Beyond Blood Ties:

Detecting Kinship in the Archaeological Record

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Abstract

This thesis explores the conceptual and methodological basis for detecting and interpreting kinship in the archaeological record. It argues that a conceptual shift is needed to produce nuanced and unbiased reconstructions of potential kin relations. This need is supported by the disconnect between sociocultural concepts of kinship and bioarchaeological interpretations of past relationships. Social anthropology recognises kinship as a flexible and socially constructed system for organising familial relationships. Nevertheless, bioarchaeological research often implements a genealogical, procreation-based kinship model, potentially representing a form of cultural colonialism that overlooks the variability of kinship systems within and between cultures.

The primary methods used for local-level kinship analysis are based on establishing patterns of genetic similarity between individuals. Developments in ancient DNA sequencing have provided a useful technique increasingly used in bioarchaeology. Underpinning the application of this technique is the prioritisation of genetic data, which implicitly supports the idea that kinship relationships are formed solely through biogenetic links, with little regard for social processes that form familial relationships. The separation of genetic data from other bioarchaeological data impedes reconstructing more complex interpersonal relations in the past.

This thesis uses a systematic review to examine the concepts and data previously used as the basis for kinship reconstruction, empirically establishing the disconnect between social anthropology and bioarchaeology while making recommendations for future research. The findings indicate that the variability of kinship systems is frequently unrepresented, and the validity of ancient DNA results can frequently be questioned, highlighting the need to move away from the essentialist argument about the role of DNA and kinship toward a more integrated, biosocial approach. This thesis also provides recommendations for future research to incorporate biological and archaeological data to interpret kinship and demonstrates how an inclusive approach might more effectively use the available bioarchaeological data to hopefully provide more comprehensive, culturally sensitive reconstructions of relationships in past populations via a simple social network analysis.

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Chapter 1. Kinship and Society

1.1 Why Study Kinship?

Kin are usually the first people encountered when we enter this world. In most societies, they represent the organisational framework through which we learn to organise our lives. Hence, these relationships are invaluable for understanding any past society (Ensor et al., 2017; Johnson & Paul, 2016). From birth, the processes of enculturation, learning and socialisation teach individuals society's cultural rules and expectations (Jenks, 2004). Thus, individuals are embedded in a web of social connections through which social norms are inherited and regulated. Daily routines and informal interactions with intimate social circles, such as close relatives, are foundational to the observation, imitation, learning, and adoption of cultural behaviours and social practices (Prescendi, 2010).

These cultural practices, values, and rituals are embedded in society, shaping how generations interact, including possible marriage partners, the inheritance of wealth, status, traditions, occupations, and the maintenance of social continuity through cultural transmission (Ensor, 2013; Johnson & Paul, 2016). Cultural transmission allows these embedded societal models of behaviour and belief to be inherited across generations. However, the consistency may vary depending on the level of conformity required for successfully navigating social environments.

The processes through which individuals acquire these societal rules and norms are generally shared between cultures, while the organisation of kin relationships based on these rules differs between cultures. Therefore, while highly variable and culturally dependent, kinship systems are essential to how people form and conceptualise lateral and vertical social bonds and groups (Carsten, 1995; Lancaster, 2005). Understanding and considering the types of kinship systems is fundamental for reconstructing kinship relationships in prehistoric populations, which might have further implications for subsequent interpretations of social organisation and structure.

Kinship, or kinship systems, can be understood broadly as the cultural logic used to organise individuals into familial relationships at different scales, subsequently shaping their expected behaviours and obligations (Bohannan & Middleton, 1968; Levi-Strauss, 1969). These scales differentiate broadly between relatives (kin) and non-relatives, organising individuals into specific categories of relatives, such as close or distant relatives. Family (i.e.

groups of relatives) is also encapsulated (Cohen, 2018; Ensor et al., 2017). In contrast, kinship is a complex and potentially abstract cultural construct made tangible in studies of skeletal remains. Therefore, the operationalisation of this construct in research determines the boundaries of how kin relationships can be perceived and interpreted.

In archaeology, the reconstruction of social life ways and identity often incorporates anthropological theoretical approaches to contextualise the potential lived experiences of past individuals (Knudson & Stojanowski, 2008). Theoretical definitions and conceptualisations of kinship and family exist on a spectrum between restricted and culturally biased to subjective and untestable. In some theories, kinship ties are reduced to the social relationships that function to produce and raise offspring (Malinowski, 1930; Davis & Lloyd Warner, 1937; Fortes, 1949; Murdock; 1949).

The nuclear family is often presented as the universal and natural social unit that functions to produce and raise offspring (Malinowski, 1930; Fortes, 1949; Murdock; 1949). It is a kinship system that determines familial group membership centres on common ancestral descent or common offspring. However, ethnographic research has identified numerous examples of kin relationships between individuals who share neither genealogical nor reproductive relationships (*e.g.,* Marshall, 1977; Merlan & Rumsey, 1991; Carsten, 1995; Bodenhorn, 2000; Nuttal, 2000). For the Ku Waru of Neliybar Valley, New Guinea, post-natal processes of bonds of family are formed by sharing *kopong* ('grease'), which is the essential matter of all living organisms (Merlan & Rumsey, 1991). *Kopong* forms important bonds between a child and the birth parents through sperm and breast milk, but it is also present in sweet potatoes and pork. Sharing food from the same land can form kin bonds as strong as those formed between individuals borne from the same biological mother (Merlan & Rumsey, 1991). Indeed, a kinship system centred on the nuclear family, or even genealogy, would be unable to capture the breadth of kinship relationships potentially connecting individuals effectively.

In contrast, Sahlins (2013) presented kinship as being shared between individuals fundamental to each other's existence. Although such an inclusive cultural logic captures the non-procreative and non-genetic forms of kin relationships, as with the Ku Waru, it is too abstract to be practically applied to non-living individuals whose relationships cannot be observed directly. Kinship alone is either too inflexible or ambiguous a concept for

bioarchaeological research, a relatively vague concept used in conjunction with the idea of relatedness, where it becