods. With so little capital in their possession, the common farmer also had to rely on a credit system to purchase provisions. Heavy debt was incurred to the merchants by most settlers during the first few years of farm inception (McCalla 1988). Those immigrants who took a mortgage for land in an old township risked everything because deeds were not issued until the debt was paid. Thus, failure of indebted farmers only put the land back into the hands of the proprietor, who was now in the possession of an improved farm for which he could ask a higher price (Howison 1821).

Quantitative Historic Evidence of Land as a Vital Resource

The Upper Canadian population grew by means of a high rate of UE Loyalist expansion and individual family immigration from the British dominions (Darroch and Soltow 1994). When surveys on new townships were completed, the best lots were grabbed at once by order of the Executive Council and under UEL rights (Landon 1974). Loyalist claims were now entering the second generation and formed a substantial part

of the land business (Moorman 1997). Evidence of this can be seen in Figures 4.1.3, 4.1.4, and 4.1.5 as initially high peaks, or rapid land alienation activity in the aggregate statistics for the various districts found in the Abstracts of the Journals of the Legislative Assembly of Upper Canada as reported by Patterson (1921: see his Appendix A). This must represent certain large patronage-derived and UEL descendent grants for the population of Upper Canada at this time was relatively small, under 100,000 people (Gourlay 1822, Wood 1988, Darroch and Soltow 1994), certainly not large enough to utilize this amount of land.

The land granted in later years represents continuing UEL descendent activity as well as grants offered to new immigrants, "...from some part of the United Kingdom, and capable, by the laws of England, of holding lands" (Strachan 1820:54). Out of the half-million British and Irish second wave immigrants who arrived at Quebec during the 1820's and 1830's, approximately two-thirds to three-quarters re-immigrated to the United States (Houston and Smyth 1990, MacDonald 1939). The prevailing socio-economic conditions would limit the increase in population to only 5 to 7% per year until 1840 (Wood 1988). In the words of MacDonald (1939:512), "No [land policy] system could be better calculated to affect the economic stagnation of a colony".

Figure 4.1.3: Total Number of Grants and Total Grant Acreage, 1798-1827

Data from Legislative Assembly of Upper Canada Journals as reported by Patterson (1921)

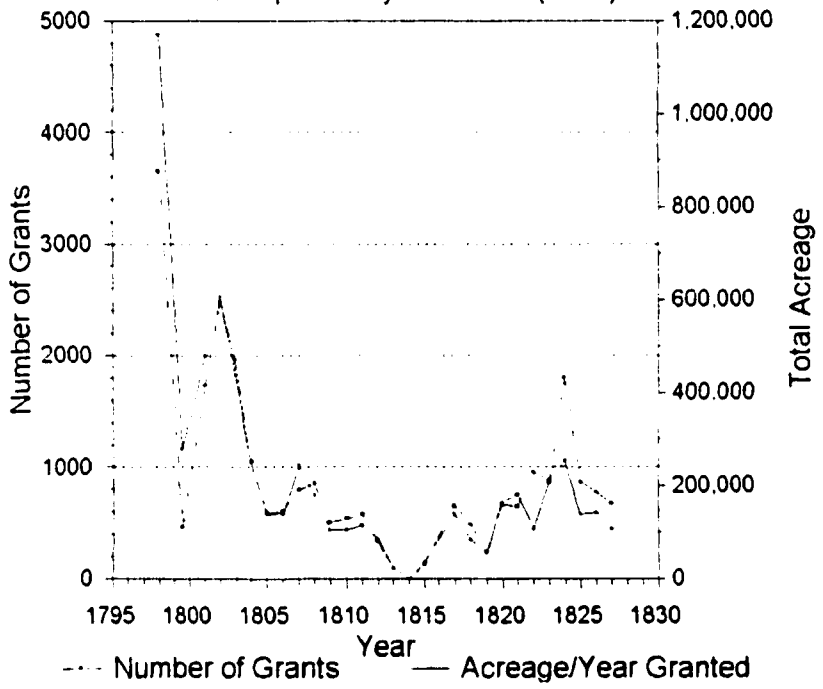
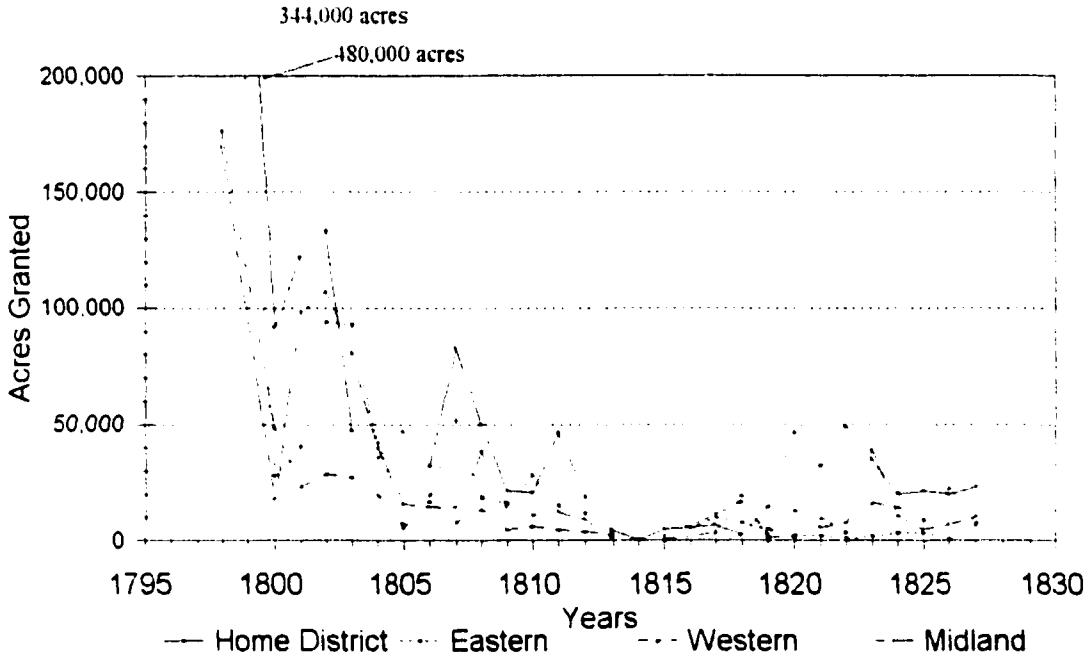
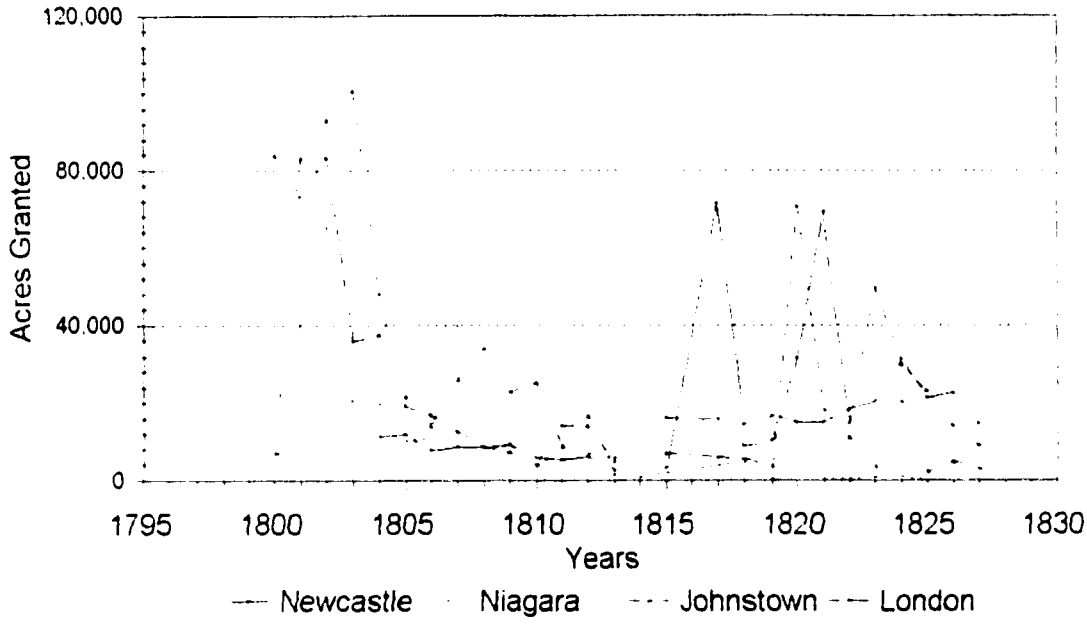


Figure 4.1.4: Acres Granted in the Four Original Districts, Upper Canada



Data from Legislative Assembly of Upper Canada Journals as reported by Patterson (1921)

Figure 4.1.5: Acres Granted in the Later Districts, 1800 to 1827

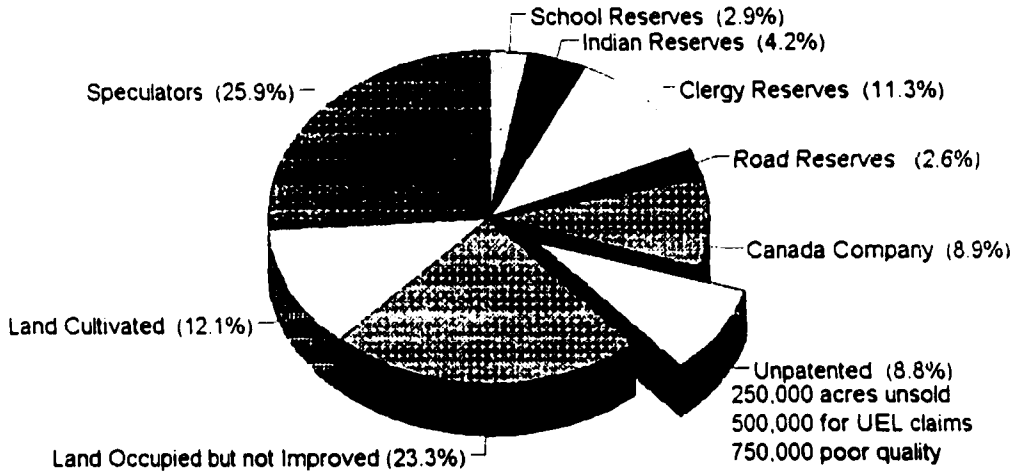


Data from Legislative Assembly of Upper Canada Journals as reported by Patterson (1921)

The perceived failure of the grant system prompted the administration of Upper Canada to abolish the granting of 'free land' in 1826 (Moorman 1997). This was despite the unrecognized fact that more than five-sixths of all grants made were to UEL descendants and a few military personnel (Gates 1968). While the land policy theoretically changed over from one of grants to public sales, UEL descendent grants continued to handicap the system of sales (Landon 1974, Patterson 1921). Wild land prices at this time rose from 30 shillings/acre in 1826 to only 60 shillings in 1835, while during the same period improved farms jumped from 50 shillings/acre to £5/acre (Hutton 1835, as cited by Houston and Smyth 1990). The period between 1826 and 1838 saw forty times as much Crown land disposed of by grant as by sale, not including the sale of land to the Canada Company (Gates 1968).

The legislation in the Act of 1837 abolished the UEL grant except to those with prior claims, established land sales at public auction, private sales of land previously offered publicly, and in certain cases private sale at valuation. UEL claimants were also permitted access to excellent locations on Crown, school, clergy, and Indian Trust lands, the consequence of which started a land rush as had not been seen since the turn of the 18th century (Johnson 1971).

Figure 4.1.6: Land Status in Upper Canada 1837*



* Out of the 17,000,000 acres surveyed in Upper Canada by 1837
Data derived from Gates (1968) and MacDonald (1939)

By the end of 1837, out of the 17 million acres within the surveyed boundaries of Upper Canada (see Figure 4.1.6) 500,000 acres were designated for school reserves, 715,740 acres for Indian reserves, 1,928,945 acres for Clergy reserves, and 450,000 acres for road reserves. Land sold or granted included 1,521,561 acres to the Canada Company, and approximately 10,500,000 in possession by individuals.

Out of the millions of acres granted, 6,060,332 acres were actually occupied of which only one-tenth, or 2,064,903 acres were cleared and cultivated, with approximately 4,500,000 acres in the possession of speculators (Gates 1968). This leaves 1.5 million acres unpatented, of which, 500,000 acres were held to satisfy claims on past UEL pledges, and the remaining land was of a poor quality (MacDonald 1939).

The United Canada in 1840

After the 'comic opera' of the 1837 Upper Canadian Rebellion, Lord Durham was commissioned to investigate the land system and how it had worked to settle Upper Canada, rather than focusing on the grievances that led to the uprising. The Public Departments at York were investigated in 1840 finding corruption, partiality, and favouritism in the surveyor-general's department dating back to the inception of Upper Canada (Landon 1974). In a statement made to the investigation, JG Chewett paints an unkind picture of the abuses of the land policies by some UE Loyalists.

> The system upon which the lands have been granted was the greatest prostitution of the sovereign's bounty ever practised in any country. The intentions of the sovereign's will evidently appear, from the instructions given for the settlement of the country, wise and guarded -- but the system pursued was corrupt; actual settlement was required upon grants, but the influence of interest obtained for individuals whose claim could not exceed 200 acres large grants to themselves and their families, dead parents as well as infants who never lived to walk out of their cradles had orders-in-council passed in their names and their families eventually obtained the lands.[sic]
> (Chewett 1840, as cited by Landon 1974:163)

No major questions of land policy had been resolved by the time Lord Sydenham had arrived to mediate the merger of Upper and Lower Canada in the Act of Union in 1840. Harmony was finally achieved with Confederation in 1867, but at that point the vast majority of Crown land fit for agricultural settlement had been disposed of long ago. Darroch and Soltow (1994) have found in their extensive analysis of the 1871 Ontario Census that the richest 1% of men owned approximately 25% of all real estate, and that the richest 10% owned 60%. They concluded that 19th century Ontario could be characterized by both a "quite steep structure of inequality in ownership and property accumulation", and by a "marked degree of opportunity for small-property acquisition" (Darroch and Soltow 1994:204).

Darroch and Soltow (1994) unfortunately fail to draw any integrative sociocultural conclusions with this data and the dramatic increase in net negative migration they also observed across the United States border after mid-century (over

350,000 people). Those Canadians seeking a new beginning were found to be the least prosperous of all U.S. immigrants, below that even of the Irish who arrived after the potato famine. Clearly some form of broad sociocultural pressure was in action in Upper Canada that had maintained the need for this 'back door' into the United States, and perceived better fortunes.

It is my argument that Darroch and Soltow's (1994) findings are also interpretable as diagnostic of a broadly based vital resource pressure amongst the later generations of post-land grant Upper Canadian families. Peasant families with maturing children and limited agricultural resources risk the economic viability of their farm if subdivided amongst siblings of the next generation (Thirsk 1976). Therefore the observed migration of Canadian-born males may be a response to land shortage within the family, as well as a lack of capital to secure land beyond the kinship property. During the economic boom of the 1840's and 1850's (McCalla 1988), the market value of land is known to have increased by more than 300% (Gagan and Mays 1973). It is likely that the arrival of countless Irish famine refugees, combined with the cessation of the UE loyalist descendent grants, resulted in a strong market demand for speculated property amongst both the native-born and immigrant settler.

Qualitative Evidence of Land Speculation in Hastings County

An ethnohistoric account of land speculation in the Midland District can be found in the chronicals of pioneer life written by Belleville occupant Susanna Moodie in 1854 (reprinted 1970). She and her husband's first hand experience with the entrepreneurial activities of "Mr. Q--", as written in Chapter XII: The Land-Jobber, provides a narrative of the methods and operation of one land speculator, and perhaps others in general. While it is realized that Susanna Moodie's account is not without prejudice, it demonstrates a qualitative approach in support of the previous statistical evidence. Parts of Moodie's (1854) chapter are transcribed here, edited only for the purpose of reducing its length.

> It is not uncommon for the land speculators to sell a farm to a respectable settler at an unusually low price, in order to give character to a neighbourhood where they hold other lands, and thus use him as a decoy duck for friends or countrymen.
>
> There was a very noted character at C--, Mr Q--, a great land-jobber, who did a large business in this way on his own account...taking advantage of the influx of emigrants, he pursued, with unrivalled success, the profitable business of land-jobbing.
>
> In his store, before taking to this business, he had been accustomed for many years to retail goods to the farmers at high prices, on the usual long credit system. He had thus got a number of farmers deeply in his debt, and, in many cases, in preference to suing them, had taken mortgages on their farms. ...instead of merely recovering the money owing to him... [he] compelled them to sell their farms nearly on his own terms, whenever an opportunity occurred to re-sell them advantageously to new comers. Thus, besides making thirty to forty per cent on his goods [from the store], he often realized more than a hundred percent on his land speculations.

> Besides a few of these unprincipled traders in land, some of whom are found in most towns, there are a large number of land-speculators who own both wild and improved farms in all parts of the colony who do not descend to these discreditable arts, but wait quietly until their lands become valuable by the progress of improvement in their neighbourhood, when they readily find purchasers - or, rather, the purchasers find them out...
>
> In 1832, when we came to Canada, a great speculation was carried on in the lands of the U.E. (or United Empire) Loyalists. ...The supply of U.E. Loyalist's lands, or claims for land, for a long time seemed to be almost inexhaustible; for the loyal refugees appear to have been prolific beyond all precedent, and most of those who held office at the capital of the province, or who could command a small capital, became speculators, and throve prodigiously. Many persons, during the early days of the colony, were thus enriched, without risk or labour, from the inexhaustible "quivers" of the U.E. Loyalists.
>
> ...my desire to guard future settlers against similar mistakes overpowers my reluctance to own that I fell into the common error of many of my countrymen, of purchasing wild land, on speculation, with a very inadequate capital. This was one of the chief causes of much suffering, in which for many years my family became involved...
>
> (Moodie 1970:181-199)

This narrative, as well as those comments by Chewitt (1840) and Canniff (1872) referred to earlier, provide qualitative support for the historic data interpreted from Patterson's primary research (1921, see his Appendix A), and that presented by Gourlay (1822), Gates (1968), Gagan (1981), and McCalla (1988). In fact, Darroch and Soltow's (1994) observations characterize native born Canadians with a reverse "J" shape population age distribution as opposed to a significantly flatter bell shape curve for foreign born settlers. They attribute this result to a high rate of

natural increase amongst the first wave of settlers, which is in perfect concordance with Moodie's (1854) account of 'prolific' UE Loyalists. That a strong young family was surely necessary to provide support on a frontier farm is not in question here; however the fact that these UEL families could rely on their genealogically derived Loyalist privileges represents an unnatural state-supported inheritance system seemingly beyond restriction until 1837.

Speculation in all forms of property, wild lands and improved farms, occurred throughout Upper Canada at all levels as seen in both the primary and secondary qualitative and quantitative sources. Land was acquired by the speculator through various means: by patronage grant, by foreclosure on mortgaged property, and less commonly by purchase of wild lands through payment of settlement fees. More than five-sixths of all land grants were secured by UEL ancestor rights (Gates 1968). This speculation epidemic occurred across all of early Upper Canada, with the best land and locations grabbed up as each new township was surveyed and opened to settlement (Landon 1974).

Land Policy Summary

The land policy that evolved in 19th century Upper Canada was a chequer-board of grant, sale, and speculation, brought on by the conflicting kinship interests of those who had arrived earliest, and those who aspired to better their

position by emigration to a new land. There was much prosperity in Upper Canada (Darroch and Soltow 1994), but little opportunity to succeed without existing capital (Johnson 1971) or previous family experience (Gagan 1981). Many pioneer families lost their farms due to foreclosures by creditors, and the land became concentrated in fewer and fewer hands.

This review provides the ethnographic vital land resource context necessary for the application of the Saxe/Goldstein theory to the analysis of Upper Canadian corporate kinship. It will be shown that control of this vital resource influenced an intragenerational consanguineous and affinal marriage strategy within persisting agricultural kinship groups in order to better manage and consolidate land holdings. Analysis of molecular genetic results will also provide evidence that kinship units used a form of intergenerational burial program, symbolically anchoring the group to ancestral land. This would have reinforced kinship stability in the face of the migration turmoil that typified Upper Canada (MacDonald 1939, Gagan 1981, Darroch and Soltow 1994, Vanderlinden 1995).

4.2: Results: Quantitative Analysis of Marriage Records

The potential violations of the statistical assumptions of the isonymy method cannot be practically determined due to the limitations inherent in the quality of the documents themselves. The settlement of Upper Canada, put in a historic perspective, is relatively young with at most times very poor records available. While many consanguineous and affinal links were discovered between some of the isonymous marriages, and therefore kinship established (see Appendix 4.2.2), conclusions drawn from this analysis must still be cautiously interpreted.

Appendix 4.2.1 lists the isonymous marriages and related information documented in the St Thomas' marriage records over the years 1821 to 1899. Figure 4.2.1 and Table 4.2.1 show measures of isonymy in the St Thomas' marriage records subdivided into decades from 1821 to 1900. Isonymous marriages seem to demonstrate an increasing trend in occurrence from the inception of the records to the end of the study period. Exceptions to this trend are observed in the 1830's with an anomalous increase in the proportion of same surname marriages, a less pronounced deviation in the 1840's, and an absence of same surname marriages in the 1880's. The number of marriages at St Thomas' demonstrates a contrasting trend of decreasing marriage totals from mid-century onwards, with a conspicuous drop in marriages occurring in the 1830's,

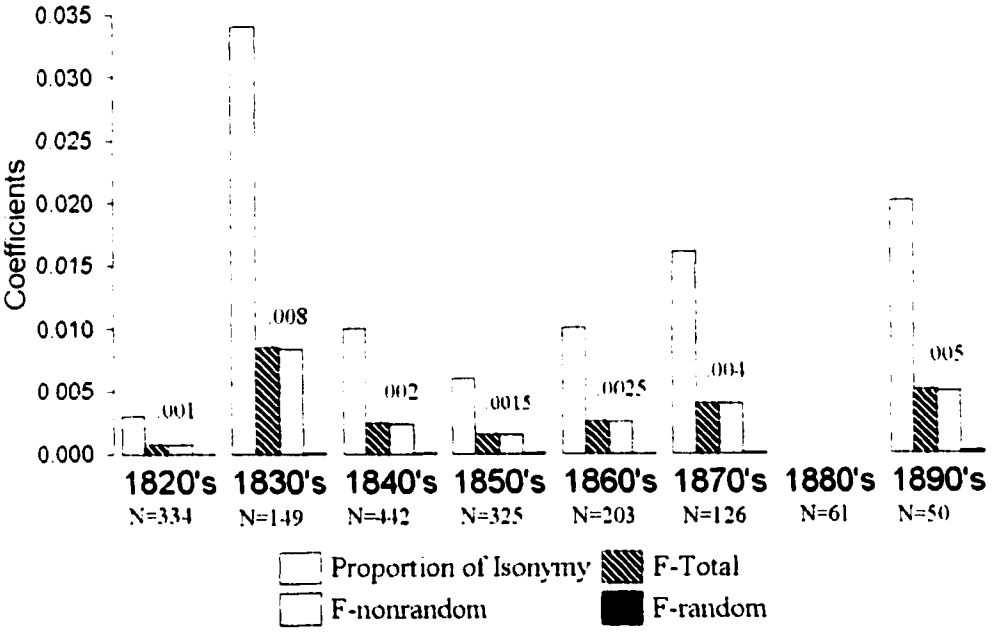
but a strong resurgence of marriage totals in the 1840's (refer to section 3.15, and Figure 3.3, Materials and Methods Chapter).

Table 4.2.1: St Thomas' isonymous marriages 1821-1900, and analysis arranged by decade

Decade 1800's	**Isonymous Marriages**	p_i	q_i	**I**	F_r	F_n	F_t
21-30 N=334	Canniff	.006	.009	.003	1.4 $x10^{-5}$	.74 $x10^{-3}$	.75 $x10^{-3}$
31-40 N=149	Haggertie McCulough Potts Reid Vance	.007 .007 .007 .007 .007	.007 .007 .013 .007 .007	.034	7.2 $x10^{-5}$	8.4 $x10^{-3}$	8.5 $x10^{-3}$
41-50 N=442	Bird Carlton Emerson Keller	.007 .002 .007 .002	.012 .005 .009 .005	.01	4.2 $x10^{-5}$	2.3 $x10^{-3}$	2.4 $x10^{-3}$
51-60 N=325	Graham Morton	.006 .015	.006 .003	.006	2.0 $x10^{-5}$	1.48 $x10^{-3}$	1.5 $x10^{-3}$
61-70 N=203	Free Haggerty	.005 .005	.005 .005	.01	1.2 $x10^{-5}$	2.49 $x10^{-3}$	2.5 $x10^{-3}$
71-80 N=126	Dunk Wilkins	.008 .008	.008 .016	.016	4.8 $x10^{-5}$	3.95 $x10^{-3}$	4.0 $x10^{-3}$
81-90 N=61	0						
91-00 n=50	McDonald	.02	.02	.02	10 $x10^{-5}$	4.9 $x10^{-3}$	5.0 $x10^{-3}$
Total N=1670	17			.01	3.1 $x10^{-5}$	2.46 $x10^{-3}$	2.5 $x10^{-3}$

p_i, q_i : frequency of the i^{th} surname in brides and grooms
I : proportion of marital isonymy
F_t : total inbreeding coefficient
F_n : non-random component
F_r : random component

Figure 4.2.1: St Thomas' Isonymy by Decade, 1821-1899



The decline in the number of marriages at St Thomas' after mid-century may reflect the migration of the native-born out of Upper Canada as observed by Darroch and Soltow (1994) and Gee (1982). Burke's (1994) study of the 1861, '71, and '81 Belleville censuses support this hypothesis by demonstrating a decrease in the number of Canadian and foreign-born Anglican children, as well as an increase in individuals over age 45. She concluded that the Anglican population of Belleville was aging and in decline. The marriage records data for the last three decades (sample sizes N=126, 61, and 50) should therefore be very cautiously interpreted because the occurrence of even a single isonymous marriage gives the appearance of a high inbreeding frequency.

Figure 4.2.1 also shows that the total inbreeding coefficient F_t, is composed mostly of the non-random inbreeding component F_n over all decades. This provides strong evidence that at least some of the isonymous marriages were non-random events, that they involved kinship unions. Table 4.2.2 summarizes the most frequently appearing surnames of the parish marriage records. Only one of the isonymous surnames, Reid, appears in this list implying that the majority of surnames are less likely to be polyphyletic (of multiple origins). Rare surnames tend to be localized and strengthens conclusions regarding the relationship between isonymy and co-ancestry (Crow 1983, Lasker 1985). This lends

Table 4.2.2: Most frequently appearing surnames by decade in the St Thomas marriage records

Decade	Males		Females		Total	
1821-1830 N=334	Bradshaw	.012	Gilbert	.012	Gilbert	.027
	Gilbert	.015	Potts	.012	Jones	.030
	Jones	.021	Smith	.018	Reid	.018
	Hill	.012	Thompson	.018	Smith	.033
					Thompson	.024
1831-1840 N=149	Jones	.013	Robinson	.020	Jones	.013
					Reid	.013
					Smith	.013
					Thompson	.007
1841-1850 N=422	Brown	.014	Campbell	.014	Brown	.024
	Elliott	.014	Elliott	.014	Elliott	.028
	Jones	.012	Reed	.028	Reed	.043
	Reed	.014	Thompson	.014	Thompson	.024
			Wilson	.024	Wilson	.038
			Wright	.012		
1851-1860 N=325	Morton	.015	Brown	.015	Brown	.022
	Wilson	.022	Smith	.022	Reed	.012
			Thompson	.015	Smith	.034
			Wilson	.018	Thompson	.022
					Wilson	.040
1861-1870 N=203	Thompson .025		Brown	.020	Brown	.034
					Smith	.020
					Thompson	.025
					Wilson	.030
1871-1880 N=126	Thompson .024		Howard	.024	May	.032
			Thompson	.032	Thompson	.056
1881-1890 N=61	Bell	.049	Wilson	.033	Bell	.049
	May	.033			May	.033
1891-1900 N=50	Smith	.02	Smith	.06	Smith	.080
			Wilson	.04	Wilson	.040

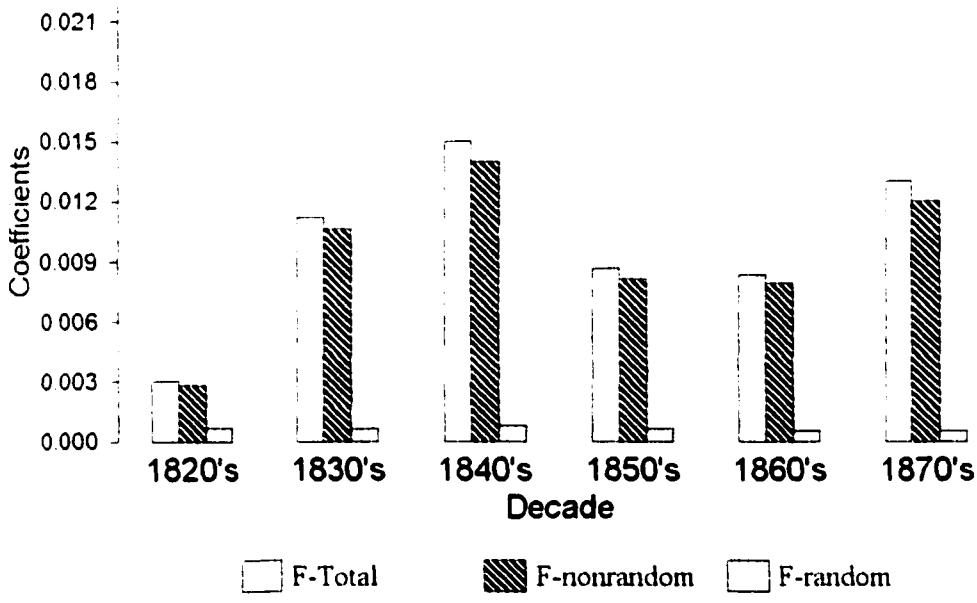
further support for the hypothesis that these surnames have monophyletic origins and are therefore kinship unions. Subsequent genealogical research has also definitively proved consanguineous or affinal kinship links between nine (or 53%) of the isonymous marriages (see Appendix 4.2.2).

The incorporation of surname spelling variants (see Appendix 3.2) in the re-calculation of isonymous surname frequencies and inbreeding coefficients did not significantly affect the results or observed trends. Only one surname, Reid, was found to have a compatible variant, Reed (see Table 4.2.2), which affected the calculation of the non-random inbreeding coefficient, dropping the contribution of the F_n value for that time period by only one percent.

Verification of the marital structure determined from the isonymy analysis can be observed in Figure 4.2.2, which presents the results of a repeated surnames analysis of the St Thomas' marriage records to the end of the 1870's (Sawchuk, unpublished results). This independent approach searches the list of marriages for all instances where the same pair of non-identical surnames occurs repeatedly, with the number of such pairs compared to the number expected at random in the population (Lasker 1985). Repeated surnames analysis can be used as a supplementary tool to isonymy by exploring other aspects of marital structure not seen in marriages of the same name (Devor 1983).

Figure 4.2.2: Repeated Surnames Analysis, St Thomas' 1820's to 1870's

(Source: Sawchuk, unpublished results)



A comparison between the two forms of analyses reveals that the non-random component of inbreeding (F_n) calculated from repeated surnames accounts for 94% of the total F_t on average over the six decades studied. This is consistent with the 95% average contribution of F_n calculated by isonymy analysis in this study. In addition, a very similar assortative mating pattern was discovered over time (compare Figures 4.2.1 and 4.2.2). The only difference in the pattern occurs in the timing of the peak assortative mating period. This peak coincides with the 1830's for the isonymy analysis, whereas it occurs in the 1840's for repeated surnames analysis; however a less pronounced elevation in repeated surnames can still be detected in the 1830's decade.

The possibility that sampling error was occurring with the small numbers of marriages in the socially abstract decade by decade analysis led to the partitioning of the marriage records into more contextually rich historic periods:

1821-1837 The land policy era of free grant activity
1838-1845 Post-grant/pre Irish potato famine migration,
1846-1864 Post-famine/pre-schism with Christ Church,
1865-1899 A post-schism St Thomas' congregation

These particular divisions correspond to major cultural and biological events that possibly altered the social fabric and perception of the parish community, perhaps leading to changes in population and marriage structure. The creation of four periods of analysis also overcomes some of the sample size

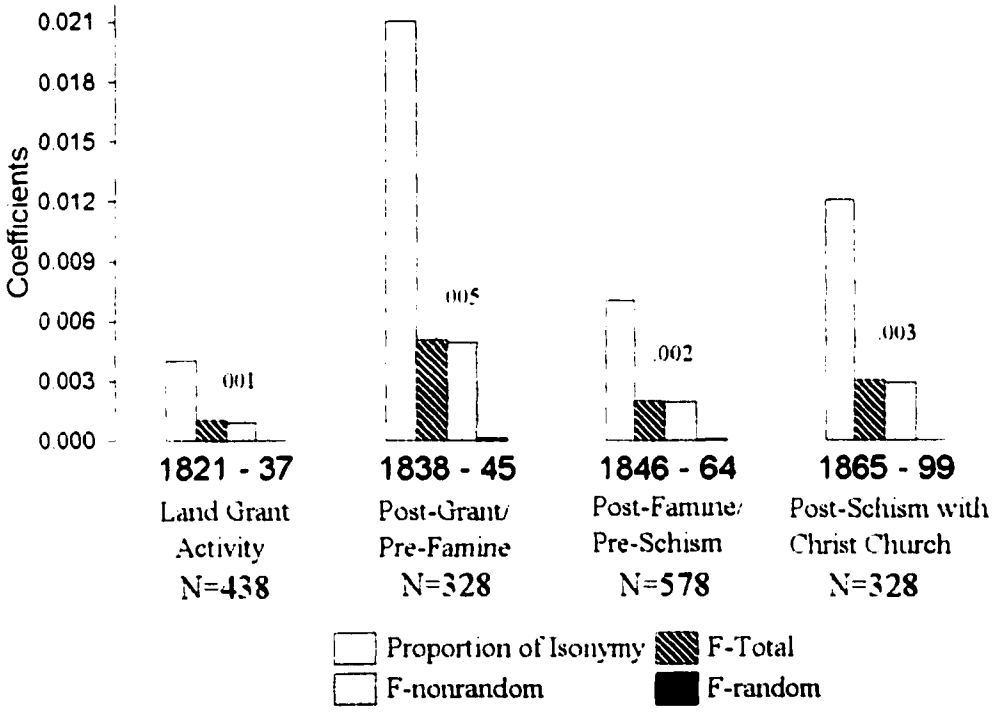
limitations of the St Thomas' records, such as the drop in marriage numbers in the 1830's. Therefore it was reasoned that these periods would provide a more accurate depiction of the trends in the St Thomas' mating pattern.

Table 4.2.3: St Thomas' isonymous marriage analysis by historic period

Period	isonymous	I	F_r	F_n	F_t	N
1821-1837	2	0.004	1×10^{-5}	0.0009	0.001	438
1838-1845	7	0.021	7.7×10^{-5}	0.0049	0.005	328
1846-1864	4	0.007	3.6×10^{-5}	0.0019	0.002	578
1865-1899	4	0.012	2×10^{-5}	0.0029	0.003	328

The historic period isonymy analysis (see Figure 4.2.3, and Table 4.2.3) yields results with more modest levels of inbreeding than that seen in the decade analysis of Figure 4.2.1. The overall pattern however has not changed from the original analysis, or that seen in the repeated surnames analysis. The first historic period has the lowest isonymous marriage frequency, inbreeding coefficient, and contribution of the non-random component ($I<0.5\%$, $F_t=0.001$, $F_n=90\%$ of F_t). This period is succeeded by an increase in isonymous marriages (over 2%) and a higher inbreeding coefficient ($F_t=0.005$), with F_n comprising 98% of the total. Following this anomalous rise is a trend to more moderate levels of isonymous marriages in

Figure 4.2.3: St. Thomas' Isonymy Using Historic Periods, 1821 - 1899



the last two divisions with F_t=0.002, and F_t=0.003, composed of F_n=95% and 97% respectively.

The overall decreased level of inbreeding discovered using historic periods is probably due to the moderating effect of larger sample sizes in each period. Nevertheless the pattern of assortative mating and strong contribution of F_n that was seen in the decade-by-decade isonymy and repeated surnames analysis remains. Lasker (personal communication) believes that the observation of consistent trends in assortative mating over time is more important than the comparison of coefficients. Therefore it must be concluded that something of social interest is happening during the 1838-1845 period to cause the increase in isonymous marriages.

Comparisons to Other Populations

The average total inbreeding coefficient calculated over the entire span of 1821 to 1899 for St Thomas' (F_t=0.0025) can be compared to that of the classic-model 'island' or 'isolate' populations (see Table 4.2.4). While the importance of the pattern of inbreeding has been established, this comparison to the literature provides a relative backdrop to interpret the magnitude of the results of this analysis. It can be seen that the F_t falls below that of the Mennonite religious isolates, yet above the geographically isolated Orkney Islanders.

Table 4.2.4: Total Inbreeding Coefficients of Select Populations (accuracy as reported by the authors)

Population	F_t
Hutterites (Crow & Mange 1965)	.049
Amish (Hurd 1983)	.025
Swiss (Morton & Hussels 1970)	.006
Mennonites (Rogers 1984 as cited by Lasker 1985)	.0036
Orkney Islanders (Roberts & Roberts 1983)	.001

If one compares the inbreeding coefficients calculated according to historic period, then the 1835-1845 division has a F_t=0.005 that sets it on the order above the Mennonites, yet below the Swiss. Comparisons to other contemporaneous pioneer populations can be seen in Table 4.2.5, placing the St Thomas' results on the order of that detected by Swedlund and Boyce (1983) in the Connecticutt River Valley of Massachusetts, and Kosten and colleagues (1983) in Oatlands, Tasmania. While this level of assortative mating is generally considered to have a negligible genetic effect (Mange and mange 1980, Swedlund and Boyce 1983), some social element may be involved in the mating structure to cause the pattern observed in isonymous marriages. But could this trend in the frequency of unions occur just by chance?

Could a possible explanation for the appearance of an assortative mating trend be found in the frontier characteristics of pioneer Upper Canada? Belleville was a comparably well serviced community with respect to transportation: weekly steamship service between Belleville,

Table 4.2.5 Inbreeding coefficients in other pioneer populations as measured by isonymy. Accuracy as reported by the author(s).

Population	Time Frame	Ft	Fn	Fr	N
St Thomas' unstandardized total	1821-1899	0.0025	0.0024(96%)	0.0001	1670
St Thomas' by historic period	1821-1837	0.001	0.0009(90%)	0.0001	438
	1838-1845	0.005	0.0049(98%)	0.0001	328
	1846-1864	0.002	0.0019(95%)	0.0001	578
	1865-1899	0.003	0.0029(95%)	0.0001	328
Oatlands, Tasmania (Kosten et al. 1983)	1840-1899	0.0046	0.0039(85%)	0.0007	759
Bothwell, Tasmania (Kosten et al. 1983)	1840-1899	0.0012	0.0011(91%)	0.0001	420
Connecticut River Valley (Swedlund & Boyce 1983)	<1770	0.0018	-0.0022(-120%)	0.0040	277
	1770-1789	0.0051	0.0025(49%)	0.0026	146
	1790-1809	0.0034	0.0016(47%)	0.0018	296
	1810-1829	0.0060	0.0039(65%)	0.0021	291
	1830-1849	0.0049	0.0029(59%)	0.0020	356

Kingston, Prescott, and York (Toronto) was established early in the 1820's stimulating trade and the export of flour, wheat, potash, and lumber (Mika and Mika 1986). Though the initial pioneer roads were impassable in bad weather, the King's Highway was a major thoroughfare through Belleville which connected it directly to York and Kingston. By the late 1820's Belleville had become an important meeting place for local farmers to sell produce in the market. Local businesses were flourishing, the population was approaching 1000 people and growing.

The early 1830's saw Belleville's population reach 1200. Exports from the wharfs of Belleville in 1834 totalled 8000 pounds of flour, 1100 barrels of potash, 1700 bushels of wheat, 250,000 staves, and 1,000,000 feet of timber (Mika and Mika 1978). Roads to outlying areas were planked, providing a route for stage lines. This improved transportation, coupled with a ferry link across the Bay of Quinte, put Belleville in the unique position to serve as trade centre for Prince Edward and Hastings Counties (Mika and Mika 1978). What this brief historical reconstruction provides is a view of a bustling frontier community with rather good transportation infrastructure for the time, certainly not an isolated backwoods settlement.

Isonymy Analysis linked to Historic Context

It has been hypothesized that assortative mating, as discovered through isonymous marriage records analysis at St Thomas', is the result of intragenerational kinship alliances. That these unions involved kin has been implicated by the significant contribution of the non-random component of inbreeding. Furthermore, this kin strategy may have been initiated to control and consolidate vital land resources in the face of changing social circumstances, but how can this be detected through the marriage analysis?

-Analysis Period 1821-1837

The first division of the marriage records spans the inception of the St Thomas' Anglican Church to the end of the free land grant policy era. While the start of the marriage records do not coincide with the formation of a settlement at Belleville, it is maintained that this historic period provides an interesting standard to compare those periods that follow. The St Thomas' marriage records during this phase are a mix of not only established family and new emigrant marriages, but also unions from many other religious denominations until the passing of the Marriage Act of Upper Canada in March 1831 (see section 3.15 Materials and Methods). How then are we to interpret the results of the isonymy analysis for this time period?

The proportion of isonymous marriages and the total inbreeding coefficient is one-fifth that of the next period, yet the non-random component of inbreeding is amongst the highest observed in any historic pioneer settlement (see Table 4.2.5). It is not unusual to observe some same surname marriages in any analysis of historic documents, in fact researchers should generally expect to see some low level of inbreeding in all populations (Lasker 1985). Therefore the isonymous unions of this period likely represents the background level of inbreeding one should expect.

-Analysis Period 1838-1845

The second historic division of this study spans the years 1838 to 1845. It begins with the official abolition of not only the United Empire Loyalist ancestor rights, but of all other forms of official free grant (Gates 1968). It is during this period that we see the highest frequency of isonymous marriages coinciding with a total inbreeding coefficient comprised mostly (98%) of the non-random component (F_n). This increase also corresponds to the escalation in repeated surnames marriages observed during the 1830's and 1840's where F_n likewise contributes approximately 94% (see Figure 4.2.2: Sawchuk, unpublished results). The discovery of analogous trends in the independent analyses supports the assertion that the cessation of land grants had an impact on the social perception of land resources amongst some pioneer families.

-Analysis Periods 1846-1864 and 1865-1899

The periods of 1846 to 1864 and 1864 to 1899 span the years of the Irish potato famine migration and the schism of the St Thomas' Anglican Church congregation resulting in the formation of the Christ Church parish. During these periods we see an overall decline in the total inbreeding coefficient, yet a significant proportion of the F_t (95% and 97%) is still composed of the non-random component, once again indicating affinal and/or consanguinous kin unions for these marriages.

A number of possible reasons may be found to explain the overall trend of reduction in assortative matings after the second historic period (1838-1845). A cogent theory can be created linking this decline with a vigorous economic growth period and incipient industrialization of Upper Canada and Belleville in the late 1840's to 1860's (Boyce 1977, McCalla 1993, Widdis 1991). Not only would subsistence agriculturalists have prospered, but the arrival of industrialization may have relaxed the sibling pressure on the family farm by providing alternative sources of income such as wage labour jobs. Farming was beginning to be challenged as the primary generator of capital in Upper Canada (McCalla 1993).

This growth also coincides with the largest single flood of immigrants into British North America and Upper Canada: the Irish potato famine migration (Houston and Smyth 1990, MacKay 1990). Swedlund and Boyce (1983) interpreted their observed oscillations in inbreeding coefficients as a reflection of instability and growth in the demographic history of the study area (see Table 4.2.5). Therefore, it is possible that the considerable influx of Irish refugees may have had some influence on the social fabric of Upper Canada, and perhaps the parish of St Thomas'. Table 4.2.6 investigates the effect that the famine migration had on the assortative mating pattern. The St Thomas' and Christ Church records were

separated into only pre-famine (1821-1845) and post-famine (1846-1899) categories. The pre-famine sample generates an isonymous marriage frequency of 1.2% and an F_t=0.003 for N=766 marriages. The post famine sample generates results slightly lower, with an isonymous marriage frequency of 0.7%, F_t=0.002, for N=1107 marriages. It appears that the famine migration had little overall effect on the existing congregation's marriage patterns, although this cannot be determined with certainty due to the sample size differences and reliability problems with the Christ Church records.

Table 4.2.6: Isonymy analysis of the St Thomas' and Christ Church marriage records by Irish Potato famine categories.

Period	isonymous	I	F_r	F_n	F_t	N
1821-1845	9	0.012	3.7×10^{-5}	0.0028	0.003	766
1846-1899	8	0.007	2.5×10^{-5}	0.0019	0.002	1107

I : proportion of marital isonymy
F_r : random component of inbreeding
F_n : non-random component of inbreeding
F_t : total inbreeding coefficient

Comparing the final historic period of post-schism St Thomas' (1865-1899) to the isonymous marriage analysis done on the Christ Church records over the same period, one can see in Figure 4.2.4 and Table 4.2.7 that there are no same surname marriages in the Christ Church congregation. This stands in contrast to the four isonymous unions observed at St Thomas' resulting in a slight resurgence of inbreeding. This marginal

increase also coincides with the economic depression seen in Belleville after the American Civil War (Boyce 1977, Mika and Mika 1986, 1975, Widdis 1991), perhaps leading to the return of old kinship marriage strategies. If the Christ Church parish was truly an offshoot of the St Thomas' congregation, then it would be logical to merge the records to form a single post-schism (1865 to 1899) data set. When this combined data set is compared to the pre-schism St Thomas' congregation (see Figure 4.2.4), one can detect virtually no difference in the inbreeding coefficients generated. This result coincides with the known chronicle of the schism: the division of the congregation into conservative religious elements, perhaps representing landed families staying at St Thomas', and more progressive liberal individuals moving off to form Christ Church (Bellstedt 1968).

It also appears that the economic depression of the 1870's, '80's, and '90's had no effect on the frequency of isonymous unions. Inbreeding results seem to have plateaued, basically returning to the ambient level seen in the first historic period. It must be kept in mind that the Christ Church records failed to pass Drake's analysis for reliability, and that the number of marriages at St Thomas' declined over the last half of the century.

Figure 4.2.4: St Thomas' and Christ Church: Pre- and Post-Schism Isonymy

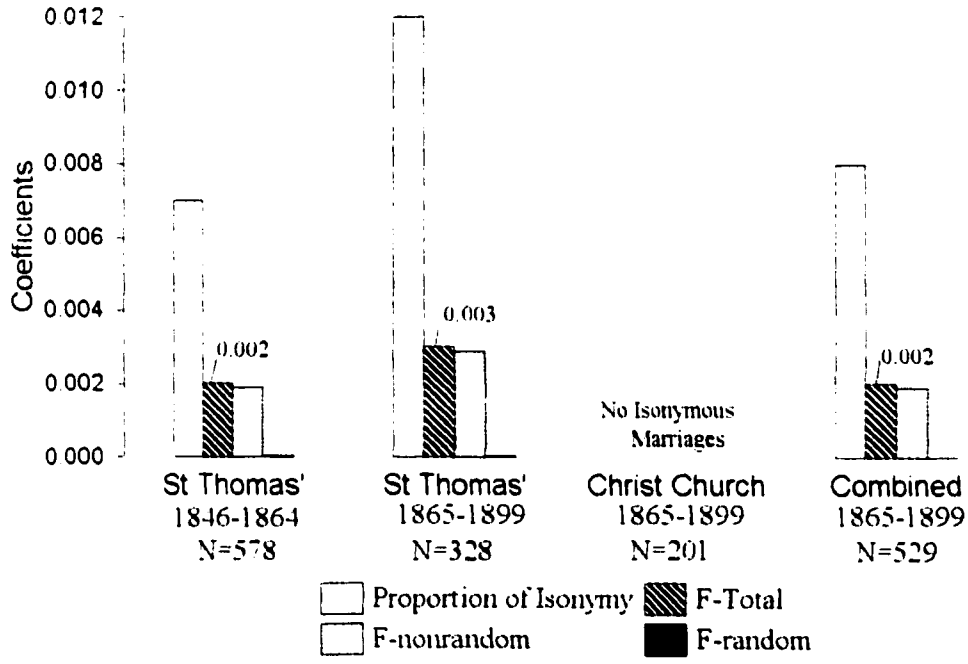


Table 4.2.7: St Thomas' and Christ Church marriage records: pre- and post-schism Isonymy analysis

Period	isonymous	I	F_r	F_n	F_t	N
1846-1864	4	0.007	3.6×10^{-5}	0.0019	0.002	578
St Thomas 1865-1899	4	0.012	2×10^{-5}	0.0029	0.003	328
Christ Ch 1865-1899	0					201
St T & ChCh 1865-1899	4	0.008	1×10^{-5}	0.0019	0.002	529

Persisting Conservative Anglicans

Table 4.2.8 presents the number of times each isonymous surname appears in the historic periods for the marriage records of St Thomas' and Christ Church. Out of the seventeen isonymous unions, eight of these surnames either appear over all four periods (Bird, Graham, Haggertie/Haggerty, McDonald, Reid), and/or have a total of ten or more marriages (Canniff, Emerson, Morton). In addition only three of the surnames involved in isonymous unions appear to have made the transition to the Christ Church congregation (Graham, McDonald, Morton). These results indicate indirect evidence that the isonymous unions represent persisting families in the region and in the parish of St Thomas' in particular. In order to explore this possibility, other historic records and genealogical data for these surnames was investigated to further support the isonymy analysis and provide qualitative

evidence of persistence and a motive for the kinship unions.

Table 4.2.8: Persistence of Isonymous Surnames in St Thomas' and Christ Church Marriage Records. (Date in brackets denotes year of occurrence of isonymous marriage for that surname)

	Time Period				Christ	
	1821-37	1838-45	1846-64	1865-99	Church	Totals
Bird*	1	5 (1843)	4	2	0	12*
Canniff*	6 (1826)	0	3	1	0	10*
Carleton	0	0	5 (1850)	0	0	5
Dunk	0	0	3	2 (1871)	0	5
Emerson*	2	6 (1841)	4	0	0	12*
Free	1	0	1	2 (1867)	0	4
Graham*	2	4	8 (1853)	1	1	16*
Haggertie/ Haggerty*	3 (1834)	4	6 (1863)	1	0	14*
Keller	0	3 (1844)	0	0	0	3
McCullough	0	2 (1840)	0	1	0	3
McDonald*	2	1	4	3 (1891)	1	11*
Morton*	2	2	10 (1854)	0	3	17*
Potts	5	3 (1840)	1	0	0	9
Reid*	6	5 (1840)	2	1	0	14*
Vance	0	4 (1839)	4	1	0	9
Wilkins	1	0	1	6 (1877)	0	8

* surnames appearing over all 4 historic periods, and/or a total of 10 or more marriages

Qualitative Surname Analysis: Genealogical Research

The historic records genealogical research involved searching the Land Records Index, Township papers, Canadian Census data, regional directories, cemetery inventories, and an array of genealogical resources. Unfortunately for the time period of interest in the Belleville region the records that are available are incomplete and of poor quality (Vanderlinden 1995). This standard extends past mid-century where one discovers that portions of all Canadian Censuses have been lost (Poleman 1998). To illustrate this point,

Lovell's Canadian Dominion Directory for 1871 includes only approximately 40% of household heads (Poleman 1998).

All isonymous surnames were investigated equally, however the assortative mating trend observed in the second historic period provided a focus for the qualitative historic documents research. Using randomly generated numbers, a group of ten marriages was also selected from the second historic period and studied in parallel to provide a control group. The control group is comprised of 19 bride and groom surnames with the Martin surname repeated by chance in two marriages (see Appendix 4.2.1).

Research was conducted in eleven different locations, consisting of various libraries, government archives, genealogical repositories, and personal collections. This led to the location of a considerable amount of information on the majority of the 36 isonymous and control group surnames. A small number of wills were also discovered with possible links to the individuals of interest, however the bulk of these documents were totally illegible due to fading, and had not archived well onto microfilm.

Research with isonymous surnames recovered nine positive identifications (or 25% of the total surnames searched), three possible links to the marriage of interest (8%), and five surnames (14%) with no indication of a match (see Appendix 4.2.2). Research with the control marriages recovered six

positive identifications (or 16% of the total), five possible identifications (14%), and eight marriages (22%) with no indication of a match. There is a strong trend of increased representation for the isonymous surnames in the genealogical and land records data collected (see Table 4.2.9). The control surname sample appears with less than half the frequency of the isonymous surnames in six out of the nine categories from Table 4.2.9. This suggests that isonymous surnames are more likely to be traceable in historic records.

It was possible to infer the arrival period of some surname lineages through the appearance of the isonymous or control group surname in the First Census of Canada 1790 (Lyon 1970). While this manuscript is based on the recorded disbanded soldier and UE Loyalist lists and claims for lost property, and is therefore not a true census, it provides the only relatively comprehensive source of information on the first wave of immigrants into Upper Canada. Whenever possible additional documents, such as other UEL resources based on Orders-in-Council and Land Records petitions, were used to link the surnames data retrieved from the '1790 census' to the individuals of interest. It was discovered that twelve isonymous surnames (70%) and eight control surnames (42%) appeared on the 1790 list. Supporting documents verified the UE Loyalist status for four isonymous grooms/brides (23%) and three control surnames (16%) (see Table 4.2.9).

Table 4.2.9: Frequency of reconstructed genealogical variables for Control and Isonymous surnames

	Control Surnames N=19	**Isonymous Surnames** N=17
Genealogy Discovered	4 (21%) Rose/Ketcheson Martin/Howard Graham/Martin	6 (35%) Canniff, Dunk Haggertie, Haggerty Morton, Potts
UEL status	3 (16%) Baker, Davis, Ketcheson	4 (23%) Canniff, Emerson Keller, Reid
Present in Lyon (1970) 'Census of 1790'*	8 (42%) Baker, Davis Froste, Graham Ketchson, Rose Warren, White	14 (82%) Bird, Canniff Carleton, Emerson, Free Keller, Graham, Reid Haggerty/ie,McCullough Potts, Vance, Wilkins
Possible Land Holdings*	6 (32%) Curran, Irwin Rose, Graham Martin, White	14 (82%) Bird, Canniff Carleton, Dunk, Emerson, Free Haggerty/ie, Keller McCullough, Morton, Potts, Vance, Wilkins
Kinship Block of Land**	4 (21%) Graham Martin Rose White	10 (59%) Canniff, Carleton, Dunk Emerson, Free Haggerty/ie, Keller, McCullough,Morton,Potts
Late Arriving Irish**	2 (10%) Martin Graham	6 (35%) Carleton, Free, Potts Haggerty/ie, Morton
Positively Identified Persister*	4 (21%) Graham, Martin Rose, White	8 (47%) Bird, Canniff, Dunk Emerson, Free Haggerty/ie,McCullough
Extra Isonymy***	1 (5%) White	5 (29%) Canniff, Dunk, Potts Haggerty/ie, Morton
Repeated Surname	3 (16%) Graham, Ketcheson White	4 (24%) Bird, Haggerty/ie Morton, Potts

* indicates approx. 2 x the frequency in isonymous surnames
** indicates approx. 3 x the frequency in isonymous surnames
*** indicates approx. 6 x the frequency in isonymous surnames

With the aid of genealogies (see Appendix 4.2.2) it was also possible to deduce surname arrival time after the first wave of European pioneers. It was discovered that six isonymous surnames (35%) and two control surnames (10%) could trace lineage roots to emigration from Ireland after the UE Loyalist period, but before the potato famine migration. Of interest is the finding that the majority of these surnames (labelled 'Late Irish' in Table 4.2.9) also appear in Lyon's (1970) 'Census of 1790', implying that they had kinship relations already present in Upper Canada.

The Land Records at the Ontario Provincial Archives provided the major source for linking isonymous and control surnames to land holdings in Upper Canada. Other sources of information such as censuses, township directories, and township papers were then used to uncover other purchased property and verify continued habitation. Out of the sample under study, thirteen isonymous grooms or brides (76%) had surname, christian name, land issue dates, and occasionally residence information, consistent with a possible link to individuals found in the marriage records. The control group marriages yielded six (32%) with possible land holdings (see Table 4.2.9).

Of equal interest was the discovery of several of the isonymous surnames associated with considerable land activity beyond that of the couple involved in the marriage (see Table

4.2.9). This was also seen in a few cases of the control group marriages. At least ten isonymous surnames and four control group surnames showed instances of large 'blocks of land' composed of many lots in close proximity found under control of the same surname and presumably representing a kinship corporate unit (see Appendix 4.2.2).

Searches for additional historic documents evidence and genealogical information yielded six positive identifications of isonymous couples from discovered genealogies (35%), and four control marriages (21% of the total, see Table 4.2.9). From the inspection of these genealogies it was discovered that a total of twelve repeated surname marriages had occurred within the positively identified isonymous families, and an additional nine isonymous marriages were uncovered beyond those found in the St Thomas' records. The genealogies of the Morton and Haggerty families displayed of the repeated, and eight of the extra isonymous surname marriages.

From the control group of genealogies only three surnames (16%) demonstrated isonymous or repeated surnames marriages beyond that found in the St Thomas' records. Two repeated surnames and one isonymous marriage was discovered in the White genealogy, and one repeated surname marriage was found in the Ketcheson, and Graham genealogies (see Table 4.2.9). Table 4.2.9 summarizes the other reconstituted genealogical variables discussed above. The isonymous surname sample

nearly doubles the frequency of occurence in six out of nine of these variables as compared to control group surnames.

Summary of Isonymy and Genealogical Research

The pattern of occurrence of same surname and repeated surnames marriages at St Thomas' appears to have been influenced by colonial land policy, particularly the cessation of the free land grant in 1837. Other historic events such as the Irish potato famine migration did not have a noticeable impact on the results, however it appears that the parish schism did involve the segregation of conservative and liberal religious elements. Some of the individuals remaining at St Thomas' were involved in further isonymous marriages, while Christ Church recorded no similar findings.

The overall total inbreeding coefficient was discovered to be moderate and consistent with other studies involving pioneer historic records. The non-random component of inbreeding at St Thomas' was discovered to greatly exceed that of other reports, significantly contributing to the total coefficient. This strongly suggests blood or affinal kinship ties were involved in these unions, nevertheless negligible genetic effects would have resulted from this level of consanguinity (Mange and Mange 1980). Therefore, subsequent analyses and interpretations of mating structure were directed towards the identification of social implications rather than biological consequences to population gene pools.

A control group of surnames drawn randomly from the St Thomas' marriage records was found to be less likely to appear in subsequent aggregate historic records. With the use of supporting genealogical research, many of the isonymous couples were directly linked to vital resources involving kinship control of large, nearly contiguous blocks of agricultural land. Other qualitative genealogical variables such as UEL status, land possessions, kinship control of land, and chronological persistence, all indicated that surname lineages involved in affinal or consanguinous isonymous marriages were more likely to have had early kinship access to land resources, and thus were perhaps able to persist in the region.

4.3: Ancient DNA Analyses

Detection of Contamination

Despite the implementation of stringent cleanliness protocols during the extraction of bone and tooth samples and PCR setup, one extract reagent blank (RB 06) and eight extracts from the St Thomas' sample, as well as six Harmony Road extracts and one extract reagent blank (RB 24), were discovered to be possibly contaminated with non-endogenous DNA (see Appendices 4.3.1, 4.3.2, 4.3.3, and 4.3.4). In the majority of these instances (93%, or 13 out of the 14 cases), the source of the contamination was traced to this author (JCD involved in 11 cases) or to a laboratory co-worker (DY involved in 2 cases). The observation of double nucleotide bands at mtDNA polymorphic sites (see Figure 4.3.1 for an example) within the aDNA sequences were consistent with the mtDNA sequences and/or the short tandem repeat profile of JCD and DY co-amplifying with the true aDNA template.

By deductive reasoning it was possible to logically remove the nucleotide and/or the STR alleles of these contemporary sources and obtain a putative endogenous aDNA sequence or STR genotype. This deductive approach was modelled after Yang's (1997) experience with ancient mitochondrial DNA sequence contamination in which he found a number of double nucleotide bands at polymorphic sites for one sample. In this situation Yang (1997) was able to deduce the

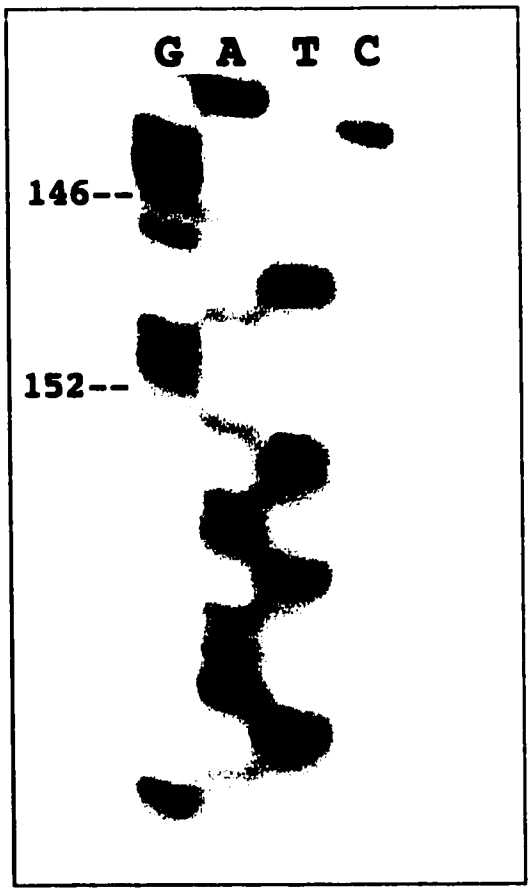


Figure 4.3.1: mtDNA sequence analysis of Harmony Road burial 5 from positions 142 to 162, with polymorphisms at 146, 150, and 152.

Note the polymorphism at 146 demonstrates contamination with two nucleotide bands, a **G** and an **A** both of approximately equal intensity, while the polymorphism at 152 shows two bands, again a **G** and an **A**, with **A** of greater intensity than **G**. The two putative sequences present (HR 5 and HR 7) share the **G** polymorphism at position 150.

putative endogenous sequence by removing the nucleotides that matched his own sequence. While the use of such deductive methods is not the perfect solution, in that it can never be proven in this manner that an individual mtDNA haplotype was the source of the contamination, it provides the most parsimonious explanation of the observations. The more complex yet definitive method for determining sources of contamination, the cloning of mtDNA PCR products to show pure amplification lines, was not accomplished due to time and budget constraints.

All of the contaminated Harmony Road samples were extracted in parallel with the same reagent blank, yet this blank did not yield any detectable mtDNA or STR PCR product with ethidium bromide staining on polyacrylamide gels. This is consistent with the appearance of the particularily insidious form of DNA contamination called 'carrier effect' by Handt and associates (1994a). A very low number of contaminating molecules within reagent blanks often adsorb to the plastic ware yielding negative PCR results, however when sample DNA is added it acts as a carrier to displace the contaminating molecules which can now serve as templates for PCR. Handt and colleagues (1994a:527) regard this type of contamination as, "a continuous source of worry for any work with extremely low copy-number sequences".

The single case of contamination that does not fit the carrier effect model within the Harmony Road sample is burial 5 (see Figure 4.3.1). This burial may have been cross-contaminated during extraction with that of another burial being processed at the same time. Contamination consistent with Harmony Road burial 7's mtDNA sequence and HUMTH01 allele 9.3 were found during analysis of burial 5, along with the putative endogenous sequence. In this instance the same contaminating mtDNA sequence (which happens to be Lineage A, or the Anderson et al. 1981 reference sequence) was also detected within the reagent blank RB 24 associated with these extracts, but not in any other tissue sample of that 'extract cohort'. The cohort extracts consist of HR 6, 9, 38, and 41, all of which have mtDNA sequences very different from the reference sequence. This leaves only burial 7 as the possible culprit for the contamination.

The eight cases of contamination involving the St Thomas' sample extracts involve six instances consistent with contamination from JCD, and two instances of contamination from DY. Unlike the Harmony Road group of samples, none of the St Thomas' cases were associated with the same extraction reagent 'cohort', and none of the other tissue samples processed in parallel appear to have visible PCR product contamination. This is consistent with the 'spot contamination' model.

Recently Herber and Herold (1998) have found that a single human dandruff particle contains between 0.8 ng and 16.6 ng of DNA. This level of modern DNA contamination introduced during the extraction step would certainly overwhelm the majority of ancient DNA samples, as is the case for burials 287, 423, and 512a which demonstrate unambiguous sequences and STR's consistent with DY and JCD. The remainder of the contaminated samples yielded ambiguous sequences suggesting that either smaller than average skin particles entered these samples, or that these extracts contained more endogenous DNA.

Other possible explanations for double nucleotide bands also exist, such as the rare occurence of mtDNA heteroplasmy (see Gill et al. 1994). However, as Yang (1997) points out it is unlikely that so many heteroplasmies would exist within the short sequence under study. Another possibility involves multiple sources of non-endogenous DNA, however the majority of ambiguous sequences were found to also have either STR profiles consistent with lab worker DNA (JCD or DY), or no detectable STR product. It is therefore concluded that the most parsimonious explanation for contamination is the random entry of airborne skin particles from laboratory personnel.

Authentication of aDNA Results

Despite the detection of modern DNA in a relatively small number of samples, the vast majority of the results obtained

from the archaeological specimens presented in Appendices 4.3.1 through 4.3.4 probably represent genetic information from a past population of Upper Canadian pioneers. Support for this supposition can be found in Appendix 4.3.5 and 4.3.6, which summarizes all of the individual aDNA results for the St Thomas' and Harmony Road burials according to similar mitochondrial DNA sequence information. One can see that even though many of the burials share the same mtDNA haplotype, they do not share the same short tandem repeat genotype, nor sex estimation. In this way 46 unambiguous individual 'aDNA fingerprints' are generated, 18 from Harmony Road, and 28 from St Thomas' cemetery. It is extremely unlikely that so many different STR genotype, mtDNA haplotype, and sex estimation combinations could be caused by any form of contamination given the restricted access to the extraction lab and the removal and/or sterilization of the outer layer of bone/tooth sample.

In a small number of cases (burials 6, 20 and 22, 23 from Harmony Road, and burials 87, 148 from St Thomas') individuals demonstrate the same mtDNA sequence, STR alleles, and either the same molecular DNA sex result, or no product. In these instances, the sample tissue was processed on separate occasions with different reagent blanks, virtually eliminating the possibility of cross-contamination between these extracts. In addition, none of the other samples processed with these

burials belong to the same mtDNA lineage, further supporting the lack of aDNA cross-over. It is thus believed that these six cases represent endogenous aDNA, and when combined with the 46 aDNA fingerprints total 52 Upper Canadian pioneers with both mtDNA and STR data over both cemeteries.

Intra and Interobserver Error for HUMTH01 and mtDNA Analyses

Intraobserver error in scoring mitochondrial DNA polymorphisms was conducted on all 69 mtDNA sequences determined in this study. Five differences, or 7.2% of the total re-analyzed, were discovered from the original polymorphism assignments. All of these involved ambiguous sequences that demonstrated contamination with a subjective error occurring in the visual allocation of band intensity to the two nucleotides at the polymorphism. An example of this type of error is found at mtDNA position 204 of St Thomas' burial 156 with the original assignment being A(G), or A band more intense than G, and the subsequent assignment being A-G, or A of equal intensity to the G band. Of these errors none had repercussions on the final haplotype estimation.

Intraobserver error in scoring the HUMTH01 STR alleles was conducted on 118 PCR reactions separated by a minimum of 3 months. Seven differences, or 5.9% of the total re-analyzed, were discovered from the original allele assignments. These involved 4 errors of not scoring a shadow band, 2 errors of not scoring a secondary allele in the first

assignment, and one error of scoring a secondary PCR product as a primary allele in the first assignment (see Harmony Road burial 33 Figure 4.3.6). Again, none of these errors had repercussions for the final estimation of genotype.

Interobserver error estimation involving STR allele assignments was conducted on a representative sample of 44 PCR reactions from both the Harmony Road and the St Thomas' sample. Six inconsistencies between the two observers were scored (or 13% of the total re-analyzed) involving only the presence/absence of secondary alleles or shadow bands. These errors would have had no effect on subsequent genotype assignments of these samples, however this error frequency is still quite high and needs explanation. The second observer (B. Eng) has experience scoring contemporary DNA short tandem repeats, but no experience with artifact-prone aDNA PCR products, while this author (JCD) was perhaps over-zealous in scoring even the faintest of Taq slippage errors. Therefore, had there been some prior consensus with respect to allele band intensity and definitions of shadow/secondary bands this error rate probably would be much lower.

Sex Typing of aDNA extracts:

Figure 4.3.2 illustrates the results of a typical aDNA amplification following Yang and coworkers (1998b) sex typing protocol. No PCR negative controls demonstrated contamination. Both male and female positive controls were

amplified and run parallel on the gels with the aDNA PCR products to assist in the determination of sex. This protocol involves the manipulation of primer annealing temperatures and the PCR cycle (as discussed in section 3.11 of the Materials and Methods Chapter), such that no X allele can be amplified in the absence of sufficient Y allele template, theoretically eliminating false female results. The creation of unequal amplification efficiencies can be observed in Figure 4.3.2 in Lane F where the Y band can be easily visualized, yet the X band is only weakly perceived due to the lesser efficiency and low copy number leading to allelic drop out. Table 4.3.1 summarizes the results of aDNA amelogenin amplifications from the St Thomas' and Harmony Road samples.

Overall, 95% of the adult male molecular sex determinations and 100% of the adult female results coincided with the historically documented or morphologically estimated sex of the skeletons. This exceeds the success rate reported for traditional indicators of sex by St Hoyme and Işcan (1989), and is comparable to Stone and colleagues molecular method (1996). The percent correct male aDNA determination rises to 96% if the single documented sex female, burial 423 from St Thomas', is removed from these calculations because it has been shown to be contaminated with male DNA from the author (JCD), and would normally be removed from further analyses. This burial was included here in order to observe

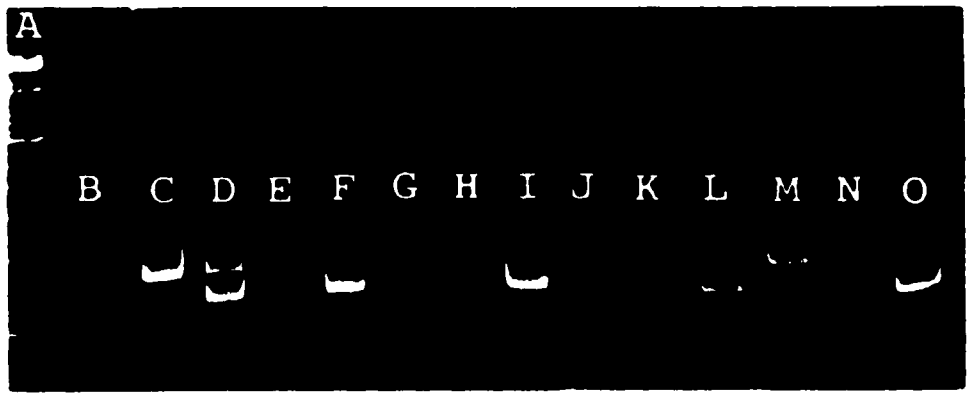


Figure 4.3.2: Amelogenin sex estimation conducted on the Harmony Road aDNA extracts. (Ethidium bromide stained Polyacrylamide gel).

Lane A) 100 bp marker; **Lane B)** PCR negative control; **Lane C)** Positive Female control; **Lane D)** Positive Male control; **Lanes E,H,J,N** have no visible PCR product; **Lane F)** demonstrates strong Y amelogenin product and weak X amplification (HR 22); **Lanes G,I,K,L,O** show only Y amplification; and **Lane M)** shows only the X amplification indicating a female skeleton (HR 38)

Table 4.3.1: Sex estimation results from aDNA amplifications of the Harmony Road and St Thomas samples

Correct Male Estimation 95%	Correct Female Estimation 100%
7 Documented Sex 12 Morphologically Estimated	3 Documented Sex 6 Morphologically Estimated
Number of Incorrect Males	**Number of Incorrect Females**
1 Documented Female shows Y band (Male DNA present) 2 Molecular Male results when Female osteology	0 Documented Males showing only X band (Female DNA) 0 Molecular Female results when male osteology
All Adult Sex Estimations:	N=56 attempts for Cemeteries
Male PCR Successful N=19 (34% of the Total) Male PCR with no result N=11 (20% of the Total)	Female PCR Successful N=9 (16% of the Total) Female PCR no result N=17 (30% of the Total)
Subadult Sex Estimations: Harmony Road N=6 -burial nos. 14, 18, 23, 24, 26, 29 -only 23 yielded Y band	St Thomas' N=3 -burial nos. 148, 321, 398 -only 321 yielded Y band
PCR Success: Harmony Road N=26 attempted, N=13 with no amplification 50% Failure of PCR	PCR Success: St Thomas' N=39 attempted N=22 with no amplification 56% Failure of PCR

the effects of known male contamination on this protocol. The remainder of the burials contaminated with JCD and DY's modern DNA were excluded from PCR analysis.

Sex determination was only conducted on aDNA extracts that had demonstrated previous success with mtDNA and/or STR analysis. It is therefore surprising that 50% of the Harmony Road and 56% of the St Thomas' sample extracts did not yield

any detectable PCR product on polyacylamide gels stained with ethidium bromide (see Table 4.3.1). A possible explanation may lie with the inherent decreased efficiency of PCR amplification of the amelogenin X allele. The successful determination of sex in nine female documented and estimated sex skeletons accounts for only 36% of the female aDNA samples and a meagre 16% of the total number of adult extracts attempted. In contrast, the successful amplification of twenty male skeletons accounts for 64% of the male samples, and 36% of the total extracts attempted, nearly twice the success rate of female PCR reactions.

Two male molecular results were found to be contradictory to the morphological sex determinations of Rogers (1991), St Thomas' burials 25 and 274. In the case of burial 25, the sex was estimated as a female from a damaged pelvis lacking the pubic region, and as a male from the cranium. Ancient DNA may therefore establish the sex of a skeleton when gross morphology yields an ambiguous result. In the case of burial 274, Rogers (1991) has unambiguously estimated this elderly skeleton as a female over two independent observations of the pelvis, and one trial using cranial indicators. A single Y band was visible for the DNA sex determination of this sample. This is a particularly interesting result as the mtDNA sequence for burial 274 is unique amongst all samples analyzed in this study suggesting that it is in fact endogenous aDNA.

In addition, the STR analyses yielded two PCR reactions in total agreement (both 6/7 genotype) suggesting that sufficient DNA was present for reliable aDNA sex determination. Thus, it may be tentatively concluded that the male molecular result for burial 274 indicates the occurrence of an elderly male skeleton incorrectly estimated as female because of a gracile appearance due to muscle marking and bone density loss.

A small sample of nine subadult extracts from St Thomas' (burials 148, 321, and 398), and Harmony Road (burials 14, 18, 23, 24, 26 and 29) were submitted to molecular sex determination. Burials 148, 398, 14, 18, 24, 26, and 29 failed to produce detectable PCR product. While no infant remains were determined to be female by this protocol, burials 321 and 23 (both demonstrating an unambiguous mtDNA sequence) were determined to be males by their aDNA. While these samples do not have supporting documentation of sex, this lends some support to the notion that this molecular approach can be usefully applied to subadult skeletal material that cannot be estimated by traditional sexually dimorphic adult traits.

Mitochondrial aDNA Sequences and Lineages

Figure 4.3.3 illustrates a mitochondrial aDNA amplification visualized on a polyacrylamide gel stained with ethidium bromide. Note that the faint band observable for reagent blank RB24 is the only contaminated reagent blank that

Figure 4.3.3: Mitochondrial DNA PCR amplification of Harmony Road aDNA extracts (Ethidium bromide stained Polyacrylamide gel).

Lane A) 100 bp marker; **Lane B)** Positive control; **Lane C)** PCR negative control; **Lane D)** RB 24 with weak contamination band; **Lane E)** Harmony Road burial 5; **Lane F)** HR 6; **Lane G)** HR 7; **Lane H)** HR 9; **Lane I)** HR 38; **Lane J)** HR 41

yielded sufficient PCR product for sequence determination. The slight contamination in RB 06 could not be analyzed despite best efforts at mtDNA sequencing protocol manipulation (see section 3.6 of the Materials and Methods Chapter). No other reagent blanks or any PCR negative controls demonstrated contamination. Due to budget constraints, mtDNA sequence analysis was repeated for only 14 extracts, or 20% of the total haplotypes generated. None of the haplotypes re-analyzed from a second PCR product demonstrated a different sequence from that originally determined.

Twenty eight sequence polymorphisms have been identified by Piercy and colleagues (1993) over the mitochondrial DNA segment amplified and sequenced in this study. A further three polymorphisms were discovered at positions 103, 131, and 193, for a total of 31 potential polymorphic sites analyzed. For convenience only the polymorphisms observed in the aDNA extracts and the positive controls (JCD and DY) appear in Appendices 4.3.3 and 4.3.4. Richards and associates (1996:186) have defined mtDNA lineages as, "...clusters of haplotypes deriving from a putative common ancestor and separated from other haplotypes by one or more mutations." Utilizing this definition, the various mtDNA sequences generated for each burial were grouped into nineteen lineages arbitrarily labelled A through S (see Appendices 4.3.5, and 4.3.6), with 'lineage A' corresponding to the reference

sequence as presented by Anderson and colleagues (1981).

The ability to compare the mtDNA sequence information from this study to other data sets was confounded by the lack of a standardized aDNA analytical approach resulting in a dearth of available published information for the hypervariable region II. Most studies of contemporary populations and archaeological human remains have utilized one of these three methods: restriction enzyme sites to generate haplotypes, the SSO probes outlined by Stoneking and associates (1991), or direct sequencing of mtDNA region I. Figure 4.3.4 compares the frequency of occurrence of the 19 Upper Canadian pioneer lineages amongst the contemporary population data available.

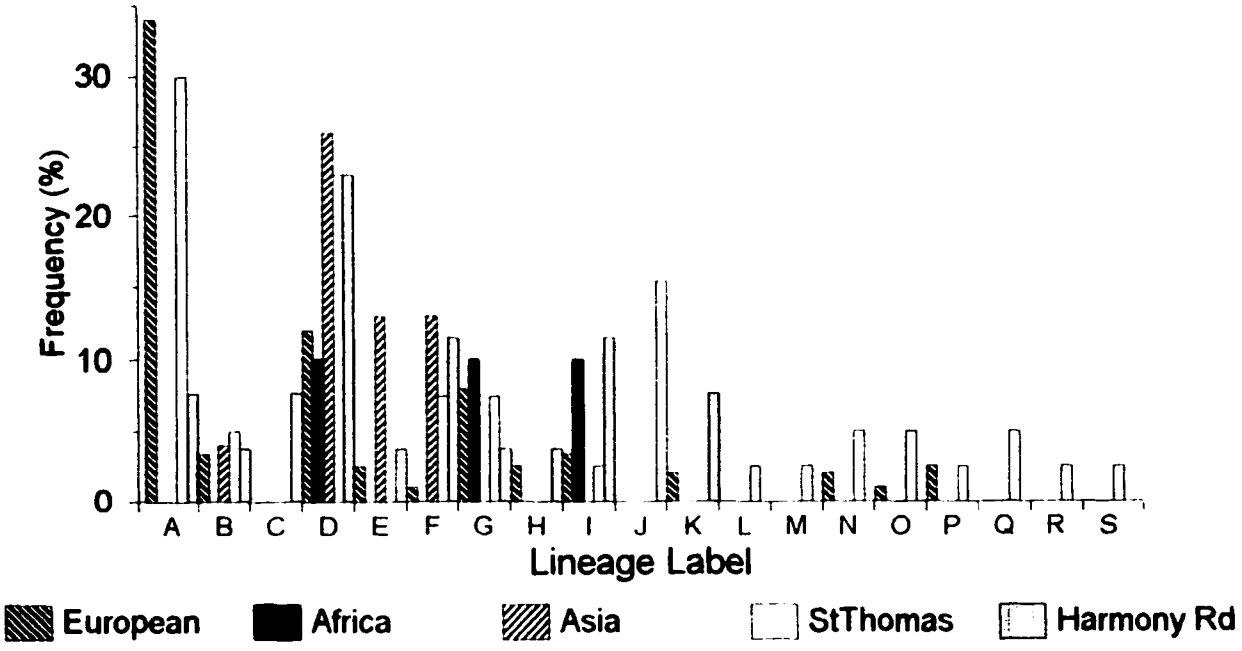
The most common sequences within the cemeteries are also the most commonly occurring lineages amongst the combined European samples (Stoneking et al. 1991, and Piercy et al. 1993, N=119 see Figure 4.3.4). The most common mtDNA sequence, lineage A or the reference sequence, is observed in 34% of contemporary Europeans and is also the most frequent in the St Thomas' cemetery (30%), but is found in only 8% of the burials at Harmony Road. This suggests that the mtDNA data collected from the Harmony Road sample may not be a random sample of European lineages, but represents instead the smaller number of families with known interment history as compared to the larger St Thomas' community parish cemetery.

Seven additional mtDNA sequences found in the pioneer cemetery samples (C, J, L, M, and Q through S) do not appear amongst the databases reviewed (Piercy et al. 1993, Stoneking et al. 1991). It is possible that these lineages represent family lines that have either completely emigrated from Europe (and thus beyond contemporary representation), have become extinct, or that mutation events have altered these archaeological sequences in living descendents. This finding also supports the rational assumption that many lineages are not yet identified within the world's populations.

BLAST homology comparisons were conducted between the nineteen lineages and the Genebank mtDNA database through the American National Institute of Health Internet server (National Institute of Health 1998). The top 100 matches generated for the vast majority of the lineages indicate ancestry consistent with European origin. Lineage J and S were exceptions perhaps because they possess polymorphisms not previously identified, thus lowering the overall homology score for all comparisons to 'racial' groups.

Out of these 19 mtDNA sequences, 6 lineages (A, B, D, F, G, I) were found in common at Harmony Road and St Thomas', and represent 61% and 75% of the respective aDNA samples. Out of the remaining sequences, 5 lineages (C, E, H, J, K, or 26% of the total lineages) were found only at Harmony Road, and 8 lineages (L through S, or 42%) were found only within the St

Figure 4.3.4: mtDNA Lineage Frequency for Contemporary and aDNA Samples



Contemporary mtDNA data from Stoneking et al. (1991) & Piercy et al. (1993)

Thomas' cemetery. The shared lineages were found more frequently at St Thomas' and a greater variety of lineages were also present within that cemetery. However, the calculation of an unbiased estimate of the mtDNA haplotype diversity revealed that Harmony Road is slightly more diverse (h=0.90) than St Thomas'(h=0.86, see Table 4.3.2). Allen and associates (1998) have determined that mtDNA diversity analyses are affected by sample size and random sampling strategies, therefore results must be cautiously interpreted.

Table 4.3.2: Unbiased estimates of contemporary population and skeletal sample mtDNA haplotype diversity*. See Nei (1987).

Location/Population	**N**	**# Haplotypes**	**Diversity(h)***
Harmony Road	26	11	0.90
St Thomas'	39	14	0.86
pre-1850 Upper Canada	33	14	0.87
post-1850 Upper Canada	32	10	0.90
Combined, Upper Canada	**65**	**19**	**0.89**
Norris Farms (Stone 1996)	52	24	0.91
Basques	61	34	0.93
Bavaria	49	35	0.98
Cornwall	69	43	0.96
Denmark	33	19	0.93
Finland	29	21	0.96
Iceland	14	12	0.96
Sardinia	69	44	0.94
Spain	30	26	0.98
Switzerland	74	42	0.96
Wales	92	45	0.93
Total (Richards et al. 1996)	**757**	**314**	**0.96**
African	129	82	0.98
Asian	74	57	0.98
Caucasian	142	99	0.98
Japanese	86	58	0.97
Mexican	94	47	0.96
Total (Stoneking et al. 1991)	**525**	**274**	**0.99**

*h=(1-Sum of x^2)n/(n-1) n=sample size x=haplotype frequency

Calculations of haplotype diversity over time for the combined Upper Canadian cemetery data set revealed h=0.87 for a sample size of N=33 pre-1850 interments, and h=0.90 for a sample size of N=32 post-1850 interments (see Table 4.3.2). This trend of increased lineage diversity over time is expected given the history of slow population growth in the first half of the 19th century, followed by increased immigration into Upper Canada at about mid-century coinciding with the Irish potato famine migration. The diversity results must however be cautiously interpreted given the likelihood of sampling error since neither cemetery was completely excavated nor analyzed for mtDNA.

Comparisons to archaeological skeletal studies is rather limited because, to this author's knowledge, only one other study has attempted to conduct comprehensive ancient DNA research on a single burial site. Stone's (1996) doctoral dissertation attempted to extract and amplify 151 precontact Indian skeletal samples from one burial mound. A total of 52 individuals were sequenced for mtDNA hypervariable region I, encompassing 27 polymorphic sites and discovering 24 mtDNA haplotypes. Only seven of these lineages appeared more than once, or amongst 67% of the total sample. A direct comparison between Stone's (1996) work and the research presented here cannot be accomplished because both studies looked at different hypervariable regions of the mtDNA genome.

The mtDNA hypervariable region II data presented in this study does however bear similar frequency characteristics to the region I studied by Stone (1996). The two mtDNA regions have been found to have similar diversity within populations (Allen et al. 1998), and therefore may allow cautious interpopulation comparison. The unbiased estimate of haplotype diversity is 0.91 for Stone's (1996) burial mound, approximately equivalent to the pioneer cemetery values reported here (see Table 4.3.2). This low mtDNA diversity may be interpreted as evidence of family burial clusters at Harmony Road, as suspected by visual inspection of the spatial relationships of the burials (see Figure 3.1, Materials and Methods Chapter). Unfortunately a complete site plan is not available for St Thomas'. Stone (1996) did not find any spatial patterning of the mtDNA lineages within the burial mound and concluded no kinship clusters.

To put the mtDNA lineage diversity in perspective, comparisons to contemporary populations with random sampling may be helpful. Richards and coworkers (1996) and Stoneking and associates (1991) found that high diversity was observed for all groups investigated with a range of 0.93 to 0.98, and totals of 0.96 and 0.99 respectively (see Table 4.3.2). This level of diversity is well above the Upper Canadian pioneer cemetery sample and the Native Americans studied by Stone

Zierdt and colleagues (1996) found with the majority of aDNA amplifications. This is likely a problem of low nuclear template number and quality of aDNA from bone tissue extracts causing a failed reaction or unequal amplification of a heterozygous genotype leading to allelic dropout. It is known that the enamel/cementum coating of teeth, as well as the hydroxyapatite inside, provide a superior environment for aDNA preservation (DeGusta 1993, Ginther et al. 1992, Lindahl 1993a, Merriwether 1994).

Heterozygosity of HUMTH01 aDNA

Heterozygosity values for the HUMTH01 locus range from 77 to 80% in contemporary forensic profiles (see Figure 4.3.8). The Harmony Road cemetery profile (see Figure 4.3.9) generates a heterozygosity value of 85% for all estimated genotypes (N=21) to 82% for confident genotype assignments (N=18). The St Thomas' profile (see Figure 4.3.9) generates heterozygosity values ranging from 69% for all genotypes estimated (N=37) to 87% for only confident genotypes (N=15). While the Harmony Road heterozygosity figures are relatively closer in approximation to the contemporary forensic values, the St Thomas' results span a much broader heterozygosity range. The low heterozygosity value (69%) for all St Thomas' estimated genotypes (N=37) likely reflects the poor STR reliability occurring with the bone extract amplifications. The allelic dropout would cause more homozygotes to appear at St Thomas'.

(1996). This difference in modern diversity may be explained by the random selection of unrelated individuals across entire regions and continents for inclusion in these forensic databases.

The comparison to the modern European databases reveals that community cemetery haplotype diversity does vary considerably from random population samples. While the reduced archaeological diversity is not unexpected, it lends support to the notion that mtDNA analysis of burial grounds and cemeteries may be able to detect population replacement, and perhaps even migration and consolidation events if accurate interment phases can be established through archaeological methods or historic documents. This result also provides a direction for further investigation into the hypothesized presence of kinship structure and lineage burial areas in the Upper Canadian pioneer cemeteries, as presented later on in this section.

Short Tandem Repeat aDNA Analysis of HUMTH01

Figure 4.3.5 illustrates a Short Tandem Repeat aDNA amplification of the HUMTH01 locus visualized on a reverse image polyacrylimide gel stained with ethidium bromide. The

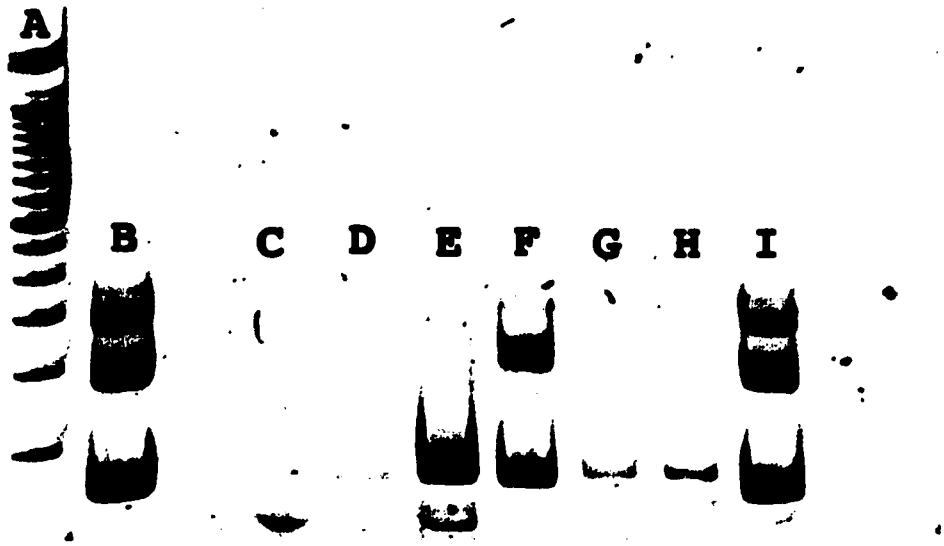


Figure 4.3.5: Short Tandem Repeat (STR) amplification of the HUMTH01 locus for Harmony Road aDNA extracts (Reverse image ethidium bromide stained polyacrylamide gel).

Lane A) 100 bp marker; **Lane B)** Positive control; **Lane C)** PCR negative control; **Lane D)** RB 24 with weak contamination band; **Lane E)** Harmony Road burial 5; **Lane F)** HR 6; **Lane G)** HR 9; **Lane H)** HR 38; **Lane I)** HR 41

faint band observable for reagent blank RB 24 is the only reagent blank that demonstrated contamination, RB 06 did not yield any PCR product in STR amplifications despite a very faint and unsequencable mtDNA contamination product. No other reagent blanks or any PCR negative controls demonstrated contamination.

Assignment of STR genotypes used the categories of 'questionable' and 'confident' previously outlined in the Section 3.9 of the Materials and Methods Chapter. In this manner 22 questionable genotypes were scored for the St Thomas' sample (60% of all genotypes estimated), yet only 3 questionable genotypes were required for Harmony Road (14% of all genotypes estimated), while 15 confident genotypes were assigned for St Thomas' extracts (40% of all genotypes estimated), and 18 for Harmony Road (86% of all genotypes estimated).

Figures 4.3.6 and 4.3.7 illustrate the HUMTH01 amplifications of tooth extracts from the Harmony Road samples (N=26). Out of this total, 23 extracts (88%) resulted in at least one amplification, and 21 extracts (91% of the successfully amplified samples) were amplified at least twice. Appendix 4.3.2 summarizes the data from both gels. Seven bone extract amplifications were attempted from a sample of the same skeletons and other burials without teeth (edentulous individuals). These bone extracts did not yield any analyzable

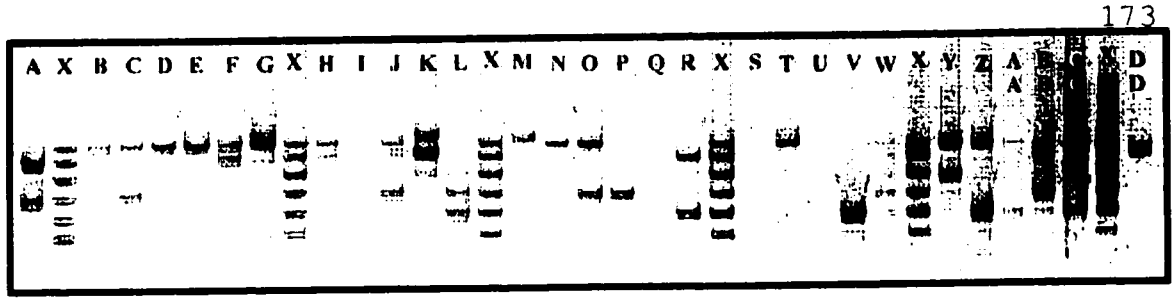


Figure 4.3.6: First PCR amplification of the HUMTH01 STR on the Harmony Road aDNA extracts.

Allelic Ladders '**X**' depict 5, 6, 7, 8, 9, 9.3 tandem repeats

Lane A) JCD positive control; **X**) Ladder; **B**) burial 5; **C**) 6; **D**) 7a; **E**) 7b; **F**) 9; **G**) 12; **X**) Ladder; **H**) 13; **I**) 14; **J**) 15; **K**) 16; **L**) 17; **X**) Ladder **M**) 18; **N**) 20; **O**) 22; **P**) 23; **Q**) 24; **R**) 25; **X**) Ladder; **S**) 26; **T**) 26; **U**) 29; **V**) 31; **W**) 33; **X**) Ladder; **Y**) 34; **Z**) 38; **AA**) 39; **BB**) 40; **CC**) 41; **X**) Ladder; **DD**) RB 24

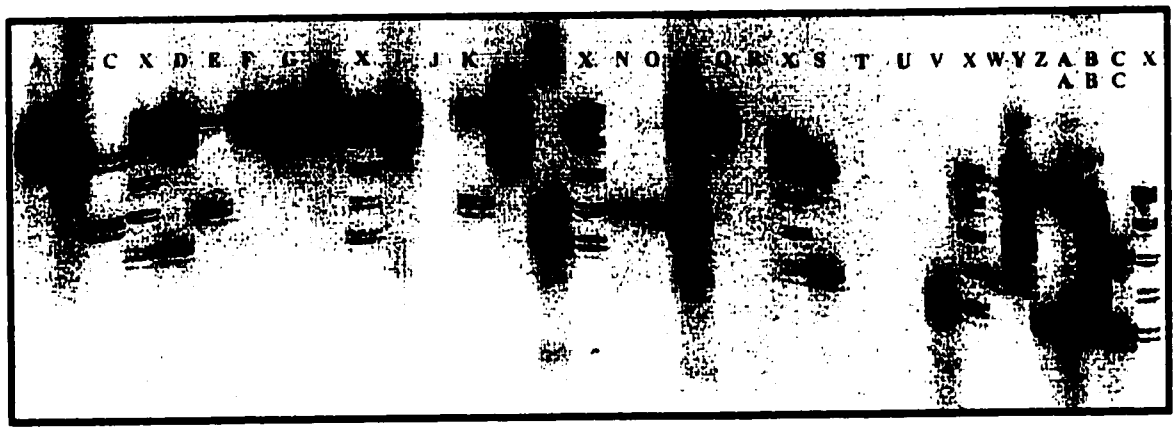


Figure 4.3.7: Second PCR amplification of HUMTH01 STR on the Harmony Road aDNA extracts.

Allelic Ladders '**X**' depict 6, 7, 8, 9, 9.3, 10 tandem repeats

A) K562; **B**) DY; **C**) JCD control DNA; **X**) Ladder **D**) burial 5; **E**) 6; **F**) 7; **G**) 9; **H**) 12; **X**) Ladder; **I**) 13; **J**) 14; **K**) 15; **L**) 16; **M**) 17; **X**) Ladder; **N**) 18; **O**) 20; **P**) 22; **Q**) 23; **R**) 24; **X**) Ladder; **S**) 25; **T**) 26; **U**) 28; **V**) 31; **X**) Ladder **W**) 33; **Y**) 34; **Z**) 38; **AA**) 39; **BB**) 40; **CC**) 41; **X**) Ladder

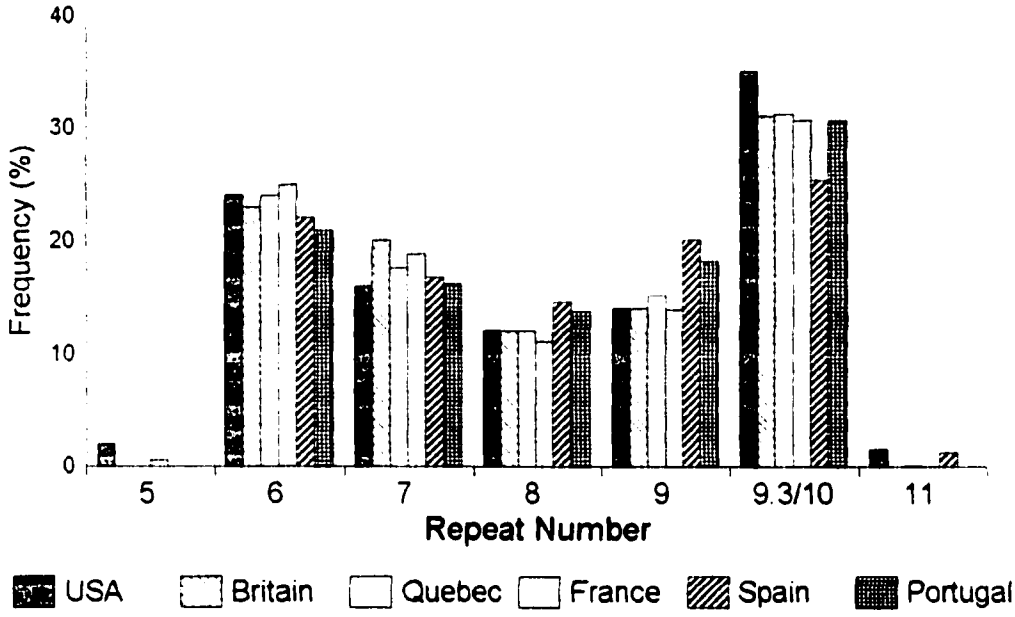
PCR products. It was surmised that the acidic clay soil and ground water observed at the Harmony Road site affected aDNA preservation in bone, therefore no further bone extracts were attempted in order to conserve research funds.

A total of 18 Harmony Road adult and subadult molar extracts, or 78% of the 23 amplifiable samples, generated the same alleles. Five problem samples (extracts HR 18, 20, 23, 26, 28) were submitted to a third PCR. Three of these extracts (18, 20, 23) had strong PCR reactions that still did not support a heterozygous genotype consistent with the alleles previously amplified, and only two extracts (26 and 28) could not be amplified a second or a third time.

The St Thomas' adult and subadult midshaft femur and clavicle extracts were successfully amplified for HUMTH01 analysis in 37 cases (or 44% of all samples, N=85), with 29 of these extracts (78%) successfully amplified twice (see Figure 3.2 of the Materials and Method Chapter, and Appendix 4.3.1). Only 15 sample extracts from these 29 cases (51%) resulted in the same alleles being repeated in consecutive PCR reactions. Therefore, 60% of the amplifiable St Thomas' bone tissue extracts demonstrate an unreliable PCR product, compared to the 22% unreliability observed with the Harmony Road tooth extracts.

The STR reliability observed with the St Thomas' samples is consistent with the results Ramos and associates (1995) and

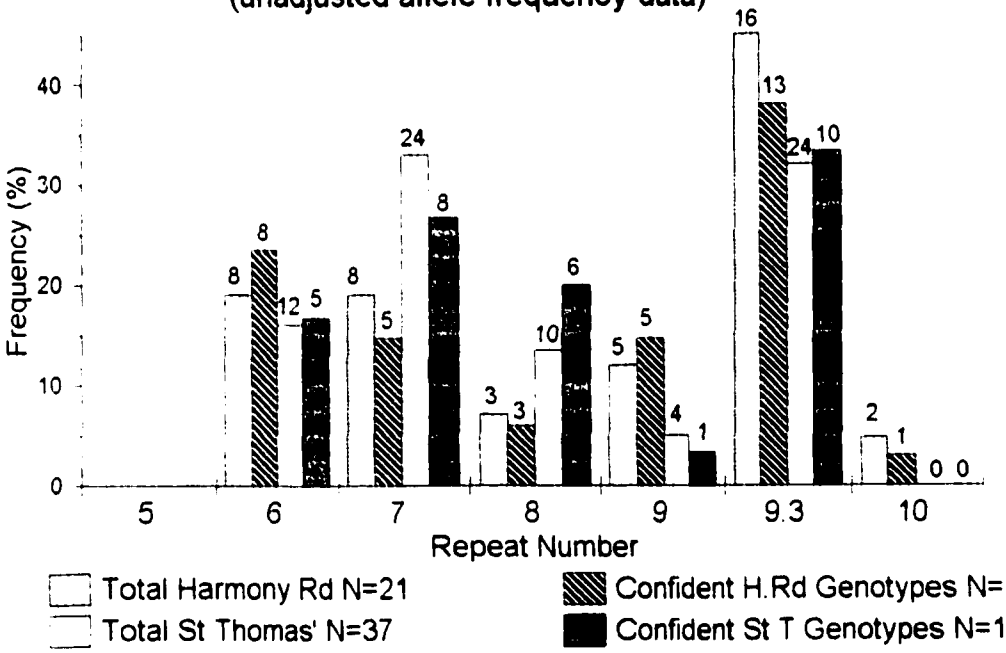
Figure 4.3.8: HUMTH01 Allele Frequency Distribution for Forensic Populations



Heterozygosity Range 77 to 80%

Figure 4.3.9: HUMTH01 Allele Patterns for Harmony Road & St Thomas' aDNA

(unadjusted allele frequency data)



82% Heterozygosity for All H.Rd Burials, 85% for Confident H.Rd Genotypes

69% Heterozygosity for All St T Burials, 87% for Confident St T Genotypes

Statistical Comparison of aDNA Short Tandem Repeat Allelic Patterns to Forensic Population Distributions

The Harmony Road and St Thomas' cemetery sample allele frequency distributions for the HUMTH01 loci can be seen in Figure 4.3.9 with Figure 4.3.8 offering a comparison to various contemporary forensic profiles from Caucasian populations (see Table 4.3.3 and Appendix 4.3.7 for data and sources). These modern allele profiles were created using randomly sampled unrelated individuals from the geographical area of interest. In some instances researchers have grouped together, or binned, alleles 9.3 and 10 due to difficulties in resolving the one base pair difference. The aDNA HUMTH01 distributions are therefore presented with these two alleles merged for convenience and statistical comparison to the forensic profiles.

A χ^2 test of goodness of fit was used to examine the Null hypothesis that the aDNA HUMTH01 allele pattern distributions generated for the Harmony Road and St Thomas' confident genotype data sets are derived from the European allele patterns established by forensic scientists (see Appendix 4.3.7). Snedecor and Cochrane (1989) advise that no expected cell frequency should be less than 1%, and that no more than one-fifth (20%) of the expected frequencies should be less than 5%. These recommendations are not violated in the aDNA data sets, therefore the use of the χ^2 test was deemed

appropriate without the need for transformation of the data sets (see Roff and Bentzen 1989). The small number of burials with allele 8 at Harmony Road (n=3), and allele 9 in the St Thomas' confident genotypes (n=1), has also lead to the merging of the individual sample profiles into a larger cemetery database for additional comparisons and interpretation.

No significant difference was found between the European forensic allele profiles and that seen in the Harmony Road or St Thomas' cemetery samples using the Chi square test at $p<0.05$ level of significance (see Table 4.3.3 for a summary of the tests). Statistically significant differences were found between the cemetery profiles and that of the Afro-Caribbean and Chinese allelic profiles. This suggests that the allele pattern generated from the archaeological confident genotypes represents a collection of Northern European interments, as expected given the immigration history of the region (see Section 4.1 of this Chapter).

A comparison between the Harmony Road and St Thomas' allele profiles yielded a statistically significant difference. While it has been hypothesized that the two cemetery samples would display significantly different allele patterns due to the genetic founder effect of the small number of first wave United Empire Loyalist pioneer families

Table 4.3.3: Summaries of the Chi Square Test of Goodness of Fit for Harmony Road and the St Thomas' Cemetery HUMTH01 allele distributions compared to forensic population patterns. Harmony Road test values appear above St Thomas' values which are in **bold text.** See Appendix 4.3.7 for allele frequencies and a sample Chi Square calculation.

Harmony Road **St Thomas' in BOLD**	Chi² Test Total	Significant Difference at p<0.05	df
vs. United States (Gill & Evett 1995)	0.54 **6.93**	no **no**	4 4
vs. United Kingdom (Gill & Evett 1995)	1.86 **5.28**	no **no**	4 4
vs. Quebec (Busque et al. 1997)	1.24 **6.38**	no **no**	4 4
vs. France (Pfitzinger et al. 1995)	1.68 **6.58**	no **no**	4 4
vs. Portugal (Pinheiro et al. 1997)	2.13 **6.86**	no **no**	4 4
vs. Spain (Lorente et al. 1994)	3.93 **7.44**	no **no**	4 4
vs. Afro-Carrib (Gill & Evett 1995)	28.8 **13.6**	yes **yes**	4 4
vs. China (Gill & Evett 1995)	49.9 **46.0**	yes **yes**	4 4

who settled, persisted, and were subsequently buried at Belleville and Oshawa, this is an unlikely explanation. The small number of burials with confident genotypes from these

cemeteries (N=18 and N=15 respectively), combined with the reported unreliability of the HUMTH01 product from the St Thomas' bone extracts, elicits little strength in this comparison. One can see in Figure 4.3.10 that the 95% confidence intervals for the aDNA allele frequency patterns encompasses both the mean allele pattern of the forensic profiles and the pioneer cemeteries. It is interesting to note that overall the allele distributions (aDNA samples and forensic populations) show the same general pattern as also seen in Figures 4.3.8 and 4.3.9.

The pioneer cemetery data set allele frequencies result in significant Chi Square test values only for the Afro-Caribbean and Chinese population profiles (see Table 4.3.3). Gill and Evett (1995) have found that the most common alleles are different in all ethnic groups. This indirectly implies that the combined cemetery database broadly reflects the Upper Canadian European immigration history, as expected, and not some other ethnic population group.

All of the short tandem repeat results demonstrate the limitations of the aDNA STR analysis when used with the small data sets typical of archaeological research. The ability to detect a reduction in HUMTH01 diversity is hamstrung by the 95% confidence intervals on the allele frequencies observed in

4.3.10: 95% Confidence Intervals for aDNA HUMTH01 Allele Distributions

95% CI = allele frequency +/- 1.96 [p(1-p)/n]^0.5
where p=allele frequency, n=number of alleles observed
(Waye et al. 1994)

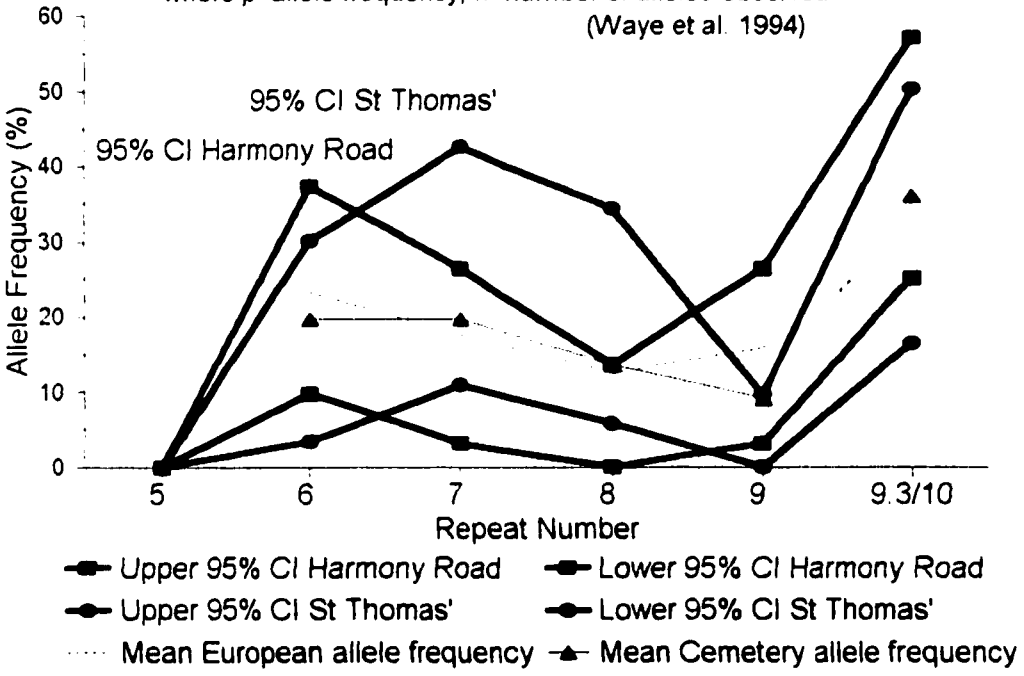


Figure 4.3.10. It is not possible to make any substantive inferences about trends in the genetic structure of these cemeteries with the STR data on hand. Therefore the testing of the hypothesized presence of kinship burial areas must implement additional data sources and perspectives to resolve this question.

aDNA and Multiplex STR kits

Multiple locus, or multiplex, PCR reactions are optimized to amplify two or more primer pair sequences at the same time, thus reducing the amount of aDNA extract used overall, the processing time involved, and post-PCR analysis. Several attempts at using commercial multiplex short tandem repeat kits (GenePrint™ CTT and FFv triplex systems, Promega 1996) were conducted on a subset of the bone and tooth extracts that had demonstrated a HUMTH01 STR product. Only five positive results were recorded (8%) out of 62 extracts that were previously successful for at least one amplification.

Out of these five positive multiplex results, four generated the same HUMTH01 genotype with different primer pairs as the singleplex reaction outlined in detail in section 3.8 of the Materials and Methods Chapter. Three of those four successful reactions amplified two other alleles from the multiplex, the TPOX and vWA tetranucleotide tandem repeat loci. All of these aDNA fingerprint genotypes differed from the profiles of laboratory personnel and each other. This

suggests that perhaps exceptional samples with enough intact aDNA template may produce multiplex PCR products, but probably with worse reliability than a single STR.

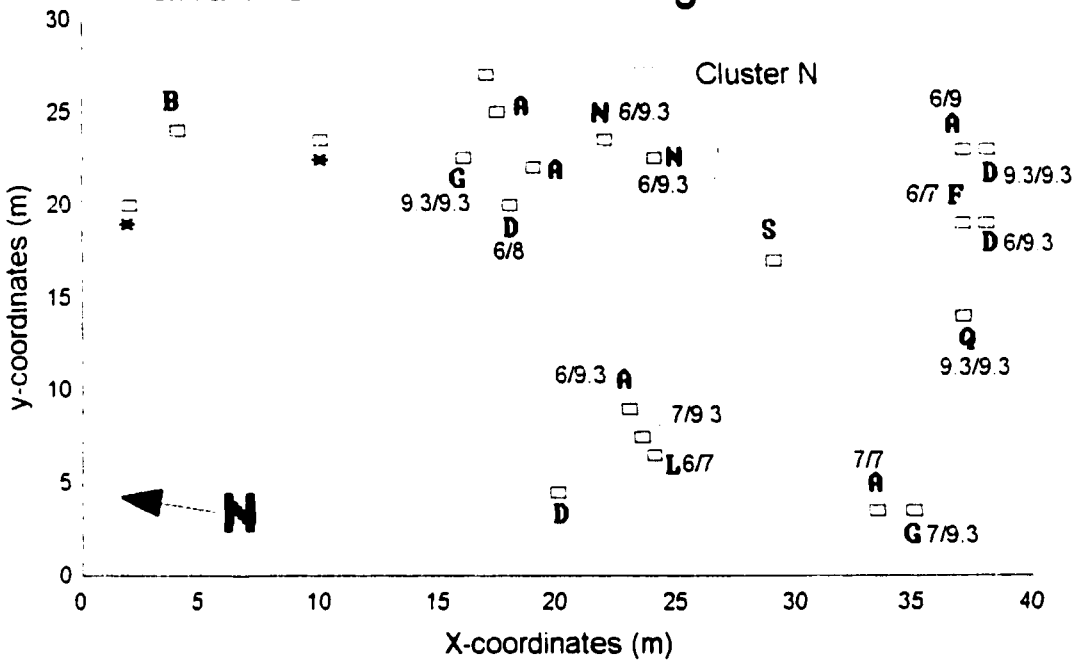
Determining Kinship Structure from aDNA

The determination of kinship structure from past peoples may be accomplished through the observation of strong patterns within aggregate archaeological genetic data. For example, with the practices of exogamy and matrilocality one would expect fewer mtDNA lineages amongst females in a community cemetery (Stone 1996). No statistical difference was found between the distribution of mtDNA lineages of females and males at Harmony Road or St Thomas' perhaps suggesting a patrilocal residence pattern after marriage given the European ethnohistoric context of male inheritance of farmland (Howell 1976, Thirsk 1976). This does not however rule out other kinship patterns such as neolocality or ambilocality of marriage partners. A more refined level of analysis involving temporal observations of mtDNA integrated with archaeological and osteological data must be used to provide support for the hypothesized patrilocal pattern.

St Thomas' Cemetery and mtDNA Lineage Patterns

Figure 4.3.11 is a schematic representation of a partially reconstructed site plan for the St Thomas' cemetery. Only interments with burial numbers up to and including the 200 range were able to be located in relative positions to each

Figure 4.3.11:St Thomas' mtDNA Lineage and HUMTH01 Clustering 1821-1874



This figure based on a partial reconstruction of the St Thomas' cemetery
All interments are historically documented, or estimated by coffin hardware, to pre-date 1850 except those marked by " * "

other. This unfortunately leaves the majority of the analyzed samples without provenience. Out of the burials that could be located (N=22), all but two have documented or estimated interment dates ranging from 1821 to 1850 placing them in the first burial phase (as described in Section 3.3 of the Materials and Methods Chapter), and therefore provide no temporal comparison.

Out of the burials located on the partial site plan 18 have mtDNA sequence information, with 9 lineages appearing in this first phase (1821-1850). Only one apparent mtDNA grouping can be identified in Figure 4.3.11, that of Cluster N involving burials 87 and 148, an adult female and a subadult both with HUMTH01 alleles 6 and 9.3. While other possible clusters may be inferred from Figure 4.3.11, such as the two burials with lineage D at the south edge of the site plan, or the two lineage A burials just on the north side of Cluster N, it must be considered that these lineages are the most common amongst the analyzed skeletons (23% and 30% respectively) and are therefore more likely to have randomly appearing clusters.

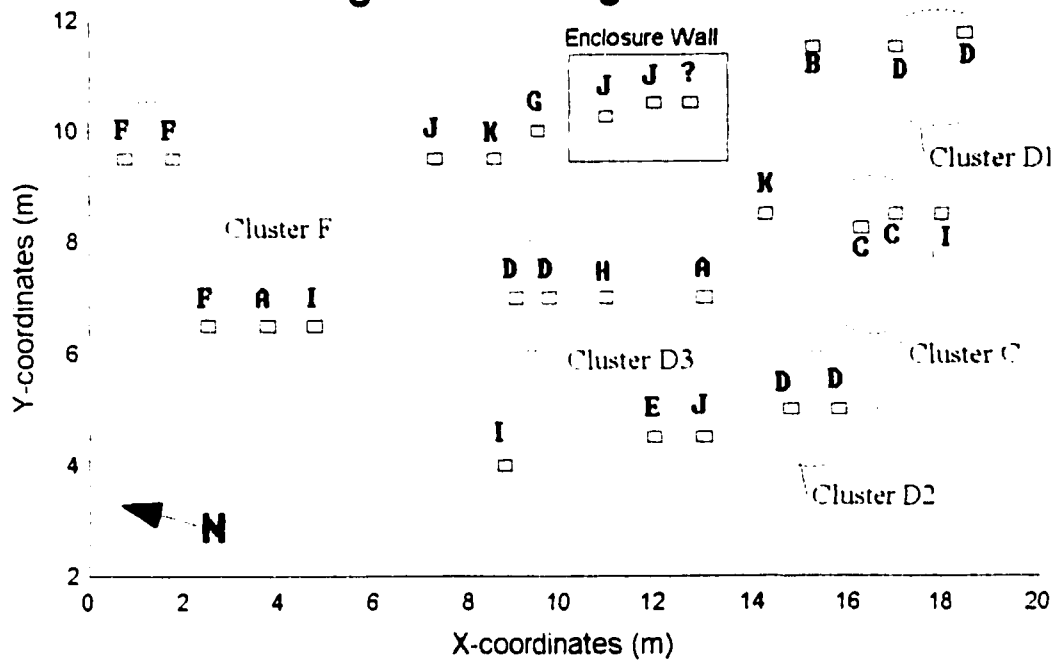
Due to the unfortunate circumstances surrounding the St Thomas' site plan, further temporal and archaeological analysis of mtDNA patterns for kinship analysis must therefore involve only the Harmony Road cemetery. Subsequent comparisons between the findings from the cemetery aDNA data sets and the historic records research is also severely limited.

Harmony Road Cemetery and mtDNA Lineage Patterns

The clustering, or grouping, of the various maternal lineages at Harmony Road are illustrated in Figure 4.3.12. This data set was too small to permit any statistical analysis of these clusters, such as multidimensional scaling or nearest neighbour cluster analysis, using the statistical package SPSS (SPSS Inc.). The six different groups of burials in close proximity with the same mtDNA lineage were interpreted by visual inspection. Five of these clusters are outlined by dotted lines, and the sixth outlined by its own mortuary feature, an enclosure wall and footstones surrounding three burials (see also Figure 3.1 in Materials and Methods chapter). Using Woodley's (1992) coffin hardware chronology for historic Southern Ontario burials it was possible to assign the Harmony Road interments into four approximate time periods, 1827-1850, 1850-1875, 1875-1900, and 1900+, as seen in Figures 4.3.13, 4.3.14, 4.3.15, and 4.3.16. Viewing the cemetery over time, one can see that almost all of the burials involved in a cluster group occur within the same time period, except Cluster F which possibly spans the periods 1827-1850 and 1850-1875.

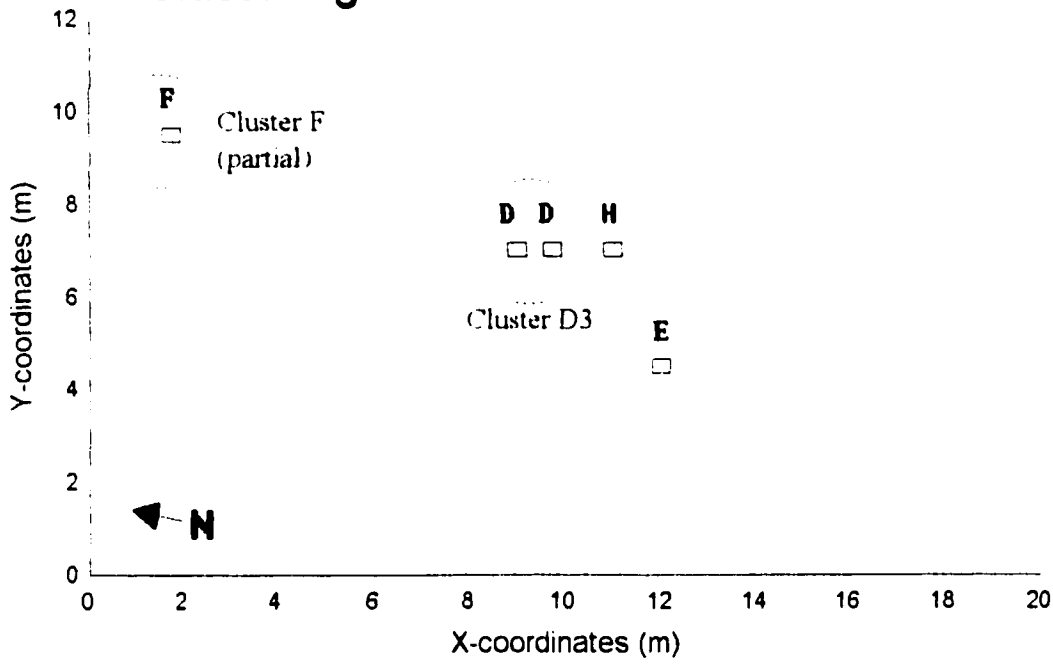
The first interment period (see Figure 4.3.13) involves 5 burials, and 4 mtDNA lineages, two D's, one E, F, and an H. The two lineage D interments, burials 12 and 13, involve an adult male and female in Cluster D3, while the lineage F burial

Figure 4.3.12: Harmony Road mtDNA Lineage Clustering 1827 to 1900+



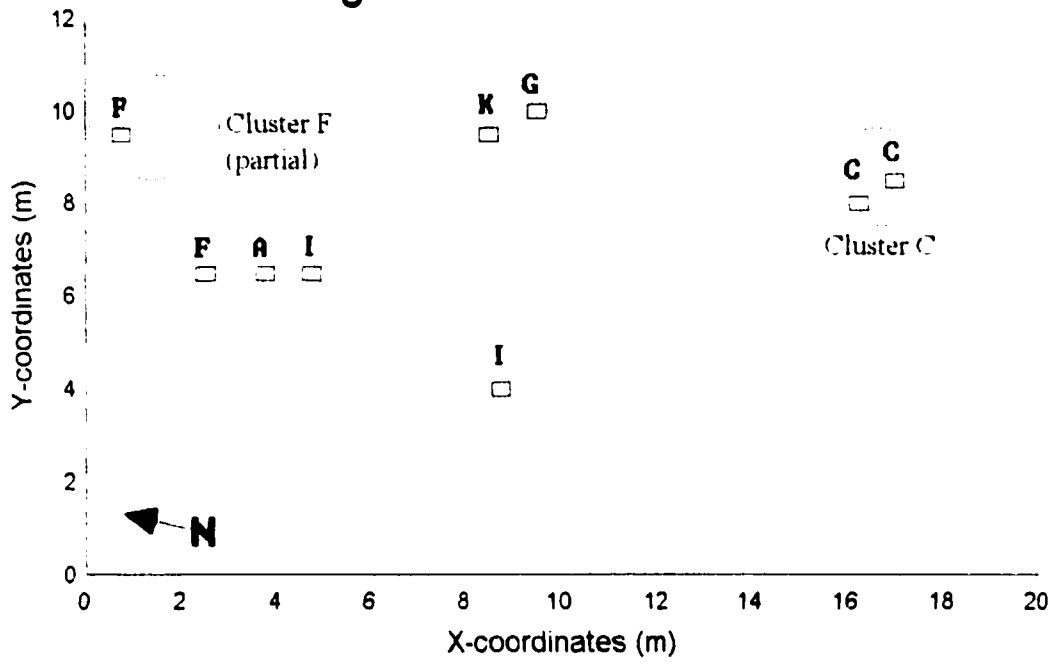
"F" = mtDNA Lineage

Figure 4.3.13: Harmony Road mtDNA Clustering for the Period 1827 to 1850



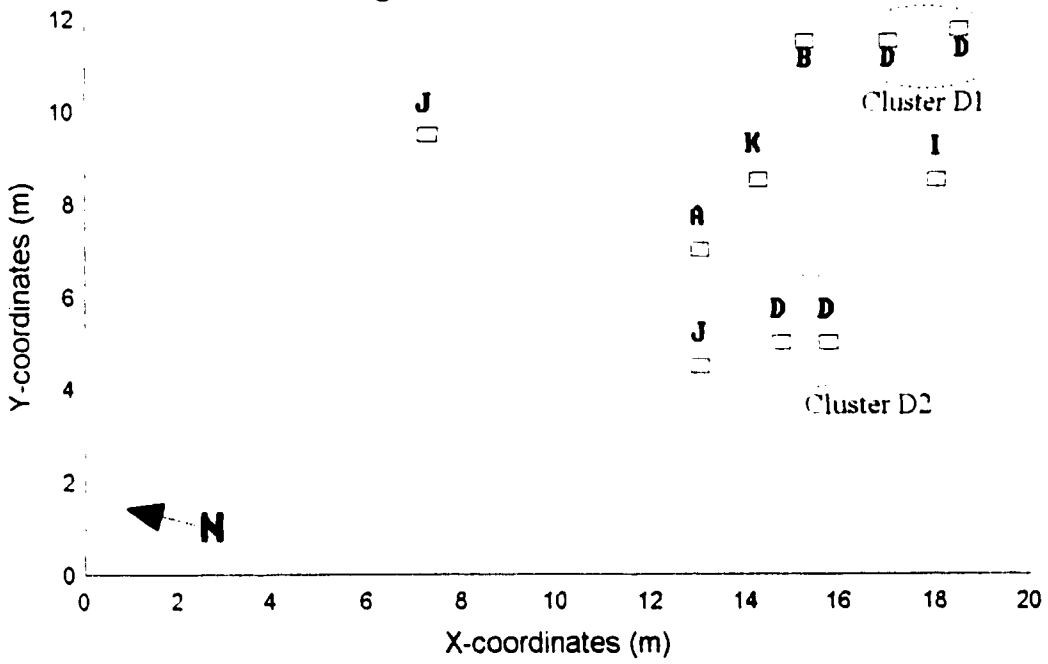
"F" = mtDNA Lineage

Figure 4.3.14: Harmony Road mtDNA Clustering for the Period 1850 to 1875



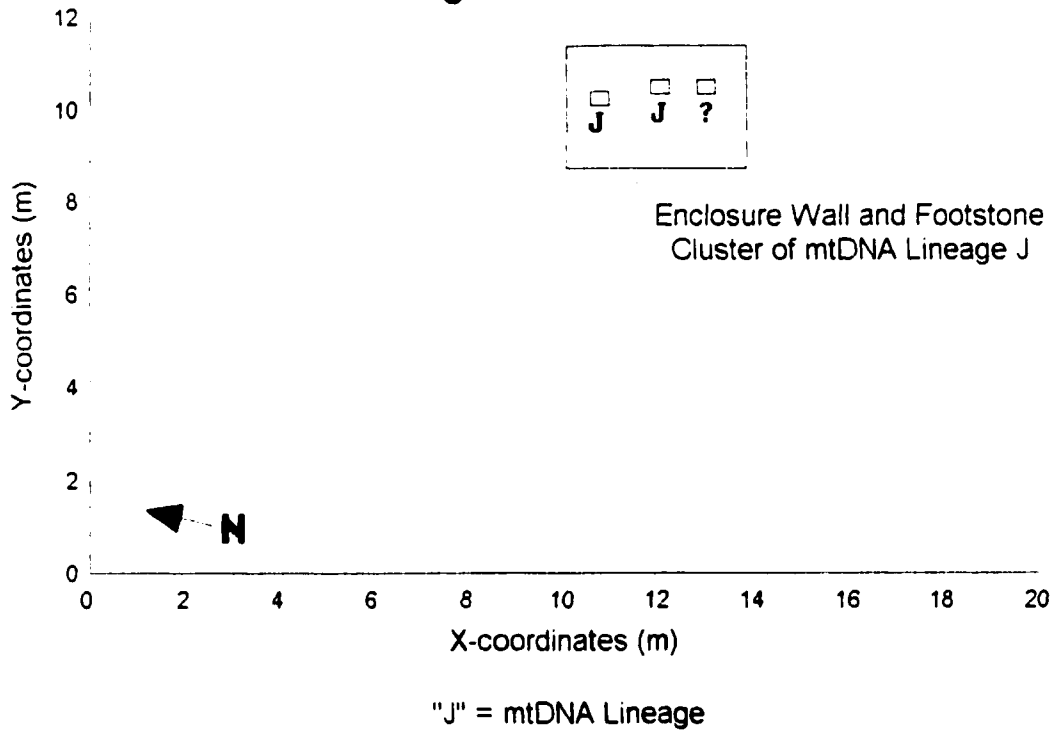
"F" = mtDNA Lineage

Figure 4.3.15: Harmony Road mtDNA Clustering for the Period 1875-1900



"D" = mtDNA Lineage

Figure 4.3.16: Harmony Road mtDNA Clustering for the Period 1900+



burial 22 (half of Cluster F) is an adult male (see Appendix 5.1 for the osteological data on Harmony Road). The majority of these burials take place in the centre area of the excavated portion of the cemetery.

The second interment period (see Figure 4.3.14) involves 9 burials and 6 mtDNA lineages, one A, two C's, two F's, one G, two I's, and a K. Lineages D, E, and H from the first phase do not appear, with lineage F being the only maternal line to be carried through to this period. Cluster F on the north-east edge of the excavated area is now completed by burial 23, a young adult male in his late teens. A second lineage F interment, burial number 16 a 30-46 year old female, is buried close by to the west but seemingly not near enough to be considered part of that cluster group. New lineages C, G, I, and K appear during this period, and all burials seem to occur on the periphery of the centre area where the first interment period burials were placed. The two lineage C burials, numbers 31 and 38, the only individuals to possess this mtDNA sequence in either pioneer cemetery or modern European database, are buried side by side on the south edge.

The third interment period (see Figure 4.3.15), from the years 1875 to 1900, involves 10 burials and 6 mtDNA lineages, one A, B, four D's, one I, two J's, and a K. Lineages A, D, and K are carried over from the second phase with D forming two new cluster groups, D1 and D2 composed of burials 34, 14

and 24, 25 respectfully, both composed of an adult male and possibly a female child. The majority of these interments are randomly arranged towards the south end of the excavated area.

The fourth interment period (see Figure 4.3.16) starts at the turn of the 19th century and involves only 3 burials, all of them purposefully arranged within an enclosure wall with footstones identifying burial locations. Only lineage J appears in this phase as seen in burials 6 and 15, burial 32 was edentulous and aDNA could not be recovered from the osteoporotic bones of this elderly female. This cluster is located to the east of the study area.

Table 4.3.4: Occurrence of Harmony Road mtDNA lineages sorted to interment period. Lineages in **bold** represent clusters.

	mtDNA Lineages Present (A to K)										
Phase 1: 1827-1850			**2×D**	E	**F**		H				
Phase 2: 1850-1875	A	**2×C**			2×**F**	G		I			K
Phase 3: 1875-1900	A	B	**4×D**					I	2×J		K
Phase 4: 1900+									**2×J**		

Overall, out of the eleven mtDNA sequences within Harmony Road, five lineages (B, C, E, G, and H) appear only within single interment periods, with lineage C forming the only pair and cluster from this group (see Table 4.3.4). The majority of these single period lineages occur in the first two

divisions, and perhaps represent individual deaths from families who later moved on, and/or hired farm hands whose documented transience never allowed them to establish families (Gagan 1981). Several surnames in fact appear only once in the Harmony Road cemetery inventory, and have no known relationship to the multigenerational family interments also listed in this inventory.

Out of the six lineages seen over two interment periods, three are involved in five clusters: F, J, and the three D clusters (lineage C composes the sixth cluster in total). No mtDNA lineages were observed over three periods, or all four interment periods. It appears that clusters generally involve mtDNA lineages that persisted for at least two interment periods, corresponding to perhaps a 50 year interval, or two generations. These maternal sequences then disappear and are replaced by lineages that again persist for two generations.

The observation of the mtDNA lineages over successive interment periods has helped reveal interesting patterns of persistance within the burials of the Harmony Road cemetery. The observations suggest an entry and replacement of mtDNA haplotypes approximately every two generations, consistent with what one would expect from a patrilocal residence structure. These results must however be cautiously interpreted with further analysis done to establish the probability of genetic relationships within the clusters.

Calculating mtDNA Probability of Kinship By Chance using Ancient DNA and Modern Forensic Databases

Table 4.3.5 presents the probability of kinship by chance (PrKBC) calculated with the equations described in Section 3.7 of the Materials and Methods Chapter for the various lineage burial clusters. It was surmised that the use of larger modern forensic databases representing random populations would over-estimate the probability of biological kinship within clusters because of their documented extensive variability (Allen et al. 1998). Since Richards and coworkers (1996) point out that the effective population size for aggregate mtDNA data is approximately one quarter that necessary for nuclear variants, the Harmony Road and St Thomas' mtDNA databases were therefore used to generate more conservative, sample specific PrKBC values.

By using the respective cemetery database from which the cluster belongs, the total cemetery database representing all pioneer mtDNA results, and the forensic database, three 95% confidence intervals (95% CI) were created. The first column and row of PrKBC values and 95% CI's reported in Table 4.3.5 depicts the kinship by chance values calculated for the burials from the relevant cemetery, and the second values in **bold face** depict the values calculated from the total pioneer database. The last column on the right reports PrKBC values calculated from the combined database of Piercy et al. (1993),

and Stoneking et al. (1991).

Table 4.3.5: Probability of Kinship by Chance for the Cluster Burials from the Harmony Road and St Thomas' Cemeteries.

PrKBC values on first row of second column are calculated from respective cemetery database, those values below in **bold** face are calculated from the combined cemetery dataset. *Third column are PrKBC values calculated from forensic databases.

Lineage & Burials	Cemetery PrKBC and 95%CI **Total PrKBC and 95%CI**	Modern Forensic Database* N=119 PrKBC and 95%CI
Cluster C: Harmony Road 31 and 38	8% 0% to 18% **3% 0% to 7%**	<0.8% 0% to <2%
Cluster D 1, 2, 3: HR 14&34, 12&13, 24&25	23% 7% to 39% **21% 12% to 32%**	12% 6% to 18%
Cluster F: Harmony Road 22 and 23	11% 0% to 24% **9% 2% to 16%**	1% 0% to 3%
Cluster J: Harmony Road 6 and 15	15% 1% to 29% **6% 0% to 12%**	<0.8% 0% to <2%
Cluster N: St Thomas' 87 and 148	5% 0% to 12% **3% 0% to 7%**	2% 0% to 4%

* Database from Piercy et al. (1993), Stoneking et al. (1991)

PrKBC values calculated from the cemetery databases generally demonstrate modest probability of kinship results. The burial clusters with the lowest probability of having the same mtDNA lineage by chance alone are Clusters C and N with PrKBC's of 3%, and 95% CI's from 0% to 7%. Clusters F and J have midrange PrKBC values of 6% and 9% with their 95% CI's falling perhaps as high 12% and 16%. The D lineage Clusters have the highest probability of occurring due to random chance

with a PrKBC value of between 21 and 23% with the high end of their 95% CI's in the 30% range.

Calculations of PrKBC from the European forensic database provide an interesting contrast. Once again clusters C and N yield low PrKBC values lending further strength to kinship conclusions, however the previously midrange clusters F and J are now amongst the lowest that can be calculated from this modern database. This reduction is a result of these haplotypes either not occurring in the database (lineage J) or at a very low frequency (1% for lineage F). The PrKBC value for Lineage D clusters also drops by approximately half of the cemetery value due to a reduced frequency of this lineage in contemporary Europeans. What this suggests is that the majority of the cemetery clusters are composed of rare or relatively uncommon mtDNA lineages, supporting the notion of a blood relationship between burials within clusters.

Modelling Parent Index Values from Recent Paternity Cases using Short Tandem Repeat Family Profiles

Table 4.3.6 presents the range of Parentage Index (PI) values generated from six short tandem repeat (STR) profiles from four contemporary families (see Appendix 3.2). In these calculations both the fathers (Parent 1) and mothers (Parent 2) were independently treated as alleged parents, with PI's generated in the absence of the other parental genotype. In this way twice as many parentage indices were created from the

Table 4.3.6: Parentage Index (PI) ranges generated from contemporary casework, and Lins et al. (1996) USA populations (values rounded to nearest whole number)

	6 Loci	5 Loci	4 Loci	3 Loci	2 Loci	1 Locus
Case 1:						
Parent 1:	19 (VyL)	24-8 (VyL-Hnt)	18-4 (L-Hnt)	10-2 (L-NU)	6-2 (Hnt-NU)	3-1 (NU)
Parent 2:	119 (ExL)	135-15 (ExL-L)	101-6 (ExL-Hnt)	50-3 (VyL-NU)	21-1 (VyL-NU)	9-1 (Hnt-NU)
Case 2:						
Parent:	1296 (PP)	1439-18 (PP-L)	514-6 (PP-Hnt)	511-3 (PP-NU)	214-1 (ExL-NU)	71-1 (VyL-NU)
Case 3:						
Parent 1:	4327 (PP)	2404-201 (PP-ExL)	1145-30 (PP-VyL)	424-11 (PP-L)	146-4 (ExL-NU)	22-2 (VyL-NU)
Parent 2:	181 (ExL)	151-10 (ExL-L)	150-5 (ExL-Hnt)	83-2 (VyL-NU)	35-2 (VyL-NU)	18-1 (L-NU)
Case 4 Child A:						
Parent 1:	29 (VyL)	29-8 (VyL-Hnt)	29-3 (VyL-Hnt)	20-2 (VyL-NU)	11-1 (L-NU)	4-1 (Hnt-NU)
Parent 2:	985 (PP)	493-126 (PP-ExL)	197-35 (ExL-VyL)	76-13 (VyL-L)	28-5 (VyL-Hnt)	8-2 (Hnt-NU)
Case 4 Child B:						
Parent 1:	26 (VyL)	29-7 (VyL-Hnt)	29-2 (VyL-Hnt)	19-1 (VyL-NU)	11-1 (L-NU)	4-1 (Hnt-NU)
Parent 2:	985 (PP)	493-126 (PP-ExL)	197-35 (ExL-VyL)	76-13 (VyL-L)	28-5 (VyL-Hnt)	8-2 (Hnt-NU)
Average Ranges:	885 (PP)	577-57 (PP-VyL)	261-14 (ExL-L)	141-5 (ExL-Hnt)	56-2.4 (VyL-NU)	16-1.3 (L-NU)

Lins et al. (1996) Paternity Index calculations on two American populations (N=unknown):

Caucasian:	27 (VyL)	13-9 (L-L)	12-4 (L-Hnt)	7-2 (Hnt-NU)	3-1 (NU-NU)	1.5-0.7 (NU-NU)
African-American:	74 (VyL)	41-26 (VyL-VyL)	21-10 (VyL-L)	10-4 (L-Hnt)	4-2 (Hnt-NU)	1.5-0.9 (NU-NU)

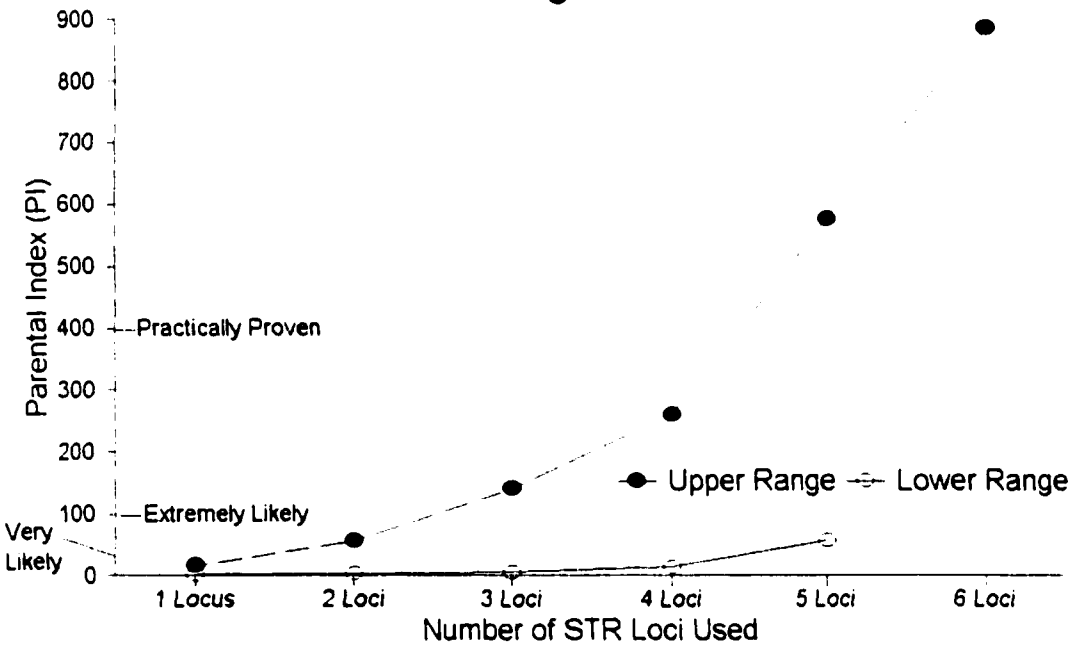
Short Forms of the Verbal Predicates:
PP=Practically Proven, **ExL**=Extremely Likely, **VyL**=Very Likely
L=Likely, **Hnt**=Hint of Parentage, **NU**=Not Useful

data set. Top range PI boundaries were calculated by multiplying the higher individual STR PI value(s), and the low boundaries utilized the lower STR PI value(s). The short form of the verbal predicate appears below these individual ranges.

It can be seen in Table 4.3.6 that a huge variety of PI values are created, with a great deal of diversity observed within individual parent PI data, and between parents and families. Individual locus data has been shown to provide little information; more discriminatory power for PI calculations is achieved by combining multiple loci (Lins et al. 1996). This is confirmed by merging all the individual upper range values, and merging all the lower range values into an average range across the data set used in this study. A clear relationship can be observed in Figure 4.3.17 between larger total PI values utilizing more STR loci, and total PI values calculated with only a few or one locus.

Lins and associates (1996) have calculated PI values from STR's for an unknown number of US Caucasians and African-Americans (see bottom of Table 4.3.6). It can be seen that these larger databases have generated average PI values closer to the lower PI range observed with the contemporary families, but still fall within the range of the data calculated in this study. The larger population database generates more conservative PI values because the vast majority of individuals share high frequency common alleles. PI calculations

Figure 4.3.17: Parental Index Ranges Calculated Using One to Six STR Loci



Upper Range PI: Average of all cases, calculated by sequentially dropping the lowest individual PI value one STR locus at a time

Lower Range PI: Average of all cases, calculated by dropping the highest PI value STR locus

utilizing rare alleles with low frequencies resulting in strong single locus PI values are exceptions, and would be overwhelmed in average calculations of large sample size.

Some of these rare allele exceptions are found in Table 4.3.6 involving Cases 2 and 3 where the parent(s) and child share a rare allele. In Case 2 the shared FESFPS allele 9 has a frequency of 0.007 and generates a PI value of 71, or 'Very Likely' parentage verbal predicate. In Case 3, Parent 1 and child share the HUMTH01 allele 5 with a Caucasian population frequency of 0.012, and Parent 2 shares the F13A01 allele 15 with a frequency of 0.014. Here one STR locus is sufficient to generate respective PI values of 22 and 18, corresponding to "Very Likely" and "Likely" verbal predicates. It is interesting to note that in these occasional instances PI values from one allele exceed or are on the order of the total PI across all six STR loci for Case 1 (Parent 1), and Case 4 (Parent 1, Child A and Child B). While it must be understood that the appearance of low frequency STR alleles are very uncommon in the general population, they can provide foci for investigation of possible biological relationships when they appear in aDNA from archaeological burial sites.

Archaeological Kinship Analysis Using Ancient Short Tandem Repeats for the Harmony Road and St Thomas' Cemetery

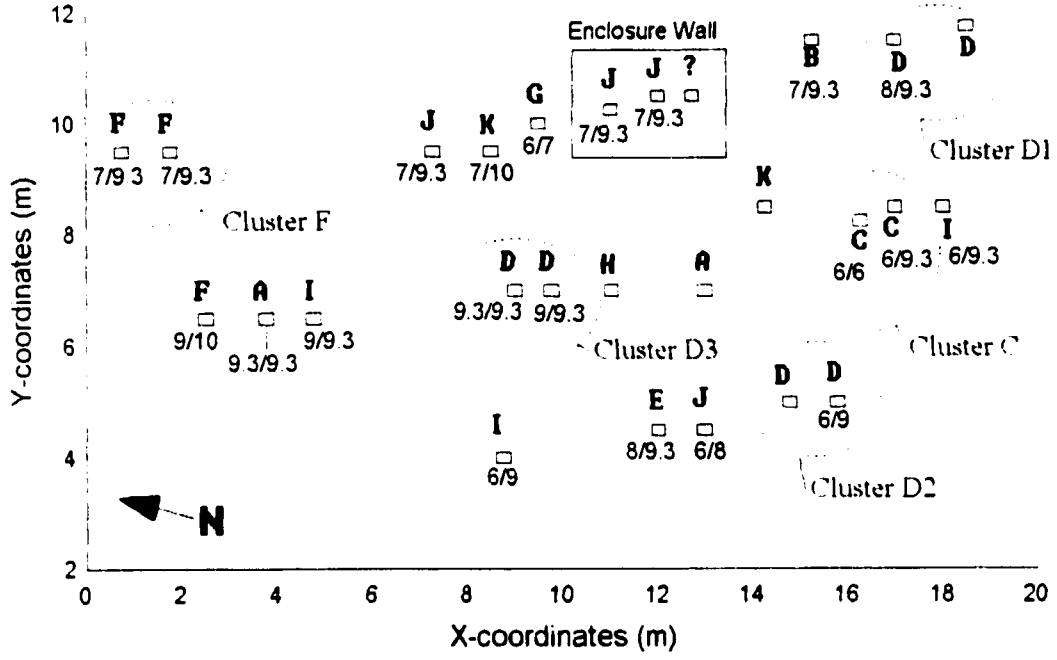
Figure 4.3.18 presents the entire Harmony Road cemetery with both mtDNA lineages and short tandem repeat (STR) data.

Within several of the clusters there is a distinct possibility of maternal relationships; the STR genotypes do not exclude any of the pairs from possible blood kinship. Since no significant difference was found between the Harmony Road HUMTH01 allele frequency distribution and the forensic Caucasian population database, the latter was used in conjunction with the parentage equations described in Section 3.10 of the Materials and Methods Chapter.

This analysis was conducted to see if the mtDNA genetic relationships could be further supported with STR data through the calculation of PI values (see Table 4.3.7). While only one STR locus was available for the calculation, it was hoped that an instance of a shared rare allele may occur within a mtDNA cluster similar to that seen in the modern cases discussed above. None of the cluster burials demonstrated parent index values above 4, that required for the 'hint' of parentage verbal predicate from Table 3.2 in the Materials and Methods Chapter.

Cluster C's PI was also calculated with burial 31 as a child and 38 as the mother (sharing mtDNA lineage C), and with burial 39 as an alleged father to complete the trio. This was deemed appropriate given the archaeological and aDNA evidence of proximity and rare mtDNA lineage. The PI generated for burial 39 was not significantly different from a random man.

Figure 4.3.18: Harmony Road mtDNA and HUMTH01 Clustering 1827 to 1900+



"F" = mtDNA Lineage, "7/9.3" = HUMTH01 STR Genotype

Table 4.3.7: Parentage Index (PI) values calculated for the Harmony Road and St Thomas' cemetery using the HUMTH01 short tandem repeat locus.

Cluster and Burials	Genotypes	Calculation	Parentage Index (PI)
Harmony Road:			
Cluster C:			
AP: burial 38	6 9.3	1/2q	PI = 2.1
burial 31	6/6	q = 0.239	Not Useful
Cluster C:			
AF: burial 39	6 9.3	X = P(AF 6)xP(M 9.3)+P(AF 9.3)xP(M 6)	
M: burial 38	6 9.3	= 0.25	
burial 31	6/6	Y = P(RM 6)xP(M 9.3)+P(RM 9.3)x(M 6)	
		= 0.17	
			PI = X/Y = 1.5
			Not Useful
Cluster D1:			
AP: burial 34	8 9.3		
burial 14	no STR results		
Cluster D2:			
AP: burial 25	6 9		
burial 24	no STR results		
Cluster D3:			
AP: burial 13	9 9.3	1/2q	PI = 1.5
burial 12	9.3/9.3	q = 0.335	Not Useful
Cluster F:			
AP: burial 22	7 9.3	1/4q + 1/4p	PI = 2.5
burial 23	7 9.3	q = 0.141 p = 0.335	Not Useful
Cluster J:			
AP: burial 15	7 9.3	1/4q + 1/4p	PI = 2.5
burial 6	7 9.3	q = 0.141 p = 0.335	Not Useful
Burials 16 & 18:			
AP: burial 16	9 10	1/4q	PI = 50
burial 18	7 10	q=0.005	Very Likely
St Thomas:			
Cluster N:			
AP: burial 87	6 9.3	1/4q + 1/4p	PI = 1.75
burial 148	6 9.3	q = 0.239 p = 0.335	Not Useful

The only mtDNA cluster at St Thomas' that may represent a possible parent/child group is that of Cluster N involving burials 87 and 148, an adult female and a sub-adult both with HUMTH01 alleles 6 and 9.3 (see Figure 4.3.16). These two alleles represent the most frequent alleles within the Caucasian distribution (see Figure 4.3.8). The resultant parent index calculation therefore did not reveal any relationship beyond random chance (see Table 4.3.7).

While none of the mtDNA clusters generated significant PI values because of the frequencies of the shared common alleles, one rare allele does occur at Harmony Road (see Figure 4.3.18). Burial 16, a 30 to 46 year old female (see Appendix 4.3.7 for osteology data) displays a confident STR genotype with HUMTH01 alleles 9 and 10 with Caucasian frequencies of 0.153 and 0.005 (Promega 1996). Alleles 7 and 10 were detected in burial 18, a 1.5 to 2.5 year old infant, but could not be repeated in subsequent amplifications leading to a questionable genotype designation. If the PI for these burials is calculated (see Table 4.3.7) a value of 50 results indicating 'very likely' parentage. Unfortunately this is a purely academic exercise due not only to the questionable genotype of burial 18, but because neither share a mtDNA lineage. The possibility still exists that these burials are related through a paternal genetic line, for example a common father/different mother step child scenario, however the

distance of the putative common ancestor with allele 10 could never be practically calculated.

Summary of aDNA Results

While a minority of aDNA extracts displayed modern contamination originating from laboratory researchers, the majority of data collected represents endogenous DNA from the pioneer cemeteries under study. This supposition is supported by the generation of 46 different aDNA genotypes through the amplification of the amelogenin sex determination locus, the HUMTH01 STR, and mtDNA lineage sequences.

Aggregate analyses of the aDNA data revealed that the mtDNA lineage diversity may be reduced from that seen in randomly sampled modern populations perhaps as a result of an immigrant founder effect and the short community history. The overall STR allele frequency distribution for the cemeteries was however consistent with the pattern seen in European forensic databases. The deviations from the modern STR pattern detected with the St Thomas' data may be a result of the amplification of a low copy number and/or damaged and unreliable aDNA template extracted from bone tissue. The Harmony Road aDNA tooth extracts demonstrated considerably better success and reliability in amplifications, perhaps leading to a more representative STR allele pattern. The calculation of 95% confidence intervals about the respective cemetery allele profiles reveals considerable overlap with

each other and with the European databases.

The use of one STR locus in the calculation of parentage index was not helpful in supporting the mtDNA probability of kinship by chance values, however the analysis did not exclude biological relationships either. This result is not surprising given the model calculations illustrating that there is a geometric decrease in the power of the analysis as fewer STR loci are used. The calculation of a rare allele PI value for the adult female interred in burial 16 and the infant in burial 18 (with no common mtDNA lineage) illustrated the need for multiple sources of data to exclude false conclusions of biological relationships. The implementation of more STR loci, in concert with amelogenin and mtDNA sequence determination, as well as other forms of analysis such as material culture and osteology, is necessary to generate stronger likelihoods of archaeological kinship.

Chapter 5: Discussion

5.1: Isonymy at St Thomas' and Christ Church

The cases of isonymous marriages can be confidently considered kinship unions due to the significant contribution of the non-random component (mean of F_n=95%) to the total inbreeding coefficient (F_t). Supporting genealogical research proves several consanguineous or affinal unions and their connection to land resources, the remainder of the marriages may therefore be similarly linked. The overall level of inbreeding would have resulted in negligible genetic effects, however the intragenerational kinship mating structure still lends itself to social interpretation within an ethnohistoric context. A comparison of the St Thomas' and Christ Church results to two other studies of pioneer mating structure have provided insights into motives behind this marriage strategy.

Interpretation of the Assortative Mating Pattern

An explanation for the anomalous increase in occurrence of isonymous unions for the second historic period (1838-1845) may be found if we return to the narrative of Susanna Moodie:

> In 1832, when we came to Canada, a great speculation was carried on in the lands of the U.E. (or United Empire) Loyalists. The sons and daughters of these loyalists,... were entitled to free grants of wild lands and of course there was a rapid rise in their value. ... The supply of U.E. Loyalist's lands, or claims for land, for a long time seemed to be almost inexhaustible; for the loyal refugees appear to have been prolific beyond all

> precedent, and most of those who held office at the capital of the province, or who could command a small capital, became speculators, and throve prodigiously. Many persons, during the early days of the colony, were thus enriched, without risk or labour, from the inexhaustible "quivers" of the U.E. Loyalists.
> (Moodie 1852:245-246)

Here it is implied that early Loyalist families took advantage of the U.E. ancestor rights by raising large families so that each of their sons and daughters could take possession of their 200 acre grant. Not only would the Loyalists have the security of the free grant when their children came of age, but families could also count on the labour pool created by a large household. So-called wild lands needed to be laboriously cleared of the endless forests, with additional daily farming chores and harvest accomplished with hand, horse, and ox. From the felling of the first trees to arable land fit for a plough took approximately ten years (Houston and Smyth 1990). McCalla (1993:29) submits that the key objective of all pioneer families must have been, "...to build wealth in the form of a productive, long-term income-earning farm whose capital value increased as the economy did." As discussed in Chapter 4.1, the most valuable land during this time consisted of improved farms in settled regions with existing roads, mills, and communities with markets.

We also discover in the Moodie narrative that the U.E. free land grant served as a primary source for funds in an economy bereft of capital. Household production and domestic exchange would have provided a very minimal income at this time (McCalla 1993). Many Loyalist descendants sold their wild land grant probably with the intention of purchasing an improved but smaller area of farm land close to the intragenerational support network of their kin relations.

What would have happened in 1837 when the free grant was basically abolished? It is hypothesized that some large agrarian families experienced a rapid transition in the vital resource of land, and in response to this stress, resorted to a kinship marriage strategy to stop the division of the established farm outside of the lineage. The individuals who employed this strategy would be the ones who persisted in the region, and were likely amongst the higher echelons of the socio-economic strata. They either had been in Upper Canada from the beginning and had the choice of the land (like the UE Loyalist's), or had recently emigrated from the old country with capital to purchase good farm land. In either case, property was central to prosperity.

It has been shown that in many instances immigrants choose to relocate to where they have established kin-relations (Kramer 1981). Gagan (1981:37) has discovered that in 1852 Peel County, Upper Canada, almost 90% of the heads of

financially secure families were foreign-born, suggesting that these immigrant families had "previous Canadian experience". The vast majority of second wave immigrants into Upper Canada came from the British Isles where impartible property inheritance was "rigorously exercised" as a means to avoid the division of property in the next generation, and thus build lineage status (Thirsk 1976:186). These late arriving immigrants may have been integrated into these successful corporate kinship systems, built on a tradition of impartible inheritance, through intragenerational consanguineous or affinal marriages.

Comparisons to other Pioneer Populations

The scenario described for 19th century Upper Canada is mirrored in the pioneer period of the Connecticut River Valley of Massachusetts as discussed by Goodman and coworkers (1988). Here they observed a traditional farming region experiencing a marked increase in market agriculture, a transition to an industrial economy with considerable off-farm employment, and a significant foreign emigration after 1850. These rapid transitions were perceived as particularly stressful, with the lament of the loss of a traditional lifestyle and an increase in religious conservatism and health complaints. Studies into the mating structure of the Connecticut River Valley pioneers by Swedlund and Boyce (1983, and see Table 4.2.5) discovered a significant high correlation between early family arrival

time in the area, wealth, and family persistence. The degree of marital isonymy within these elite families was approximately 19 times higher than expected by chance.

Additional research by Swedlund and colleagues (1983) has shown that these elite families tended to persist and asserted control over a disproportionate amount of the town's wealth. Those families that persisted grew fewer in number but maintained a considerable portion of the wealth. No correlation was found between family wealth and marriage frequency, yet a consistently high negative correlation was discovered between growth and marriage frequency. This prompted the interpretation that,"...a low frequency of marriages in an earlier period is a good predictor of a high marriage frequency in the next because there would be more resources (particularly farmland) to go around for the next generation." (Swedlund et al. 1983:127, bracketed portion in original text). The overall conclusion of these studies was that derived genealogies tend to represent an elite sub-class of persisting families, not the general population.

An interesting comparison to another 19th century frontier region is seen in the pioneer populations of Oatlands and Bothwell, Tasmania (see Table 4.2.5). Kosten and colleagues (1983) found that these marriage records generated inbreeding coefficients not dissimilar to those reported in other mainstream communities, however unlike other studies,

they discovered that the non-random component predominated. They uncovered an explanation for this difference in the history of the region: a class division between transported convicts and free settlers, and an assortative mating strategy within the newly landed gentry. Of the fourteen isonymous marriages in Oatlands, eight involved only two surnames, and there was strong evidence that these surnames were monophyletic. Reynolds (1969 as cited by Kosten et al. 1983) has documented that by 1850 seventeen families owned 46 of the 100 largest estates in Tasmania, and by 1875 extensive intermarriage had further enmeshed the land-owning families in an intricate web of kinship.

The marital pattern detected in Tasmania parallels what is occurring at St Thomas' after the cessation of the land grant era as seen in the isonymy and the repeated surnames analysis. In all three analyses the total inbreeding coefficient is moderate, yet the non-random component accounts for a significant proportion of F_t. Sabean (1976:97) maintains that,"...variation in family and kinship structure over space and over time is often intimately linked to the way resources, particularly land, are held and passed from generation to generation." If land resources figured so prominently in Tasmania, and are implicated in the Connecticut River Valley, then clearly kinship property figures highly in the explanation of the marital structure seen with the St

Thomas' pioneers.

If we integrate Swedlund and colleagues (1983) and Kosten and associates (1983) conclusions with Swedlund and Boyce's (1983) finding that isonymous unions occur more often within elite landed families, then a marriage strategy unfolds involving the maintenance of vital land resources by consanguineous and/or affinal unions of persisting kinship groups. In this way, the intragenerational kinship support alliances detected at St Thomas' would be able to prosper in the face of the mounting resource pressure documented for Upper Canada. By further integration of land records and reconstituted genealogies, it was possible to directly establish the link between some of these families and the vital kinship property they owned and managed.

Historic Records Research involving Isonymous Surnames

If the St Thomas' families associated with the kinship unions are persisting regional lineages, then one would expect that they would generate a focus of genealogical interest in the area, or provide some historic records evidence over time. Using Swedlund and colleagues' (1983) overall conclusion of derived genealogies tending to represent an elite sub-class of persisting pioneers, a tool is made from the historic documents research that was used to infer the socio-economic status of the isonymous couples and their relations. The χ^2 test of goodness of fit between the randomly selected control

surnames historic document appearances, and the isonymous surnames appearances discovered a statistically significant difference in the frequency of records 'hits'. It can be concluded that some of the surnames involved in kinship unions at St Thomas' must represent wealthy persisting families of the region.

The isonymous couples demonstrate a complex pattern of genealogical and historic documents data (see Table 4.2.9 and Appendix 4.2.2). The location of five genealogies (Haggertie/Haggerty count for two marriages, thus six unions in total) allowed the determination of consanguinity (usually first cousins) and affinal links (brother/sister-in-law, and father/daughter-in-law) between these kinship unions. These families controlled large blocks of land, and each demonstrated extra isonymous and repeated surnames marriages beyond those recorded at St Thomas'.

A curious finding is that only two individuals involved in isonymous unions, Abraham A. Canniff and Asa Keller, were definitively linked to UEL ancestor-derived land resources. Other couples could only be implicated through appearances on land grant records, or Loyalist lineage status from Lyon's (1970) pseudo-census circa 1790 (see Table 4.2.9). Yet from this list of thirteen surnames, ten lineages were found to control substantial kinship land holdings over several generations, and eight isonymous individuals were documented

as persisting (see Appendix 4.2.2).

Approximately half of the genealogies represented protestant Irish immigrants arriving sometime during the 1820's to the early 1840's, with almost all of these surnames (except Morton) listed in Lyon's (1970) First Census of Canada. It therefore appears that these lineages had what Gagan (1981:37) described as, "previous Canadian experience". Both Houston and Smyth (1990) and MacKay (1990) characterize the majority of these pre-Irish potato famine immigrants as relatively affluent members of large farming families from the Anglican ruling class. In fact by the 1840's protestant Irish represented the largest ethnic group in Upper Canada (Houston and Smyth 1990). Whether they were precognisant of the growing crisis in Ireland, or just willing to make a new start, MacKay (1990:191) believes that,"Family loyalty, more than anything else, transported hundreds of thousands of Irish across the Atlantic."

If this was the positive kinship experience of the pre-famine migrants, why did we not see a mating pattern effect once the potato famine started? Presumably the 1.5 million Irish refugees (Daly 1996) needed kinship support more than their pre-famine counterparts. An explanation can be found in the characterization of the famine immigrants themselves: a greater proportion were Catholic and/or of poorer socio-economic status (Houston and Smyth 1990). Of the 250,000

refugees who arrived in Quebec in 1847, 90% were Catholic (MacKay 1990) with only approximately 40,000 reaching Upper Canada, and of these 36,000 were sent further west or re-emigrated to the United States (Houston and Smyth 1990). Burke's (1994) study of the Belleville 1861, '71, and '81 censuses showed a significant increase in migration-prone poor Irish Roman Catholics, perhaps associated with the lumbering industry in the region. It is therefore likely that famine refugees did not have established relations in Protestant-dominated Upper Canada.

It appears that the persistence of all ten lineages controlling blocks of land in Hastings County can be ascribed to either their early arrival as UE Loyalists or, more tentatively, as second wave Irish Protestant immigrants with other relatives already present in Upper Canada. The sole exception to this pattern of success is the Dunk lineage, being late immigrants from Texas in 1831 and not listed in Lyon's (1970) First Census of Canada 1790 (see Appendix 4.2.2). Despite their uncharacteristic origin, this family maintained a modest farm of three part lots and two town lots in Campbellford, but nevertheless displayed one extra affinal isonymous marriage and two repeated surnames marriages involving a neighbouring family. Clearly intragenerational kinship alliances played an important role for many types of families dealing with the perceived stress on land resources.

Summary of Surnames Discussion

Thompson (1976) has theorized that particular social groups devise practices to provide for and guarantee the security of future generations. The model of UE Loyalist's acquisition of land resources through political policy manipulation must certainly be an extreme example of these practices, and what Weaver (1996:982) identified as "frontier settlers... contriving low-cost methods of grabbing land". They managed to obtain, for a brief period, the unprecedented security of a state-sponsored inheritance program. According to Moodie's (1852:245-246) narrative, they most certainly took advantage of it, "...for the loyal refugees appear to have been prolific beyond all precedent...". This allowed the families of subsequent UE offspring the opportunity to produce large numbers of children for several generations, all with the guarantee of 200 acres of land free of all fees, to farm or to sell.

During this time many second wave immigrant families from the British Isles, particularly Ireland, entered Upper Canada and attempted to establish families. The majority failed and moved on to better prospects in the United States; however many immigrants with existing kinship ties married into these landed families to persist and prosper. Amongst peasant societies of historic Western Europe, Sabean (1976) has noted that where there is no property, there is no basis for

individuals reinforcing their ties of blood or affinal kinship. While his observations hold in European societies with land and population density problems, the scenario in early Upper Canada is seemingly one of limitless land resources combined with a marginally sized population prone to migration. Should we see kinship alliances in Upper Canada given this set of circumstances?

In fact, very few kinship unions are found at St Thomas' during the initial period of marriage records analysis. This has been attributed to a variety of factors including: the growth of Belleville in the 1820's, the inclusion of many non-Anglican marriages in the records prior to 1831, and the presence of a land grant policy which acted to ameliorate the stress amongst certain privileged lineages. Only after the cessation of the free land grant and the growth in economy and immigration in the second period of analysis do we observe the rapid rise in kinship marriages. Sabean's theoretical predictions are empirically demonstrated in Upper Canada once the ethnohistoric context alters the perception (and perhaps the reality) of the inhabitants.

It must be concluded given the various lines of historic evidence that arrival time, previous kin relations, and most certainly land resources, are all connected in a marriage structure that for some families involved intragenerational kinship alliances. The rapidly changing social milieu must

social milieu must have placed an extreme pressure on corporate kinship property. With the hypothesis of a vital land resource stress now firmly established (hypothesis #2), it becomes possible to challenge the Saxe/Goldstein theory and its application to the archaeological and molecular aDNA evidence. Did the perceived stress on vital land resources lead to a symbolic intergenerational kinship mortuary practise involving lineage burial areas at the St Thomas' and Harmony Road cemeteries?

5.2: Interpretation of aDNA Results

Contamination and Recommendations for Contamination Control

When considering that over 120 aDNA extracts were conducted with 20 different reagent blanks, the 14 specimens and 2 reagent blank cases of contamination represent a minority of the total work. It must be concluded that systematic contamination was overcome. This leaves only random spot contamination and carrier effect as possible explanations for non-endogenous template molecules appearing in aDNA PCR reactions. No PCR master mix amplification reagent blanks demonstrated contamination during the collection of the data presented here, and reamplification of ambiguous PCR products resulted in the same ambiguous sequences. It is therefore concluded that contamination, either spot or carrier effect, occurred during the relatively

extensive manual handling required during the extraction protocol.

The fact that even seasoned aDNA researchers still report contamination experiences (Krings et al. 1997, Lindahl 1997, Stone 1996, Handt et al. 1994b) indicates that some element of random chance is involved, and that large studies involving many specimens should expect some contamination and carrier effect. It is thus unlikely that the ideal situation of zero contamination would have occurred in this study. In addition, considering the budget constraints involved in the creation of the extraction facility and the completion of this research study, and that just over one-tenth of the total work demonstrates contamination, this level is considered acceptable for the purposes at hand.

When pondering the ubiquitous presence of sloughed human skin particles and the static attraction that seems to surround the disposable plastic-ware, it is difficult to envision affordable solutions not already implemented. The more extensive use of protective hair nets, lab coats with longer sleeves, face masks, and large goggles to retain eyebrow dandruff are necessities. We (JCD and DY) have also recently recovered two plexiglass baby incubator covers from our University Hospital that are currently being used to contain extraction and PCR set up areas. Unfortunately, these areas were created too late for use in this study, but promise

to be an inexpensive way to control outside sources of airborne human dust. More expensive 'clean rooms' with positive air ventilation are generally not a realistic option for most anthropology departments or individual researchers.

Authentication of aDNA

Regardless of the lack of aDNA quantification data, it is asserted that the results obtained from the archaeological sample extracts do, in fact, represent genetic information from a past group of Upper Canadian pioneers. This conclusion is substantially supported by the occurrence of 46 different genotypes, 18 from Harmony Road, and 28 from the St Thomas' cemetery. It is extremely unlikely that so many different STR genotypes, mtDNA haplotypes, and sex determination combinations could be caused by any form of contamination given the sterilization and/or physical removal of the outer layer of the specimen, the cleanliness protocols implemented during extraction and amplification, and the restricted access to the extraction lab.

This finding provides support for further investigation into the use of new aDNA methods such as the sex determination, and mtDNA and STR kinship analyses outlined in the Materials and Methods Chapter. It also allows interpretation of the data set within the ethnohistoric context established, as well as testing of the population history and archaeological theory hypotheses outlined in the

Introduction. Does the interpretation of aDNA results reveal viable conclusions about kinship and population history without the integration of other lines of evidence?

Ancient DNA Sex Determination

The molecular determination of sex using the protocol of Yang and associates (1998b) resulted in the correct sex result for 100% of adult females, and 96% of adult males if burial 423 from St Thomas' is not included due to its identified contamination. This exceeds traditional morphological success frequencies, and is comparable to that observed in the more complex molecular technique of Stone et al. (1996). The two adult cases uncovering probable incorrect osteological sex estimation, combined with the moderate success of the subadult analysis, demonstrates that molecular determination of sex can be accomplished from problem skeletons.

While the amelogenin protocol virtually guarantees no false females due to the decreased PCR amplification efficiency of the X allele, a compromise is created whereby a significant proportion of female PCR reactions will not yield product. Yang and associates (1998b) have determined the X allele to drop out at approximately 50 pg of DNA template, with continued Y allele amplification to nearly the 5 pg range of a modern dilution series. This low level of sensitivity for the amelogenin gene has also been observed by Stone (1996), and Stone and colleagues (1996) with as little as 5 to

10 pg detected after their first round of PCR. It can be concluded that the female extracts successful for mtDNA and STR analysis but failing to amplify in sex determination, must have less than 50 pg of aDNA per PCR reaction. Future use of this protocol should be aware of this limitation.

Yang and colleague's (1998b) method has nevertheless permitted the accurate determination of sex from the St Thomas' and Harmony Road skeletal remains. When sex determination was combined with corresponding mtDNA and STR results, the 46 individual aDNA fingerprints created led to the confident conclusion that endogenous pioneer aDNA was in fact being analyzed. This permitted further investigation of genetic structure, archaeological mortuary patterns, and the determination of kinship in this study.

Short Tandem Repeat Analysis of Ancient DNA

Several aDNA researchers have attempted to amplify short tandem repeats with little reliable success, prompting one group to subtitle their aDNA STR paper, "more inconsistency than usefulness" (Ramos et al. 1995:205). The research undertaken in this study encountered similar reliability issues when working with cortical bone tissue. Only 40% (15 confident genotypes out of 37 extracts with PCR STR results) of St Thomas' bone extracts repeated the same HUMTH01 alleles in independent reactions. With the use of Harmony Road teeth as a source for aDNA template the reliability more than

doubles to 78% (18 confident genotypes out of 23 extracts with PCR STR products). The generation of confident HUMTH01 STR genotypes allowed further analyses to be conducted on the use of multiplex STR systems, ancient population allele pattern profiles, and individual genetic relationship analysis from skeletal remains.

Multiplex Short Tandem Repeat Amplifications from aDNA

It must be concluded that even though sufficient aDNA was present for the amplification of a single STR locus, the compromise of PCR conditions inherent in any multiplex caused a lowering of amplification efficiency for all loci. Only eight percent (5 out of 62 attempts) of the aDNA extracts successful for at least one HUMTH01 reaction yielded PCR product from the commercial multiplex kits. Yet in this small number of cases there was 80% agreement between the genotype results for the HUMTH01 multiplex reaction and the single locus protocol described in the Materials and Methods Chapter. It is recommended that if several STR loci are required, such as with biological kinship determination, then several PCR protocols for each locus must be individually optimized.

HUMTH01 aDNA Allele Frequency Distributions

It was hypothesized that family relationships within the Harmony Road sample would produce a statistically different STR profile when compared to a population database created from biologically unrelated individuals (hypothesis #1). The

HUMTH01 STR results do not support this hypothesis despite the strong historic, archaeological, and mtDNA lineage evidence showing several probable kinship burial zones (see Figure 3.1, and Figures 4.3.12 to 4.3.16). The statistical analysis does however reveal an allele frequency pattern consistent with the known immigration of Northern Europeans to the Oshawa region and interment history at Harmony Road. It therefore appears that considerable nuclear DNA variability is maintained in even small family cemeteries. This is likely a result of exogamous mating and recombination at every generation, and/or the interment of several affinal kinship members, such as unrelated god-parents or allied farmhands.

The comparison of the St Thomas' confident genotype profile to that of the forensic allele profiles does yield significant differences for all populations of Northern Europe, the Iberian peninsula, Africa, and Asian origin. The possibility exists that persisting families within the parish of St Thomas' are over-represented in the aDNA sampled from the cemetery. This theory is advanced by the marriage records and mtDNA diversity analysis, however it is not supported by the available St Thomas' mtDNA lineage cluster data which shows only one when compared to the six clusters at Harmony Road. It must be kept in mind that the partially reconstructed St Thomas' site plan only locates 20 burials with aDNA data from a limited temporal and spatial portion of

sharing isonymous surnames are buried in the cemetery including: Burd [Bird], Canniff, Haggerty, McCullough, Potts, and several Reed/Reid interments; however only one individual involved in an isonymous marriage is known to be buried at St Thomas', that being Mary Haggerty, wife of James, daughter of William Haggerty.

It is believed that the lack of a reliable STR product for the St Thomas' bone extracts led to the creation of a confident genotype allele frequency distribution with an increased representation of heterozygous genotypes (67% observed verses 77 to 80% expected). This led to an over abundance of the common alleles 7 and 8, as one can see in Figure 4.3.9. Zierdt and colleagues (1996) blamed considerable aDNA allelic dropout for their underestimation of STR heterozygosity in their total sample (41% observed verses 77% expected). If the St Thomas' confident and questionable genotypes are considered together the heterozygosity is observed to drop to 69%. The overlap of both cemetery allele frequency 95% confidence intervals with the mean European allele distribution (see Figure 4.3.10) also dispels the notion of a real pattern difference with the St Thomas' STR data.

The observed St Thomas' HUMTH01 allele pattern is therefore more parsimoniously explained by the problem of STR PCR reliability leading to the inaccurate portrayal of allele

frequencies. The possibility of persisting St Thomas' lineages in the parish cemetery has not been disproved by this conclusion. One must recall that both cemeteries did not display a significantly different STR allele pattern when compared to European forensic profiles.

Mitochondrial Sequence Analysis of Ancient DNA

It was hypothesized that the limited number of family interments at Harmony Road would exhibit reduced mtDNA lineage diversity when compared to the parish community cemetery of St Thomas' (hypothesis #1). Lineage diversity was also expected to increase overtime due to substantially increased immigration and population growth at Belleville after mid-century (see Herring et al. 1991: Figure 4).

Mitochondrial DNA analysis revealed a slightly higher diversity at Harmony Road (h=0.90) verses St Thomas' (h=0.86), seemingly in direct opposition to hypothesis #1. The moderate trend in increased diversity observed after mid-century in the combined cemetery data set (h=0.87 pre-1850, h=0.90 post-1850) suggests an influence from the Irish potato famine migration. It has in fact been documented that at least nine famine refugees were interred at St Thomas' (Boyce 1990). However, the small sample sizes involved in both comparisons must be considered, as well as the potential confounding problem of combining two data sets with different diversities. Allen and associates (1998) have determined that mtDNA diversity

analyses are affected by factors such as sample size and random sampling strategies. Any trends can therefore be only cautiously suggested without further aDNA sampling and analysis. This prompted further investigation with other approaches and comparisons to data sources.

While the contrasts between the pioneer cemetery data sets were necessarily limited, it was maintained that some insight into the genetic structure of the cemeteries could be uncovered, and perhaps the population history as a whole. This was attempted through comparisons to published mtDNA studies with samples of approximately similar size. When the cemetery diversity results were compared to contemporary population data (Table 4.3.2), it was discovered that both cemeteries have a lower diversity than randomly sampled populations (range of 0.93 to 0.98). This suggests that the populations represented in the cemeteries may also have had a lower diversity perhaps due to a founder effect or population bottleneck event.

Stone's (1996) comprehensively studied Native American community burial mound displayed a mtDNA haplotype diversity of h=0.91. This may also indicate the effects of genetic drift on the population that created this mound. The observed haplotype diversity for the pioneer cemeteries is on the order of that observed in Stone's (1996) archaeological sample. With these results and a superficial historical review, it may

argued that these Upper Canadian settlements, and the associated cemeteries, were carved out of the forests by a relatively small number of individuals in a short period of time. The pioneer cemetery mtDNA data is therefore reflecting the diversity of a community with a relatively brief population history. This is consistent with the known formation of Upper Canadian communities by UE Loyalist refugee families at the turn of the eighteenth century. Founder effect seems to have played a significant role in their population history.

This conclusion is not however supported by the ethnohistoric context nor the historic records research conducted on the cemeteries. The historiography presented earlier documents that Upper Canada was in fact initially settled by a small number of people (Darroch and Soltow 1994, Gourlay 1822, Wood 1988), but immigration and internal growth were substantial in the succeeding decades, especially over the period under study (Darroch and Soltow 1994, Herring et al. 1991, Wood 1988). While the majority of the population was prone to re-emigration, a minority of landed families persisted from census to census (Gagan 1981, Gagan and Mays 1973, Wood 1988). The interment inventory for Harmony Road documents that it was a multigenerational kinship burial area, and the analysis of the St Thomas' parish marriage and burial records show that persisting families were located in the

Belleville region, and buried in the cemetery. What this demonstrates is an alternative perspective to the founder effect interpretation of the aDNA results.

The mtDNA data takes on a new light when considering the social context of the communities that created the cemeteries. The presence of persisting kinship lineages would, over time, result in the increased frequency of some mtDNA lineages in the cemeteries. This would mimic the effects of founder effect, which now loses its power of explanation when the cemeteries are viewed as many interments over generations rather than as a snap shot of the pioneer population.

One must also be aware that both this study and Stone's (1996) research retrieved mtDNA data from only a sample of the possible interments. While every effort was made to extract aDNA over the complete period of cemetery use at St Thomas', and from the entire collection of individuals disinterred at Harmony Road, sampling error is a confounding possibility. This could further mimic the effects of random genetic drift, leading to inaccurate conclusions of genetic structure and population history. Future aDNA research may observe even lower haplotype diversity if the entire burial area under study is not analyzed or randomly sampled due to the possible presence of lineage burial clusters.

Archaeological Kinship Structure

If the persisting pioneer families followed the general European ethnohistoric custom of male inheritance of the family farm (Goody 1983, Howell 1976, Thirsk 1976), and by extension a patrilocal residence practice, we would expect mtDNA lineages to appear for only two generations. These generations would correspond to the entry of a maternal mtDNA lineage into the family through a bride, transfer of the lineage to both male and female children, and then the cessation of appearance in the cemetery as the female carriers of the lineage were wed and moved off. Male carriers would in all probability take wives with a different mtDNA lineage. If females of the second generation were never wed and were interred in the family cemetery, they would likely not have passed on the lineage to a third generation, and would appear indistinguishable from their male siblings. This pattern is in fact what we see at the Harmony Road cemetery (see Table 4.3.4, and Figures 4.3.12 to 4.3.16). Therefore we may accept hypothesis #3 as correct, the spatially associated aDNA evidence reflects the kinship structure of the pioneers who created the cemetery.

While this reconstruction of a kinship system does fit the available aDNA evidence and ethnohistory, this scenario is not generalizable to all pioneer families. It must be kept in mind that kinship systems are not rigidly maintained in

cultures (Peletz 1995), therefore this result cannot exclude the possibility that other patterns were also in operation. We cannot conclude archaeological kinship structure without further supporting evidence, such as individual historic documents linking some of these clusters with land and persistence in the region.

Integration of Multiple Data Sources to Confirm Kinship

It has been demonstrated that aDNA results can yield many interpretations, however with the creation of a social context through the incorporation of diverse data sources, rival theories were eliminated. Rouse (as reported in Siegel 1996) has referred to this process as consilience: a logical process whereby two or more independent yet supporting lines of evidence are used to draw conclusions of greater confidence. The following discussion presents the integration of osteological and archaeological data, multiple historic documents inquiries, and the aDNA research. In this manner informed decisions regarding the possible kinship of individual burials were made, and overall conclusions drawn on the ability of aDNA to answer anthropological questions.

- **The George W. Hinkson Family and the Cluster D1**

Support for the hypothesis of land as a critical resource in Upper Canada is found by considering the lineage D burials at Harmony Road which only occur in clusters (hypothesis #4). One of these skeletons from Cluster D1, burial 34, has been

positively identified as George W. Hinkson by a white metal name plaque. The other interment in this cluster is a 7 to 9 year old child, possibly a female due to a partially preserved braid of hair. The osteological and molecular analysis determined burial 34 to be a male, aged 32 to 44 years at death. The coffin hardware places this burial in the third interment period, 1875 to 1900. The cemetery inventory only lists one George W. Hinkson, born Oct 22 1785 died Oct 17 1857 age 72. This is not in agreement with the estimated age at death, nor the coffin hardware date. Other Hinksons listed in the inventory include Polly (wife of George), Daniel, Mary (wife of Daniel), Thomas (son of Daniel and Mary), and three Hinkson babies.

A resolution to this dilemma was discovered upon searching the 1871 Census where a farmer named George Hinkson was listed, age 25, working lot 14 BF (or Broken Front, a lake shore location), alongside Daniel Hinkson age 50, and Thomas age 21, who worked lot 12 BF. The cemetery inventory compiled from extant headstones must have missed the younger George W. Hinkson. The large Hinkson lineage headstone at the Harmony Road cemetery is very poorly preserved with the soft limestone inscriptions worn and considerable lichen and moss growth. Whether George the younger's death was inscribed on this stone, or on yet another stone which did not survive, is unknown. The connection between the two Georges is

established through the farm on lot 14 Broken Front.

The Whitby Township Census of 1822 (Oshawa Vindicator 1864) establishes that only 742 people lived in the region, and lists George Hinkson as heading a family of 8. Warton's (1836) Directory places George on lot 12 BF, with another or possibly the same George Hinkson possessing lot 2 conc. 8. Later inhabitant lists place a mis-spelled Daniel Hinkinson (son of George) on lot 12 BF, with George Hinkinson now on lot 14 BF, Ransom Hinkinsom on lot 13 BF, William Hinkinson on lot 17 conc. 7, and Henry Hinkinson on lot 18 conc. 8 (Crowder 1988, McLeod and McLeod 1989). That these men are part of the same Hinkson family is established in the 1877 Alphabetical Index for the Ontario County Townships of Whitby and East Whitby which shows a D. Hinkson as owner of lots 12, 13, 14, 15 BF, and lot 17 conc. 5. It appears as though the valuable Hinkson broken front property was consolidated in the hands of one descendent, Daniel son of George the elder.

The connection is thus made, George W. Hinkson the elder first farmed lot 12 BF and then lot 14 BF, passed on lot 12 BF to his son Daniel sometime before he died and was buried at Harmony Road. Daniel passed this property on in turn to his son George who was working it in 1871, and father and son were subsequently interred at Harmony Road as indicated by the cemetery inventory and the white metal coffin plaque in burial 34, composing part of the mtDNA lineage D clusters.

One of the Hinkson branches must therefore be mtDNA lineage D, and perhaps the two other lineage D clusters are in some way maternally related to George junior's mother Mary, wife of Daniel son of George the elder. Lineage D is one of the most common sequences amongst the Upper Canadian pioneers and the forensic database, as can be seen in the PrKBC values calculated in Table 4.3.5. Cluster D genetic relationships could conservatively occur by chance alone 12% to 23% of the time. An association has been made however between the Hinkson family, their land, and a formal burial Cluster D1. Unfortunately Mary Hinkson's maiden name remains unknown, and connections to the other D group burial clusters is therefore only suggestive given the PrKBC values.

• **The Brown Family and Cluster J**

An example of using aDNA to indicate mother/child pairs in the archaeological record is found in the analysis of Cluster J from Harmony Road. This cluster was itself defined by a mortuary feature, a low retaining wall and footstones which surrounded burials 6, 15, and 32. Ancient DNA was extracted from 6 and 15 using adult molars, but burial 32 was edentulous and no aDNA could be recovered from the cortical bone tissue of this elderly female.

Mitochondrial DNA analysis revealed that both possessed lineage J, which did not appear in the forensic database and thus generates a PrKBC value of <0.8%. The Harmony Road

cemetery database generates a PrKBC value of 15% because four burials were typed with this sequence. This contrast in values suggests that all lineage J burials are more likely to be maternally related. HUMTH01 allele frequencies for burials 6 and 15 yielded a PI value not useful for parentage determination. While the PrKBC and PI do not definitively indicate biological kinship, the presence of a strong formal mortuary symbol in the retaining wall surrounding these burials does supply convincing archaeological evidence that burials 6 and 15 are kinship related, possibly a mother/child by interment dates.

Burial 6 was identified as Robert James Brown (b. 1857 d. May 27 1937 age 80) by a white metal coffin plaque with the name "Robert James Brown", and a footstone engraved with "Robert J.B.". Burial 15 was identified as Sarah (née Cooper) Brown (b. 1824 d. 1907 age 83) by the footstone engraved with "Sarah B.", osteological findings, and coffin hardware consistent with this interment date. The 1871 census lists Sarah A. Brown age 46, Robert J. Brown age 14, Sarah 6, Deliah 4, and James Brown (deceased) their husband/father who died in 1868. From this evidence, as well as the family headstone, it was possible to definitively determine the genetic relationship between burials 6 and 15 as mother and child.

A large family, or families, with the surname Brown was well established in the pioneer Oshawa region. Abraham Brown

appears alongside the names of other early pioneers in the Whitby census of 1822 as a head of a family of 5 at the time. Abraham appears later in the 1836 Warton's Directory as an owner of a farm on lot 35 concession 1, that road being part of the Kingston Road which connected York (Toronto) in the west to Kingston in the east. Several early pioneers settled along this road taking advantage of the established transportation route (Hood, no date). Brown's Toronto City and Home District Directory 1846-47 (McLeod and McLeod 1989) and Rowsell's Directory for 1850-51 (Crowder 1988) now places a Bartholomew Brown on Abraham's farm on lot 35 conc. 1, and also lists eight other Browns (Alex, Archibald, George, Ira, John, Michael, Nicholas W., and William) on farms not more than 2 to 3 concessions away.

The first documented appearance of the Brown family branch found at Harmony Road is the 1871 census previously discussed which places the widow Sarah Brown on lot 19 conc. 4. The 1877 alphabetical index of Whitby and East Whitby shows that five lots from the Brown family property listed in the 1850-51 directory were now concentrated in the hands of Ira Brown and a D. Brown. Unfortunately, no historic link could be substantiated between the various Brown lineages. Bloomfield and Bloomfield (1991) have studied the formation of Canadian industry in 19th century Southern Ontario and list several Brown family businesses in the Oshawa area in 1871

ranging from harness, wagon, and sash and door manufacture, to weaving, tanning, and foundry operations. Whether these various businesses were linked by kin relations cannot be substantiated either as the Brown surname becomes a very common one in records of the later half of the 19th century.

The Brown burials within the enclosure wall feature does demonstrate a clustering of mitochondrial lineage J. Two other lineage J burials precede the appearance of Cluster J in the 1900's. Burial 20 is found within three meters of the enclosure wall and has been estimated to be a 30 to 55 year old female, perhaps the remains of Sarah Brown, daughter of Sarah the elder who died in 1886 at the age of 21 years. The other lineage J interment (burial 41) is not consistent with any documented relation of Sarah the elder. This burial can be suggestively linked through Sarah Brown's maternal line, or the Cooper family, which does not appear in the earlier historic records. It may be that this branch of the Cooper family was late to arrive in the Oshawa area, with lineage J appearing only in the last quarter of the 19th century.

This theory is substantiated by the 1871 census which documents that Sarah (née Cooper) Brown was in fact born in England and therefore must have emigrated sometime near mid-century. She married into the established Brown family, had three children, and was widowed by the death of her husband James in 1868. That Sarah must have had the independent means

and/or kinship support to persist in the region with her young children may be inferred by her occupation listing as 'none' in the subsequent census records.

• **The Weekes and Baldwin/Drew Families and Cluster C**

Mitochondrial DNA Lineage C does not appear in the forensic database but is found only in Harmony Road burials 31 and 38, an adult male and elderly female skeleton interred beside each other. The PrKBC value for this cluster ranges from 8% for the Harmony Road mtDNA database to less than 0.8% for the forensic database. This evidence alone strongly suggests that some form of maternal genetic relationship exists between these two individuals. The parentage index value for the HUMTH01 locus was calculated between burials 31 and 38, and 31, 38, with burial 39 as the alleged father, but the PI value was determined to be not different from that for a random unrelated person. The shared alleles between alleged parents and child were not rare within the frequency database.

Burial 38 was tentatively identified from a white metal coffin plaque engraved with "Patience". The cemetery inventory lists only one individual with this first name, Patience (née Baldwin) Drew (b. 1789 d. 1870 age 81). Burial 31 had a series of 46 brass studs arranged to form the initials "G W" separated by more brass studs used to form the outline of what appears to be an inverted 'valentine' heart shape. Only two individuals in the inventory share these

initials, George Weekes senior (b. 1800 d. April 8 1888 age 88), and George Weekes junior (b. 1827 d. Nov 17 1858 age 31 years 3 months). Burial 31 had an osteological age at death estimate of 25 to 36 years old, therefore it appears that George junior is more likely to be the skeleton interred in feature 31.

The mother of George Weekes junior is documented from the Weekes family headstone to be Margaret Weekes (b. 1799 d. April 1 1870 age 71), wife of George senior. This is confirmed from the obituary of Margret Weeks [sic] (Oshawa Vindicator, reprinted 1994), and the 1871 census which lists George senior as 70 years old, and his wife Margaret as recently deceased at age 71. If burial 31 is George junior, this would eliminate the possibility of burial 38 being Patience (née Baldwin) Drew as his mother or sister.

The historic document sources used to trace other surname lineages were not helpful in tracing the family of George Weekes. The Weekes family does not appear in the Whitby Township census of 1822 or 1836. A Gilbert and David Weeks ages 26 and 24 are listed in the 1846-47 Home district directory but have no known connection to the Weekes family. A George Weeks [sic] finally appears on lot 4 conc. 3 in Whitby Township in 1850-51 (Crowder 1988), however it is unclear if this is any relation to the "G W" of interest, for in 1877 a T. Weeks now is listed as the occupant of lot 4 conc

3 in the Whitby and East Whitby Alphabetical Index (no author 1877).

Once again we are confronted by a quandary, if "Patience" is not Patience Drew, yet burial 38 shares mtDNA with "G W", this leaves only the possibility that "Patience" may be some other relative of George Weekes such as an undocumented sister or maternal aunt. The other permutation is that "G W" is not George Weekes, but some maternal relative of Patience (née Baldwin) Drew such as a child or brother in which case the last initial "W" makes no sense if it represents the initial for a family surname. Therefore the only possibility is that "G W" may be a maternal uncle. Of course we have been assuming that the initials "G W" are symbolic of the dead man's name, which may not be the case at all.

No matter what the scenario, the cemetery inventory has been shown to be incomplete and therefore limits the resolution of the identification of these particular burials. It does however raise our consciousness to the false sense of security we place in the written word, whether from historic documents or inferred from brass studded initials. Multiple sources of information must be used in order to collectively support any inferred genetic relationship.

• Cluster N from the St Thomas Cemetery

The only mtDNA cluster from the St Thomas' cemetery involves burials 87 and 148, an adult female and subadult with

lineage N, interred sometime during the first half of the 19th century. The probability of kinship by chance for these burials is 5% or less for the pioneer and forensic databases. The parentage index calculated from the HUMTH01 loci does not yield a useful value because the shared alleles are both common in the population database. Unfortunately, no historic evidence exists that would aid us in linking these two burials, and without further aDNA analysis such as multiple STR loci, we must with caution only suggest a mother/child relationship.

Recommendations for Anthropological Analysis of aDNA

The use of aDNA analysis to determine kinship from the pioneer burials at Harmony Road has been moderately successful. The parentage index calculated from one STR locus was not as helpful in resolving biological links as the mtDNA lineage data. It is nevertheless predicted that the reliable use of more STR loci in single PCR reactions will likely establish its use as a standard procedure for determining archaeological genetic kinship. It is recommended that a minimum of six STR loci should be attempted to generate allele profiles for each extract involved in putative biological relationships. The possible range of PI values for six loci spanned the verbal predicates of "Very Likely Paternity" to "Practically Proven" based on contemporary paternity cases, while population data reveal a more moderate "Very Likely"

(see Table 4.3.6). As fewer loci are incorporated in the PI calculation, the probability of paternity declines exponentially. By attempting to amplify six loci the likelihood is also increased that at least a few will generate usable aDNA data for the statistical analysis.

The use of mtDNA lineage data proved to be more helpful than expected in resolving putative biological kinship with the implementation of the PrKBC calculation. Despite the use of more conservative PrKBC estimates from sample specific databases, several clusters generated probabilities that indicated strong evidence of a maternal relationship. The comparison PrKBC values calculated from a modern database also provides some indication of how rare or common the lineage is in a large unrelated population sample. It is recommended that future use of this calculation likewise use the mtDNA frequencies from both the study sample and a forensic database to provide a comprehensive PrKBC confidence range.

Considerable diversity was observed within the mtDNA region analyzed even amongst the small archaeological groups in this dissertation. This is the result of a higher nucleotide substitution rate promoting the creation of many novel haplotypes (Allen et al. 1998), while the effective population size is one-quarter that for nuclear variants (Richards et al. 1996). Other benefits of mtDNA observed in this study include its reliability, its high PCR success rate,

and a product that is virtually immune to the effects of jumping PCR and allele dropout. This last point is particularly salient when considering the variety of STR products and artefacts observed for some extracts that had produced unambiguous mtDNA sequences. Unfortunately individual kinship relationships are limited to the identification of maternal lines with mtDNA.

In order to overcome this limitation, future studies of archaeological kinship should consider the use of Y-chromosome haplotypes (Epplen 1994, Jobling et al. 1996). In this way both maternal and paternal lineages could be identified over time within a burial group. Limitations to using Y loci would include: the loss of the robust nature of mtDNA analysis because Y-markers are single copy nuclear genes, as well as entail the ability to detect these markers only in male interments.

Summary of Discussion

It has been shown that multiple interpretations are possible for aDNA data in the absence of a social context and historical documentation. The small sample sizes of the cemeteries and burial phases precluded the reliable assessment of drift and temporal genetic trends, however comparisons to other studies seemed to support a model of a community with a short population immigration history perhaps involving a founder effect event. In contrast, marriage records analysis

and in depth ethnohistoric research provided a social context indicating the persistence of a minority of landed kinship lineages in the presence of continued overall population mobility. Placing the aDNA data in a temporal and contextual framework proved that the persisting lineage theory fits the observed results better than a random genetic drift explanation. Therefore hypothesis #5 is accepted: multiple lines of evidence involving an ethnohistoric context revealed a more confident population history for the Upper Canadian pioneers than could be created from the aDNA data and a cursory investigation of immigration history. This conclusion also reinforces Cadien and colleagues (1974) assertion that aggregate skeletal collections cannot be considered samples of the populations from which they are derived.

When the aDNA kinship analysis was integrated with the socio-historic and isonymous marriage results indicating a land resource stress, the application of the Saxe/Goldstein theory to the Upper Canadian pioneers was found to be justified. Only through the logical process of consilience was it possible to make confident conclusions about the associations between the isonymy and aDNA results, the connection to land resources, and the nature of the observed kin relationships.

Chapter 6: Conclusions

Ancient DNA researchers have observed the need to implement sources of information other than mtDNA sequences in order to test questions of biological kinship. This study has used a variety of genetic markers ranging from nuclear STR loci, X and Y sex-linked amelogenin alleles, as well as a hypervariable region of mtDNA, to show that individual aDNA profiles can be generated from skeletal remains. Two statistical methods used in forensic and paternity analyses were adapted to suit the unique needs of archaeological kinship determination, and found to provide robust results when good quality data are retrieved.

Past investigations into the efficacy of using STR loci on archaeological tissues have generally been plagued with a reliability problem. Part of achieving good STR results hinges upon the retrieval of an adequate amount of DNA template that is free of inhibitors and contamination from non-endogenous sources. The extraction protocol developed by Yang and co-workers (1997, 1998a) proved to provide a sufficient amount of inhibitor free aDNA extract for use in the amplification of nuclear markers. Even though this protocol was created to reduce the number of manipulations of the sample, and therefore decrease the possibility of

contamination, some modern DNA was observed upon analysis. Extreme care must be taken during all steps of any extraction protocol to avoid the entry of contaminating airborne dust from possible human sources. It must be further cautioned that Yang and coworkers' (1997, 1998a) protocol generates a concentrated aDNA extract at the expense of extract volume. Therefore, researchers must take care that all PCR protocols are completely optimized before costly PCR reagents and precious aDNA template is lost on failed reactions.

Another key factor for nuclear aDNA success is the use of a tissue location with a high proportion of hydroxyapatite in order to maximize DNA molecule preservation. Teeth have been acknowledged to possess the most hydroxyapatite, and were shown in this study to provide a superior source of aDNA template. The HUMTH01 STR data collected from the Harmony Road molars was shown to greatly exceed the reliability found from bone tissue sources at St Thomas', and the reliability generated by previous investigators using loci of this type. The use of commercial multiplex STR protocols was investigated with the bone and tooth extracts and found to fail in the vast majority of cases. Therefore, these commercial kit protocols are not recommended for generating DNA profiles from archaeological skeletal samples. It is advised that individual PCR STR loci should be optimized and analyzed separately in order to collect the data necessary to achieve

robust kinship probabilities using the equations provided.

Much debate has flourished over the use of archaeological skeletal series in the determination of population history and structure data. The concern lies in the fact that burial collections generally span a length of time inappropriate for the estimation of population variables. Attempts to draw conclusions about the population history of past peoples from the aDNA data in this study revealed that there were a number of possible explanations for the observed results. A reduced number of lineages were observed in a larger proportion of the pioneer interments when compared to similarly sized archaeological and modern samples. This prompted interpretations of a population founder effect/genetic drift event.

An alternative theory for the reduced diversity was revealed in the over-representation of persisting regional lineages. This would mimic the effects of genetic drift as generations of persisting lineages were buried in the same area over the time period of cemetery use. This is analogous to the repeated appearance of certain persisting family surnames in the aggregate historic documents research. It must be concluded that the aDNA extracted and analyzed from the historic cemetery skeletal remains do not represent the past population of pioneers. It is also maintained that other burial collections may not represent their associated

population for similar reasons, and others as yet unidentified. Therefore, conclusions about population history and past kinship structure derived from aDNA should be taken as only suggestive without other lines of supporting evidence.

Future research with aDNA must break from the realm of a 'novelty act' and pursue questions meaningful to anthropologists. This would involve the use of larger samples from single sources, analyzed with a variety of different nuclear and mtDNA markers from standardized loci. It must also involve the comprehensive research and integration of a number of other culturally relevant sources of information to create a social context from which the molecular data will be further interpreted. This would include historic records whenever possible, oral traditions of indigenous peoples, and archaeological and osteological analyses. These recommendations will encourage the orderly collection of data and promote the systematic comparison of databases across cultural, chronological, and geographical boundaries. Only then will anthropological theory be truly tested.

The creation of interment periods, through the use of time-dependent material culture, significantly assisted in the integration of all forms of analysis in this study. The establishment of a burial chronology in skeletal research, while not a surrogate for a statistical population sample, may act as a sort of imperfect proxy by limiting any skeletal

analysis to a shorter period of time. This may reduce the number of confounding cultural and biological variables acting on any single burial 'cohort' and aid in a more refined analysis of individuals who died during that period. This in turn would allow the further comparison of associated periods both within the collection and between other skeletal samples, perhaps revealing trends that would be lost in an aggregate approach.

Integrating aDNA and Social Context in Upper Canada

The isonymy and historic records analysis indicates that some of the landed and persisting families of Hastings County made a practise of marrying biological or affinal relatives, either endogamously within the region, or exogamously from family left in their ancestral country. These marriages seem to have been encouraged in order to maintain vital family resources within the corporate kinship unit after the cessation of the UE Loyalist free land grant was accompanied by a surge in foreign immigration. Farm land, particularly corporate kinship control over large nearly contiguous blocks, was positively associated with the persistence of lineages over time, and thus indirectly with the wealth of the family. Ownership of land was, and still is, synonymous with prosperity. Historical evidence to support this conclusion was observed in the marriage records, land and census records, and in the reconstituted genealogies.

Molecular genetic evidence has also been found to support this hypothesized relationship between persisting lineages and vital land resources. Biological kinship relationships were established between several burial clusters within the historic cemetery of Harmony Road, and links made between some of these clusters and the control of agricultural holdings. It appears that the mortuary practices of 19th century Upper Canadian subsistence agriculturalists reflect the vital resource pressure on corporate kinship property perceived by the inhabitants. This was observed by the symbolic link these pioneers made to dead ancestors by the formation of kinship burial grounds in the manner theorized by Saxe (1970) and Goldstein (1976): the presence of a spatially non-random burial program as detected by the ancient DNA and archaeological data.

Therefore it appears that within the context of pioneer Upper Canada, the hypothesized application of the Saxe/Goldstein theory has merit. This conclusion is strongly supported by the independent lines of archaeological and molecular evidence collected in this study, and by the establishment of a social setting through historiographical, genealogical, and isonymy analyses. Without the use of these various lines of evidence combined through a logical process of consilience, the aDNA genetic analysis would only have generated sterile probabilities without any meaningful

context.

It has been recommended that future ancient DNA studies must competently integrate more anthropological sources of theory and information in order to avoid the methodological virtuosity that new technological approaches seem to engender. On a broader scale then, this study may serve as a model for possible future transdisciplinary research with aDNA. While not all skeletal collections are fortunate enough to have associated historic documents, several archaeological methods have been established to infer past social and biological environments such as archaeobotany and dietary analysis.

Future Research

While strong evidence has been presented to support and reject the hypotheses outlined in the introduction, many avenues of research are left open to pursue. It still must be empirically demonstrated that the practice of family burial areas is linked only to persisting families who controlled land resources. The possibility exists that this was just a tradition carried on from Europe, and/or part of the socioeconomic competition seen in the mortuary 'fashion cycle' theorized by Cannon (1989, 1995). For definitive proof of the link between land, kinship, marriage, and burial practice, it is necessary to repeat this analysis with a larger control group in at least one, if not a series of study areas in Southern Ontario (née Upper Canada). Perhaps of more

challenge to the hypothesized links would be to conduct analogous research in a location with a similar context. Historically this could include anywhere in the United States or Australia, however we need not limit ourselves to the past.

Goodman and associates (1988) have made an analogy of the historical conditions in the Connecticut River Valley to recent development in industrializing Third World countries. Here the opportunity to conduct anthropological field research interviews in a subsistence agricultural society with kin groups experiencing land crisis would provide first hand narrative behind the decision-making processes involved in encouraging consanguineous unions and burial practices.

If the cemetery observations made in this study have any general application, it is to caution physical anthropology researchers working with skeletal collections to be conscious of the possibility of the unrepresentativeness of their sample. If Swedlund and associates' (1983) finding that derived historical genealogies are not accurate portrayals of the larger population but of a persisting social elite, then any burial ground may contain a confounding array of select individuals. While it is true that we in fact look for such differences to explain as anthropologists, we must be aware of the broader social context involved in all research, and this involves knowledge beyond a single discipline.

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Appendices

Appendix 3.1: Contemporary paternity case data used to model archaeological parentage index situations, including a sample calculation involving the second case.

Case:	Locus HUMTH01	TPOX	CSP1P0	F13A01	FESFPS	vWA
Parent 1	6/7	8/11	11/12	5/5	10/10	16/18
Parent 2	7/9.3	8/11	9/11	6/16	10/12	16/18
Child	7/9.3	8/11	9/11	5/6	10/12	16/18
Parent 1	9/9.3	9/11	11/12	6/6	9/11	15/16
Child	9/9.3	8/11	11/11	5/6	9/12	16/16
Parent 1	5/7	9/9	11/11	3.2/5	10/10	19/19
Parent 2	7/7	8/9	10/10	4/15	11/11	16/17
Child	5/7	8/9	10/11	3.2/15	10/11	17/19
Parent 1	6/7	11/11	10/11	5/7	10/11	16/18
Parent 2	7/7	11/8	10/9	6/6	10/11	17/18
Child A	7/7	11/11	9/11	6/7	10/11	17/18
Child B	7/7	11/11	9/11	5/7	10/11	17/18

Case 2 Sample Calculation:

HUMTH01: PI = 1/4q + 1/4p
AP **9** **9.3** = 1/4(.153) + 1/4(.335)
Child **9** **9.3** = 2.38 Not Useful

TPOX: PI = 1/4q
AP 9 **11** = 1/4(0.285)
Child 8 **11** = 0.90 Not Useful

CSF1P0: PI = 1/2q
AP **11** 12 = 1/2(0.306)
Child **11** = 1.63 Not Useful

F13A01: PI = 1/2q
AP **6** = 1/2(0.285)
Child 5 **6** = 1.75 Not Useful

FESFPS: PI = 1/2q
AP **9** 11 = 1/2(0.007*)
Child **9** = 71.4 Very Likely
***allele 9 very rare**

vWA: PI = 1/2q
AP 15 16 = 1/2(0.212)
Child 16 = 2.4 Not Useful

Range of Total PI Values

One Allele: 71.4 - 0.9

Two Alleles: 214 - 1.4

Three Alleles: 511 - 2.5

Four Alleles: 514 - 6.0

Five Alleles: 1439 - 18.1

All Six Alleles: 1295

Appendix 3.2: Alternate Surname Spellings derived from Mormon Family Library database

Bird: Bard, Bardo, Bardy, Birdsey, Birdseye, Burd, Byrd, Burda

Canniff: Cannif, Canniffe, Cannuff, Conduff, Cundiff, Kannawarf, Kannawurf

Carleton: Carlton, Carltone, Carltown, Carltowne, Karlton, Karleton

Dunk: Danke, Danka, Dankow, Dunnick

Emerson: Emberson, Emmerson

Free: Freeh

Graham: Graeme, Grahame, Grayham, Grayum, Groom, Groome

Haggerty/Haggertie: Eigert, Haegert, Hagarty, Hagerty, Haggardt, Haggart, Haggert, O'Hegarty, O'Hegerty, Haggith, Heygate, Hoggart

Keller: Kelder, Kellar, Kelleher, Kildare, Quiller

McCullough: MacCall, MacClaughery, MacCleary, MacCullough, Machulla, Mackall, Makhoul, Mackelduff, McCaw

McDonald: MacDonald, Macdonald, MacDonnell, McDannald, McDanold, McDonnold, Mcdonald

Morton: Maaten, Maerten, Maertin, Maten, Matern, Maton, Matoon, Mayton, Molton, Mortan, Morten, Mortin, Morden, Moulton, Mourtin

Potts: Patters, Pothiers, Pots

Reid: Raid, Reidy, Reild, Road, Rode, Rodee, Ruid, Reed, Read, Reade, Rede, Reede, Ryde, Wrede

Vance: Van Gass, Van Ness, Van Nice, Vanness, Vannice

Wilkins: Wickens, Wilckens, Wilckins, Wilkens, Wilkie

Appendix 4.2.1: Isonymous and Control Marriages for Qualitative Surname Research

Isonymous Marriages:

Maryr	Groom name	Gres	Gocc	Bride name	Bage	Bres
1826	canniff abraham	thurlow	farmer	canniff anne		thurlow
1834	haggertie james	huntingdon		haggertie mary		huntingdon
1839	vance james	hungerford	farmer	vance sarah		hungerford
1840	mccullough william	tyendinaga		mccullough margaret		tyen.
1840	potts william	hungerford		potts ann		hungerford
1840	reid jeremiah	belleville		reid ann		belleville
1841	emerson robert	hungerford		emerson mary		sophiasburg
1843	bird stephen	hungerford		bird ann		huntingdon
1844	keller asa	madoc		keller sarah		madoc
1850	carleton james	hungerford		carleton elizabeth		hngrfd
1853	graham arthur	huntingdon		graham mary ann		hungerfrd
1854	morton thomas	hungerford		morton elizabeth		hungrfrd
1863	haggerty james	huntingdon		haggerty elizabeth		hntgdn
1867	free rice 61	seymour		free eleanor 36		seymour
1871	dunk charles 23	seymour	grdenr	dunk elizabeth 17		seymour
1891	mcdonald c	thurlow	labour	mcdonald mary c		thurlow

Control Marriages:

Maryr	Groom name	Gres	Gocc	Bride name	Bage	Bres
1840	warren christoph	belvil	innkeep	justice jane	26	belvil
1841	baker john	ernstwn	gentman	briscoe mary anne		ernstwn
1841	froste henry	belvil		payne mary ann		belvil
1841	white webster	sidney		davis eliza ann	31	sidney
1841	rose george w	sidney		ketcheson amy		sidney
1841	graham francis	thurlow		martin ann		thurlow
1843	martin william	thurlow	yeoman	howard jane		huntingdon
1844	curran david	tyendinaga		foster esther		belleville
1845	irwin william	sidney		dixon mary jane		sidney
1845	prest leonard	huntingdon		reed emma		rawdon

Appendix 4.2.2: Summary of Genealogical Research for Isonymous Surnames

-Stephen Bird and Ann Bird

Stephen and Ann Bird were married in 1843 at St Thomas' Anglican Church, listing Hungerford and Huntingdon as their residences. Lyon's (1970) First Census of Canada 1790 lists a disbanded British soldier, Henry Bird, as a resident of Marysburgh and thus there is an indication that the Bird family is an early arrival in Upper Canada.

Stephen (age 31) and Ann Bird (age 28) and four children appear in the 1851 Sidney Township Census, and 1860 and 1878 directories. However the Provincial Land Records list a Stephen Bird being issued a lease on Crown Land lot E5 con 4 Hungerford in 1843, consistent with his stated marriage record residence in 1843, and in fact he is issued a deed for this land in 1853. Two other Stephen Birds were found in the historic documents, one residing in Elzevir Township in 1860, and another in Rawdon from 1860 to 1891. Given that Stephen and Ann appear to be stable in Sidney Township in 1851, and that two other Stephen Birds are in Midland during this period, it is possible that another Stephen Bird could be involved in the property lease and purchase in Hungerford. However this does not resolve the overwhelming coincidence of the statement of marriage residence.

Nevertheless, it does appear that the Bird family does have an association with property, an early history, and a wide distribution in the Hastings County appearing in Sidney, Rawdon, Hungerford, Elzevir, Huntingdon, and Marmora. Additional repeated surname unions were also discovered between two Bird brothers and two possible Sine sisters.

-The Canniff Family

> "...an established and prestigious eastern Ontario family of Irish Huguenot origin." (Swainson 1971)

Probably the best example of a persisting lineage in the Hastings County is the Canniff family. Lt Governor J.G. Simcoe's District Loyalist Rolls for the years 1796-1803 (Fitzgerald 1985) provides the first document evidence of the Canniff family as United Empire Loyalists in Upper Canada. This Loyalist roll includes a John Canuff relocating to Prince Edward County. Canniff (1872) also places Richard Canniff amongst the first loyalists at Foxboro in 1789. John Canniff's other brothers, James and Abraham came to Upper Canada soon afterwards (Wannamaker and Wannamaker 1995). Abraham must have arrived prior to the War of 1812 for he appears to have leased land on clergy reserves in Sidney

Township in 1812. These three Canniff brothers form the trunk of the family tree that would later prosper in Thurlow Township.

John and James moved their families to Thurlow in 1806, where John purchased lots 5 and 6, conc. 3. Abraham operated as a farmer, and leased his brother John's land, "...for as long as they [he and his wife] lived and no longer." (Will of John Canniff Sr. as cited in Canniff 1987). James would later purchase lots 5 and 6, concession 2 in Thurlow and construct a damn, and build grist and saw mills on either side of the Moira River. It is interesting to note that the capital costs of constructing two mills on the Ottawa River in 1823 were £235 and £244, this at a time when average farm production barely grossed £30 per year (McCalla 1993). The well located mills comprised the nucleus for the small town that would later form around these large capital investments of the Canniff family. This town was subsequently named Cannifton.

Land records at the Provincial Archives document John Caniff (Canniff), a resident of Adolphustown, as receiving free land grants via old regulations, U.E.L., and military service, for two lots in the township of Sidney, one town lot in Belleville on Pinnacle Street, and several other lots in unspecified township locations. Abraham Caniff (Canniff) received two free grants from his military service of 200 arces each in Dawn Township. Similarily, James Canniff received at least two free land grants in unknown locations. In total, the related members of the Canniff family would receive at least 21 free grants of land comprising approximately 4200+ acres from their UE Loyalist descendent grants.

The Thurlow census for 1851 places 11 Canniff families totalling 55 individuals on 6 lots of land, yet many of these Canniffs had received free grants of land in other townships. This is indirect evidence that many of the Canniff's 21 free grants had been sold to finance the purchase of property around Cannifton and construct the mills.

The isonymous marriage involving the Canniff surname occurred at St Thomas' in 1826 between Abraham A. Canniff, and Nancy [Ann] Canniff (née Dulmage). Abraham A Canniff Jr, one of Abraham senior's 21 children, arrived relatively late in Upper Canada, about the year 1820 (Wannamaker and Wannamaker, 1995). Nancy descends from the established Loyalist Dulmage family (Morgan 1984), and was the widow of Abraham's first cousin John McBride Canniff who drowned in 1825. While investigating deeper into the Canniff genealogy, it was further discovered that after Abraham A.'s wife Nancy (née Canniff, née Dulmage) passed away in 1868, he was remarried to his brother's widow Elizabeth (née Guy) Canniff sometime between 1868 and 1871. Abraham's brother, John Weeks Canniff,

was listed as a farmer in the 1851 Thurlow census, then as a butcher in Cannifton in the 1861 census. He died shortly after in 1864. The widow Elizabeth was subsequently listed as a grocer at Cannifton in the township directory of 1867. After the marriage of Abraham A. and Elizabeth, Abraham A. took over the business as he was now listed as a grocer in the 1871 census of Thurlow.

By wisely investing capital generated from the sale of their property in Adolphustown (and possibly some other UE grants), the Canniff brothers created a niche for their family and formed a centre of activity at the mills at Cannifton. They thus managed to diversify their holdings, expand their influence, and assure the prosperity of the family by providing a sound business base, employment, and a community with strong kinship ties for the next generation. In many ways this resembles the "feathered nests" that Landon (1974:154) used to describe the miniature regional Family Compact.

While isonymous marriages do not form the keystone to the strategy of Canniff prosperity, they seem to be indicative of a high level of altruism within the family. After Abraham Canniff Sr died, the land he had leased from his brother John would have returned to that branch of the tree. What would Abraham A. have been left with? We in fact know that when Abraham A. died in 1890, his Last Will and Testament does not include the disposal of any land (Wannamaker and Wannamaker, 1995). His possessions at the time of his death included: his horse and buggy and share in a cutter which he left to his step son and nephew Guy, and $500 dollars and furniture which he left to his step daughter and neice Sarah, also from the marriage with Elizabeth.

-James and Elizabeth Carleton

James and Elizabeth were married at St Thomas' Church in 1850, James listing Hungerford as a residence, Elizabeth Hillier Township. Lyon's (1970) First Census of Canada 1790 lists a Dean, Dennis, MJR Christopher, and a Sir Guy Carleton in Upper Canada. The surname Carleton appears only in the Township of Hungerford, with six male heads of families in 1860 (ages 61 to 26) all born in Ireland. No information was found that could make a link between the isonymous couple, but it appears that many of the Carleton's in Hungerford were late arriving immigrants with perhaps previous UEL kinship support.

-Charles Dunk and Elizabeth Dunk

Charles and Elizabeth Dunk were married in 1871 at St Thomas' Anglican Church, both with residences in Seymour

Township, outside of Hastings County. No Dunks were found in any early Upper Canadian Loyalist lists, but one branch of Loyalist Dunks landed in PEI in 1784 (Dunk 1967).

Samuel Dunk left Great Britain in 1816 to go to the United States to work as a carpenter and jointer. In 1831 he and his entire family left Texas to immigrate to Upper Canada where he purchased lot 15 con 2 in Seymour Township for $100. Samuel later purchased and moved to lot 16 con 8 and lot 18 con 7 in 1845.

Charles Dunk, son of Alonzo, grandson of Samuel, married Elizabeth, daughter of William (brother to Alonzo), granddaughter to Samuel in 1871. Yet another isonymous marriage occurred in the Dunk Family under tragic conditions. Samuel Dunk junior, son of Lewis Weller Dunk, grandson to Samuel Senior, broke through river ice and drowned widowing his wife Isabella. Samuel junior's mother, Mary (née Locke) wife of Lewis Weller Dunk, was so distraught by her son's death that she herself took her life leaving behind her husband and four daughters. Dunk (1967) documents that subsequently at a later date Lewis Weller and his son's widow Isabella were married.

The will of Samuel Dunk senior was also found at the Provincial Archives and supplies an interesting insight into the egalitarian attitudes of this family with respect to inheritance. Samuel left to his son Alonzo the S½ lot 16 con 8 in Seymour, to his son Lewis Weller went lot 12 E½ Front Street in Campbellford, to William he left the property at 17 Market Square St., and to his youngest son George he left S½ lot 18 con 7 in Seymour Township with the provision that he must pay £50 to each of the two daughters who did not receive any property. While Samuel was certainly trying to be equitable to all of his children, it cannot be helped but notice that the true valuables, the land, only went to his sons.

-Robert and Mary Emerson

Robert and Mary were wed at St Thomas' in 1841, Robert listing Hungerford as his residence, Mary as Sophiasburg. On Lyon's (1970) First Census of Canada appears a John, and an ANC of Thomas (?) Emerson in Upper Canada. The Emerson surname has a wide distribution in the Hastings County: Hungerford, Huntingdon, Thurlow, and Tyendinaga. A large block of 600 acres on concession 8 in Thurlow was held by five Emersons, with documents discovered that one of these lots was a UEL free grant.

A Robert Emerson of unknown residence, was issued a deed in 1834 for lot E 2 con 1 in Hungerford, followed by a purchase in 1840 of S 10 con 2 also in Hungerford. This is

consistent with Robert's listed place of residence in the marriage record, yet a Robert Emerson appears as a yeoman on lot 12 con 9 in Huntingdon in 1860. That two Robert Emersons exist is not unlikely.

While no proof of a kinship union was found between Robert and Mary, a connection has been made between this surname, UEL status, and persistence in the region.

-Rice Free and Eleanor Free

Rice and Eleanor were married in 1867 at St Thomas' Anglican Church, both listing Seymour Township as their residence, and both natives of Ireland. A William Free appears in Lyon's (1970) First Census of Canada 1790 in Upper Canada, but no relationship was established between him and either Rice or Eleanor. The surname Free is found only in the Townships of Seymour and Hungerford.

A Henry Free (residence Parkham, near Ottawa) appears in the Land Records as receiving a full fees paid land grant in 1825, with subsequent location tickets in 1834 for Seymour Township on ajoining lots NWpt2 con 7, and SEpt3 con 8. One month later Henry purchases lots W½ 2 con 7, and SE½ 3 con 8 to complete his block of land.

The 1861 census for Hungerford lists a Henry and Jane Free (both born in Ireland) on lot E4 con 4, and a daughter Eleanor, b. 1844 age 17 indicating a likely match to the bride. However, further information was discovered in Hancocks (1980) concerning the isonymous Free union, in which Eleanor is listed as age 36 (born 1831) with parents William and Alice Free. Rice Free is listed as age 61, son of William and Mary Free. The discrepancy between age at marriage for the groom and bride suggests an affinal marriage, but no further documents were uncovered. It appears though that the Henry Free with land in Seymour must be a relation of either, or both, Rice and Eleanor. Since only 2781 people lived in Seymour Township at 1871, the surname is just too rare to be a coincidence.

-James and Mary Haggertie, James and Elizabeth Haggerty

James and Mary Haggertie were wed in 1834, and James and Elizabeth Haggerty were wed in 1864, both marriages occurred at St Thomas' Anglican Church, all four people resided in Huntingdon Township. Lyon's (1970) First Census of Canada lists an Edward and a Hugh Haggerty in Upper Canada. The surname Haggertie is interchangable with Haggerty, the first being the Olde English spelling which was used for a time by the Haggerty's living in Huntingdon (Haggerty 1944).

The family of James Haggerty and Anne Morley originates

in County Cork Ireland where James descends from a long line of prominent protestant James Haggerties dating back to 1682. James and Anne left Ireland for Upper Canada in 1827. Soon after arrival the Ontario Records list a Jas and John Haggertie purchasing a Crown Land lot 3, con 4 in Huntingdon Township, the deed for this property being issued in 1837 when Jas and Johnathan Haggertie split the lot into east and west halves. Jas and John/Johnathan are in fact James the elder (wife of Anne Morley), and his son John born in Ireland 1822 (Haggerty 1944). This farm becomes a focus of activity for this family branch.

During this time James Haggertie (likely the son of either Mike, Tim, John, or George all brothers of James the elder) and Mary Haggertie (daughter of William Haggertie, brother to James the elder) are married in 1834. Perhaps James was a new emigrant from one of the Haggertie Irish branches that did not cross the Atlantic. His residence is listed as Huntingdon Township in the marriage records suggesting he was related to the family branch living there.

In 1842 a James Haggerty is found in the Land Records with an assignment for lot E5 con 4 Huntingdon. No deed was discovered and no Haggerty appears to be living on that lot later on. By 1860 the Directory for Hastings County lists the Haggerty family with a block of four lots in Huntingdon Township consisting of lots 1 con 3, 3 con 3, 2 con 4, 3 con 4, totalling 800 acres, with another farm on lot 16 con 8. Additional Haggerty's are found only in Rawdon Township where they have lots 1 and 2 on con 7, and lot 13 con 3.

At this time another isonymous union is formed between a James Haggerty and Elizabeth Haggerty in 1864 both of Huntingdon. However it is unclear who they are descendents of, and it becomes even more murky when it is discovered that John Haggerty (son of James the elder and part owner of lot 4 con 3) weds his cousin Elizabeth Haggerty(daughter of George, brother of James the elder), no date discovered (Haggerty 1944). It is possible that John's marriage has been confused with James in the genealogy, but unlikely as it seems that James the elder had sired three sons by the name of James, two dying as children, the third born in 1833. James and John are distinctly different names in this family. It appears that three consanguinous unions have occurred in the Huntingdon Haggertys, with the isonymous union of 1864 possibly being that of James, son of James the elder.

It can be concluded that the intragenerational kinship demonstrated by the Haggerty's allowed this large immigrant family to persist, prosper, and grow without the concommitant subdivision of the farm that sustained them. That the family security revolved around the block of farms is best characterized by a Haggerty in the following passage:

> This farm is still in the family, having been owned and farmed by the original owner [John Haggerty] until his death, and then by his son, James as long as he lived... The last member of the family to live on the homestead was Barton Haggerty, son of James... I have been told, on good authority, that there has never been a mortgage, or even a note, against this farm at any time.
>
> (Haggerty1944:5)

-Asa Keller and Sarah Keller

Asa and Sarah Keller were married in 1844 at St Thomas' Anglican Church, both reporting Madoc Township as their residence. The name Frederick Keller, a disbanded Hessian soldier, is listed in Lyon's (1970) First Census of Canada 1790, as living in Kingston. The Keller family alone (without spelling variants) has a list of 48 free land grants including several old regulations, UE, son of UE, and daughter of UE Loyalist. Asa Keller, son of Frederick (order in council for UE grant 1808), grandson of Frederick (Reid 1973) appears in the Provincial Land Records with an order in council in 1843 issuing him a son of UE loyalist land grant for an unknown location. This is corraborated by Reid's (1973) list of marriages and orders in council for UE Loyalists. Granpa Fredrick has been documented as having 15 children, 14 of them receiving their order in council for a land grant. Despite all of the documented indications of persistance, no other links could be made been between Asa and Sarah, and thus no definitive kin relationship could be established.

-William and Margaret McCullough

William and Margaret McCullough were married at St Thomas' in 1840, both of them listing Tyendinaga as their place of residence. Lyon's (1970) First Census of Canada 1790 lists Charles, John and Mary, and Jane McCullough in Upper Canada. The surname McCullough was only discovered in Tyendinaga where a block of 1400 acres centered on concession 4 was controlled by nine different male heads of households in 1860. A William McCullough appears to have purchased one of these concession 4 lots in 1836 with a deed issued in 1854. He still appears on this lot in 1860. Given the limited distribution of this surname in the Hastings County to Tyendinaga, specifically to seven lots on concession 4, and that both William and Margaret were both residents of that Township, it must be concluded that they were at least affinally related if not consanguinously. Their marriage surely involved some aspect of an intragenerational kinship structure motivated by the family property.

-Thomas Morton and Elizabeth Morton

Thomas and Elizabeth were married in 1854 at St Thomas' Anglican Church, and report their respective residences as Hungerford and Huntingdon townships. The surname Morton, and possibly the families of the isonymous couple, appears to have a long history in North America as Alexander Morton is listed as residing in Upper Canada in Lyon's (1970) First Census 1790. This is not a true census, but a compilation of the United Empire Loyalist rolls at the time, and therfore provides only a provisional link between the Morton Family and likely UE Loyalist heritage.

Thomas Morton (son of James Morton born 1795 Ireland, son of Alexander Morton) was born in 1828, possibly in County Fermanagh Ireland (Alexander Morton Family Genealogy, no author, no date). He is seen in the Provincial Land Records as receiving a location ticket in 1847 for the purchase of a clergy reserve lot E4, concession 5 in Hungerford when he was 19 years old. The 1860 Hungerford directory shows Thomas on lot 1 con. 3, with five other Mortons on lots 33, 4, 3, con. 1, 5, 7 respectively. The 1861 census places Thomas (age 32) and Elizabeth (age 28, born Canada, Church of England) in Hungerford with four children. Thomas died in 1908 and is buried in Thomasburg Cemetery, Hungerford (Piper, no date).

Elizabeth Morton (daughter of William, son of Alexander) was born in 1833 possibly near Belleville. She married her cousin Thomas (see above), lived in Hungerford and had seven children (Alexander Morton Family Genealogy, no author, no date). She died in 1916 and is buried in Thomasburg Cemetery, Hungerford (Piper, no date).

The link between the Alexander Morton in Upper Canada 1790, and the Alexander Morton grandfather to both Thomas and Elizabeth has not been determined, however it is likely that some form of kinship existed between them. It is documented that this protestant family originated in Ireland, County Fermanagh, and gradually moved to Upper Canada, sometimes individually other times in sibling groups generation after generation (Alexander Morton Family Genealogy, no author, no date). Perhaps as many as five additional isonymous and four repeated surname marriages were discovered in the records, but the exact nature of the kinship link of these unions is unknown. It therefore appears that many Mortons followed the 'previous experience' model of immigration to Upper Canada. The Morton surname was widely distributed in Hastings County, appearing in Rawdon, Hungerford, Huntingdon, and Sidney Townships.

-William Potts and Ann Potts

William and Ann Potts were married in 1840 at St Thomas' Anglican Church, both reporting Hungerford as their residence. Lyon's (1970) First Census of Canada 1790 lists a Jacob and a Reynard Potts in Upper Canada, but their relationship to the Potts of interest is unknown.

William (son of Thomas) was born in 1819 in Ireland, but it is unknown when this Potts branch immigrated to Upper Canada. William's older sister Anne (b. 1811) was married to Ben Reed and living on lot 3 con 11 Hungerford in 1831, so it can be assumed that younger brother George (b. 1823), and William Potts emigrated some time before his marriage to Ann Potts in 1840. Ann Potts was born in 1826, but her lineage is unclear, and in fact her maiden name does not appear in the Potts family genealogy (Potts 1901) for unknown reasons, perhaps to avoid confusion with her sister in law Anne. William (42) and Ann (35) appear in the Hungerford 1861 census on lot 4 con 11, and have no listed children. It is documented that they adopted a girl Mary Ann Sample born in 1862 (Potts 1901).

The farm(s) on lot 4 concession 11 Hungerford (adjacent to Anne (née Potts) Reed's farm) has an interesting history in that it was divided and the N½ 100 acres sold to George Potts in May 1848 from William Weir (brother-in-law of George's wife Jane née Elliot). The SEþ lot 4 con 11 was sold to William Potts in December 1848, followed by the remainder of the S½ to George in 1854. The SWþ of lot 4 con 11 was sold to another brother-in-law of George's in 1858. It appears that the snuffle and division of this property never left the management of the extended kinship group during the period observed.

The Potts surname has a wide distribution in the Hastings County, appearing in Sidney, Rawdon, Hungerford, Huntingdon, and Thurlow townships. The genealogical research uncovered two more additional isonymous marriages involving the related Elliot and Lovat families, and two repeated surnames unions involving Elliot and Potts.

-Jeremiah and Ann Reid

Jeremiah and Ann Reid were married at St Thomas' Church in 1840 both listing Belleville as their residences. There are too many instances of Reid/Reed/Read appearing in Lyon's (1970) First Census of Canada in 1790, and throughout Hastings County to list here. Canniff (1872) documents the persistence over four generations of one branch of the Reed [Reid] family on a 600 acre block of land in Thurlow.

While an extant genealogy was anticipated given the widespread and persisting nature of the family, none was discovered that could definitively link the isonymous couple. That they are both from Belleville, a documented place of Reid persistence (Reid's Dairy still sells the best milkshakes in Belleville, Boyce personal communication) must give some support to the notion that there is a kinship link in place.

-James and Sarah Vance

James and Sarah Vance were married at St Thomas' Church in 1839, James listing Hungerford as a residence, Sarah's is unknown. No documents establish the early presence of the Vance family in Upper Canada, and the surname was only discovered in Rawdon Township. A James Vance appears to have received two (gratuitous) free land grants (no dates) for Wollaston and Faraday Township both in the far reaches of north Hastings County where the rocky Canadian shield does not permit any form of agriculture (Richards 1958). These lots may have been used for their potential timber resources, and then this Vance couple must have moved on, for no other documentation was found of them.

-John Henry and Eleanor Clarissa Wilkins

John Henry and Eleanor Clarissa Wilkins were married at St Thomas' Anglican Church in 1877, John Henry listing the nebulous term 'Canada West' as his residence, and Eleanor C. listing Belleville. Many Wilkins are listed in Upper Canada in Lyon's (1970) First Census of Canada 1790, however only one family is found in Sidney Township in 1851. This consists of Charles (39) and Clarissa (33) Wilkins and their daughter Eleanor Clarissa age 5 years. The Provincial Land Records list a John Henry Wilkins, a resident of Stratford (Canada West) as leasing four lots of land in 1845 in the Stratford area. Given that John Henry and Eleanor may have taken up residence in Canada West out of the study area, it is not surprising that no other documentation was found for this couple. However a strong association can be made for a kinship union between this couple given the great distance John Henry travelled to wed Eleanor. Swedlund and Boyce (1983) found a strong association between kinship isonymous marriages and distance.

Appendix 4.3.1: Results of Sex Typing and Short Tandem Repeat analysis from the St. Thomas' aDNA extracts.

Bur. No. is burial number, **PCR Sex Products** refers to amelogenin X and Y chromosome specific alleles amplified, **1st PCR** and **2nd PCR** refer to repeated analysis of the Humth 01 STR: **Prime** refers to primary or dominant allele(s) present, **(sec)** to secondary or weaker PCR products.

Bur. No.	PCR Sex Products	1st PCR Prime(sec)	2nd PCR Prime(sec)	Geno-type	Comments
2	no rxn	7(6)	6(7)"5"	6/7 ?	allelic dropout?
19*	Y	7/9/9.3(6)	6/7/9/9.3	6/9.3?	JCD contam of 7/9*
25	XY	8/9/9.3	8/9.3	8/9.3	9 is PCR artefact
28	Y weak	no rxn	6/9	6/9?	allelic dropout?
37		no rxn	no rxn		mtDNA successful
56	no rxn	6/9.3(7)	"5"/9.3	?	(7)&"5" artefacts?
58	Y	no rxn	6/(8)	6/8?	partial dropout 8?
67	no rxn	no rxn	9.3("9")	9.3/9.3?	allelic dropout?
68	no rxn	(6/9)	no rxn	?	mtDNA successful
87	X	6/9.3	6/7/9.3("5")	6/9.3	confident genotype
97	no rxn	no rxn	7	7/7?	low template?
98	Y	9.3	9.3	9.3/9.3	confident genotype
108	no rxn	6/9.3(7/8)	no rxn	6/9.3?	bad template/contam?
111		7	7("6")	7/7?	"6" is artefact
132	Y weak	6/8	8(6/7)	6/8	partial dropout 6
137	no rxn	no rxn	7 ("6")	7/7?	allelic dropout?
148	no rxn	6/9.3	no rxn	6/9.3?	allelic dropout?
156*	Y	9.3 (7/9)	9.3	9.3/9.3?	JCD contam of 7/9*
165	Y weak	7/9.3	7	7/9.3?	allelic dropout?
179	no rxn	7	no rxn	7/7?	allelic dropout?
210a		9.3	no rxn	9.3/9.3?	allelic dropout?
211a	X weak	6/9.3	6/9.3("5/9")	6/9.3	confident genotype
231		9.3	no rxn	9.3/9.3?	allelic dropout?
268		7/9.3	7/9.3("6")	7/9.3	"6" is artefact
272*	no rxn	9.3(7/8/9)	7/8/9/9.3("6")	?	spot contam 7/9*?
274	Y	6/7	6/7	6/7	confident genotype
287**		9**(8)	9**	9/9**	DY contamination**
303	XY	8/9.3(9)	8/9.3	8/9.3	(9) is artefact
304	X weak	7/9.3(6)	9.3(7)	7/9.3	partial dropout 7
311		9.3	7("11")	?	poor template?
317		6/7(8)	no rxn	6/7?	allelic dropout?
321	Y	7/9(8)	7/9	7/9	confident genotype
336	Y	7/9.3	7/9.3	7/9.3	confident genotype
351a	no rxn	7	7/9.3	7/9.3?	allelic dropout?
361		no rxn	7(6)	7/7?	"6" is artefact
362	no rxn	6/9.3	7("6")	?	poor template?
375*	XY	7/8 (9)	7/9*(8)	*?	JCD contam of 7/9*

continued on next page

Appendix 4.3.1 (continued)

376	no rxn	no rxn	no rxn		mtDNA successful
397	no rxn	7/9	no rxn	7/9?	allelic dropout?
398	no rxn	7	8"7"	7/8?	allelic dropout?
405	Y weak	no rxn	no rxn		mtDNA successful
412	Y	6/8(7)	(6/8)	6/8	confident genotype
423*	Y weak	9(6/7/8)	(9)*	*?	JCD contam of 7/9*
467	no rxn	no rxn	no rxn		mtDNA successful
470*	Y	8/9/9.3(7)	7/9*/9.3	8/9.3?	JCD contam of 7/9*
472	Y	7/8	7/8	7/8	confident genotype
494	X	9(8)	9	8/9 ?	allelic dropout?
512	no rxn	no rxn	no rxn		mtDNA successful
512a*		7/9 (8)	no rxn	*?	JCD contam of 7/9*
514	no rxn	8/9.3(5/6/7)	8/9.3	8/9.3	template artefacts
527a	no rxn	no rxn	no rxn		mtDNA successful
548	Y	7(6)	7	7/7	confident genotype

Total extracted N=85, All estimated genotypes N=37, those genotypes with relative confidence N=15

'( )' = less intense secondary PCR products, or shadow bands
'?' = not a confident genotype assignment
'*' = contamination from JCD (19, 156, 272, 375, 423, 512a)
'**' = contamination from DY (lab co-worker), burial 287

Appendix 4.3.2: Results of Sex Typing and Short Tandem Repeat analysis from the Harmony Road aDNA extracts.

Bur. No. is burial number, **PCR Sex Products** refers to amelogenin X and Y chromosome specific alleles amplified, **1st PCR** and **2nd PCR** refer to repeated analysis of the Humth 01 STR: **Prime** refers to primary or dominant allele(s) present, **(sec)** to secondary or weaker PCR products.

Bur. No.	PCR Sex Products	1st PCR Prime(sec)	2nd PCR Prime(sec)	Geno-type	Comments
5$	no rxn	6/9.3$(9)	9.3$(6/9)	6/9	9.3$ contam from 7
6	Y	7/9.3(6)	7(9.3)	7/9.3	partial dropout?
7	no rxn	9.3	9.3	9.3/9.3	contam in 5
9	no rxn	9/9.3	9/9.3	9/9.3	
12	XY	9.3(6/7/8/9)	9.3	9.3/9.3	Ladder spillover
13	X	9/9.3	9/9.3	9/9.3	
14	no rxn	no rxn	no rxn		deciduous tooth
15	X	7/9.3("9")	7/9.3	7/9.3	PCR artefact "9"
16	X	9/10(8)	9/10(8/11)	9/10	PCR artifact 8/11
17	Y	6/7 (8)	6/7 (5)	6/7	PCR artifact 8&5
18	no rxn	10 (9)	7	7/10?	7 for 3rd PCR
20	no rxn	9.3	7 (9.3)	7/9.3?	9.3(11) 3rd PCR
22	XY	7/9.3(6/9)	7/9.3(6)	7/9.3	PCR artefact 6/9
23	Y	7 (6)	9.3	7/9.3?	9.3 for 3rd PCR
24	no rxn	no rxn	no rxn		deciduous tooth
25	Y	6/9(5/8)	6/9(5/8)	6/9	PCR artefact 5/8
26	no rxn	9.3	2 x no PCR	?	allele dropout?
28	no rxn	10 weak	2 x no PCR	?	allele dropout?
29	no rxn	no rxn	no rxn		deciduous tooth
31	Y	6 (5)	6 (5)	6/6	5 is artefact
33	no rxn	7(6/8/9/9.3)	7/9.3	7/9.3	Ladder spillover
34	Y	8/9.3(6/7/9)	8/9.3(7)	8/9.3	Ladder spillover
38	X	6/9.3(5/"9")	6/9.3	6/9.3	PCR artefact 5/"9"
39	no rxn	6/9.3	6/9.3	6/9.3	
40*	no rxn	7/9(6/8/9.3)	7/9(8/9.3)	8/9.3	7/9 is JCD contam
41	Y	6/8 smudgy	6/8 clean	6/8	
RB24$		"9.3" contamination from Harmony Road burial 7$			

Total extracted N=33 (26 teeth, 7 metacarpals), all estimated genotypes N=21, those genotypes with confidence N=18

'( )'= less intense secondary PCR products, or shadow bands
'?' = questionable genotype assignment
'$' = probable contamination from burial 7
'*' = contamination from JCD (burial 40)
RB24 = is a contaminated reagent blank for burial extracts 5, 7, 9, 16, 18, 26, 40

Appendix 4.3.3: Mitochondrial DNA Sequence Analysis of Harmony Road Samples (sequence positions after Anderson et al. 1981)

	Sequence Polymorphisms and positions														
	228	207	204	199	195	193	188	185	152	150	146	131	103	93	73
REF	C	C	A	A	A	T	T	C	A	G	A	A	C	T	T
DY	.	.	G	G	.	.	.	.	.	.	G	G	.	.	C
JCD	.	.	.	.	G*	.	.	.	.	.	.	.	.	.	.
1	no reaction														
5$	.	.	.	.	.	.	.	.	A/G	.	A-G	.	.	.	C/T
6	.	T	G	G	.	.	.	.	G	.	.	.	.	.	C
7 R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
9	.	.	.	.	.	.	.	.	G	.	G	.	.	.	C
12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
15R	.	T	G	G	.	.	.	.	G	.	.	.	.	.	C
16	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C
17	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.
18*	T	.	.	.	G-A*	.	C	T	.	.	.	.	.	.	C
19	no reaction														
20	.	T	G	G	.	.	.	.	G	.	.	.	.	.	C
22R	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C
23	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C
24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
25R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
26	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.
28*	.	.	.	.	G/A*	.	.	.	.	.	.	.	.	.	.
29	T	.	.	.	.	.	C	T	.	.	.	.	.	.	C
31R	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C
32	no reaction														
33*	.	.	.	.	G/A*	.	.	.	.	G(A)	.	.	.	.	C-T
34*	.	.	.	.	G/A*	.	.	.	.	.	.	.	.	.	C
38R	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C
39R	.	.	.	.	.	.	.	.	G	.	G	.	.	.	C
40*	.	.	.	.	G/A*	.	.	.	.	.	A-G*	.	.	.	C/T*
41	.	T	G	G	.	.	.	.	G	.	.	.	.	.	C
RB24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

'R' = repeated analysis
'?' = unreadable polymorphism
'.' = polymorphism identical to reference sequence
'G/A' = 'G' dominant over 'A'
'G-A' = 'G' or 'A' band of equal intensity
'*' = Probable contamination from JCD
'$' = RB 24 and extract 5 are consistent with contamination from burial 7 (RB 24 is a contaminated reagent blank for extracts 5, 7, 9, 16, 18, 26, 40)

Appendix 4.3.4: Mitochondrial DNA Sequence Analysis of the St. Thomas' Samples (sequence positions Anderson et al. 1981)

	Sequence Polymorphisms and positions															
	228	207	204	199	195	193	188	185	152	150	146	131	103	93	73
REF	C	C	A	A	A	T	T	C	A	G	A	A	C	T	T
DY	.	.	G	G	.	.	.	.	.	.	G	G	.	.	C
JCD	.	.	.	.	G*	.	.	.	.	.	.	.	.	.	.
2	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C
19*	.	.	.	.	G(A)*	.	.	.	.	.	.	.	.	.	C
25	?	.	.	.	.	.	.	.	.	.	.	.	.	.	.
28	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
37R	.	.	G	G	.	.	.	.	.	.	.	.	T	.	C
56*	.	.	.	.	A(G)*	.	.	.	.	.	.	.	.	.	.
58	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
67	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.
68	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
87	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C
97	no PCR product, see Short Tandem Repeat analysis														
98	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
108	.	.	.	.	.	.	.	.	.	A	.	.	.	.	C
111	no reaction sequencable for 3 different extracts														
132	T	.	.	.	.	.	C	T	.	.	.	.	.	.	.
137	.	.	.	.	.	.	.	.	.	A	.	.	.	.	C
148	?	.	.	.	G	.	.	.	.	.	.	.	.	.	C
156*	?	.	A(G)*	.	G(A)*	.	.	.	.	.	.	.	.	.*	T(C)
165R	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.
179	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
210a	no PCR product, see Short Tandem Repeat analysis														
211aR	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
231	no PCR product, see Short Tandem Repeat analysis														
268	PCR product too weak to sequence, see STR analysis														
272	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
274	.	.	.	.	.	C	C	.	.	.	.	.	.	.	.
287$	.	.	G$	G$	.	.	.	.	.	.	G$	G$	.	.	C$
303	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
304	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
311	PCR product too weak to sequence, see STR analysis														
317	no PCR product, see Short Tandem Repeat analysis														
321	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C
336	?	.	.	.	.	.	.	.	.	.	.	.	.	.	C
351a	?	.	.	.	.	.	.	.	.	.	G	.	.	.	.
361	no PCR product, see Short Tandem Repeat analysis														
362	?	.	.	.	.	.	.	.	.	.	.	.	.	.	C
375*	.	.	.	.	G*	.	.	.	.	A/G	G	.	.	.	C(T)
376R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
397	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
398	no PCR product, see Short Tandem Repeat analysis														

Appendix 4.3.4 (continued)

```
405R .  .  .  .  .  .  .  .  .  .  .  .  .  .  .
412  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .
423* .  .  .  .  G* .  .  .  .  .  .  .  .  .  .
467  .  .  .  .  .  .  .  .  .  .  .  .  .  .  .
470R ?  .  .  .  .  .  .  .  G  .  .  .  .  .  .
472  .  .  .  .  .  .  .  .  G  .  G  .  .  .  C
494  .  .  .  .  .  .  .  .  G  .  .  .  .  .  C
512  .  .  .  .  .  .  .  T  .  .  .  .  .  .  C
512a$.  .  G$ G$ .  .  .  .  .  .  G$ G$ .  .  C$
514R .  .  .  .  .  .  .  T  .  .  .  .  .  .  C
527aR.  .  .  .  .  .  .  .  .  .  .  .  .  .  .
548  .  .  .  .  .  .  .  .  .  .  .  .  .  .  C
```

```
 'R'   = repeated analysis
 '?'   = unreadable polymorphism
 '.'   = polymorphism identical to reference sequence
'G/A'  = 'G' dominant over 'A'
'G-A'  = 'G' or 'A' band of equal intensity
 '*'   = Probable contamination from JCD
 '$'   = Contamination from DY
```

Appendix 4.3.5: Summary of all aDNA results for the Harmony Road sample sorted by mtDNA lineage

Lineage A:	Humth01 Genotype	Sex Estimation
7R	9.3/9.3	-
28	-	-
RB24	9.3?	-
Lineage B:		
33	7/9.3	-
Lineage C:		
31	6/6	Y
38	6/9.3	X
Lineage D:		
12	9.3/9.3	XY
13	9/9.3	X
14	-	-
24	-	-
25	6/9	Y
34	8/9.3	Y
Lineage E:		
40	8/9.3	-
Lineage F:		
16	9/10	X
22	7/9.3	XY
23	7/9.3	Y
Lineage G:		
17	6/7	Y
Lineage H:		
26	-	-
Lineage I:		
5	6/9	-
9	9/9.3	-
39	6/9.3	-
Lineage J:		
6	7/9.3	Y
15	9/10	X
20	7/9.3	-
41	6/8	Y
Lineage K:		
18	7/10	-
29	-	-

Appendix 4.3.6: Summary of all aDNA results for the St Thomas' sample sorted by mtDNA lineage

Lineage A:	Humth01 Genotype	Sex Estimation
25	8/9.3	XY
28	6/9	Y
56	-	-
68	-	-
179	7/7	-
211a	6/9.3	X
397	7/9	-
405	-	Y
412	6/8	Y
467	-	-
527a	-	-
Lineage B:		
108	6/9.3	-
137	7/7	-
Lineage D:		
19	6/9.3	Y
58	6/8	Y
98	9.3/9.3	Y
272	-	-
304	7/9.3	X
336	7/9.3	Y
362	-	-
376	-	-
548	7/7	Y
Lineage F:		
2	6/7	-
321	7/9	Y
494	8/9	X
Lineage I:		
472	7/8	Y
Lineage G:		
67	9.3/9.3	-
165	7/9.3	Y
470	8/9.3	Y
Lineage L:		
274	6/7	Y
Lineage M:		
303	8/9.3	XY
Lineage N:		
87	6/9.3	X
148	6/9.3	-
Lineage O:		
512	-	-
514	8/9.3	-
Lineage P:	Humth01 Genotype	Sex Estimation
351a	7/9.3	-

Lineage Q:		
156	9.3/9.3	-
Lineage R:		
132	6/8	Y
Lineage S:		
37	-	-

Appendix 4.3.7:Chi-Square Test of Goodness of Fit between aDNA HUMTH01 cemetery allele distributions and modern forensic databases adjusted to cemetery sample size. (Harmony Road adjusted value above, St Thomas' below)

Repeat Number	6	7	8	9	9.3/10
Harmony Road N=17	n=8	n=5	n=3	n=5	n=14
St Thomas' N=15	n=5	n=8	n=6	n=1	n=10
Combined data	13	13	9	6	24

Contemporary Forensic Databases:

Repeat Number	6	7	8	9	9.3/10
USA (Gill and Evett 1995)	8.4 7.2	5.6 4.8	4.2 3.6	4.9 4.2	12.8 11
Britain (Gill and Evett 1995)	8.1 6.9	7 6	4.2 3.6	4.9 4.2	10.8 9.3
Quebec (Busque 1997)	8.4 7.2	6.2 5.3	4.2 3.6	5.2 4.5	11.3 9.7
France (Pfitzinger et al. 1995)	8.8 7.5	6.6 5.6	3.9 3.3	4.9 4.2	10.7 9.2
Portugal (Pinheiro 1997)	7.4 6.3	5.7 4.9	4.8 4.1	6.4 5.5	10.7 9.2
Spain (Lorente et al. 1994)	7.7 6.6	5.8 5	5.1 4.4	7 6	9.3 8
Afro-Carib (Gill&Evett'95)	5.2 4.5	14 12	7 6	5.2 4.5	4.6 3.9
Chinese (Gill and Evett 1995)	4.6 3.9	9.1 7.8	1.8 1.5	16.8 14.4	3.2 2.7

continued on next page

Appendix 4.3.7 (continued)

Chi-square Test of Goodness of Fit Sample Calculation:

Repeat Number	6	7	8	9	9.3/10
Harmony Rd **(observed)**	8	5	3	5	14
France **(expected)**	8.8	6.6	3.9	4.9	10.7
$(obs-exp)^2/exp$	0.07	0.39	0.21	0.002	1.02

Chi^2=Sum of$(obs-exp)^2$/exp=1.68 with 4 df's, $p<0.05=9.49$

Outcomes of the various Chi^2 Test of Goodness of Fit are summarized in Table 4.3.2 on page 180

Appendix 5.1: Summary of osteological data collected on the Harmony Road skeletal remains (Austin and Dudar 1993).

Burial	Age & Sex Estimates		Comments
Backfill 31-65		f	skull from burial 2 (?)
1	31-54	m	disturbed by past trenching
2	31-65	f	disturbed by past trenching
3	yng adult	m	disturbed by past trenching
4	adult	f	disturbed by past trenching
5	32-48	m	
6	60+	m	Robert James Brown d. 1937 age 80
7	33-52	m	
8			remains removed in the past
9	33-53	f	
10	0.5-1.5	-	
11	NB-0.5	-	
12	60+	m	
13	38-63	f	
14	7-9	f?	braid of hair preserved
15	60+	f	Sarah (nee Cooper) Brown d. 1907 age 82
16	30-46	f	
17	31-46	m	
18	1.5-2.5	-	
19	42-68	f	
20	30-53	f	
21	1.5-2.5	-	
22	34-51	m	
23	16.5-18	m	
24	11-12	-	Elizabeth Mitchell(?) d.1863 age 12
25	32-49	m	
26	10-12	-	
27	0.5-1.5	-	
28	20-22	f	
29	14-16.5	f?	
30	0.5-1.5	-	
31	25-36	m	George Weekes Jr (?) d. 1858 age 31
32	60+	f	edentulous, no successful aDNA
33	30-42	m	
34	32-44	m	George Hinkson (see discussion chapter)
35			Telephone Cable Trench
36			Telephone Cable Trench
37	NB-0.5	-	
38	47-67	f	Patience (nee.Baldwin) Drew d.1870 age 81
39	52-62	m	
40	35-42	f	
41	42-62	m	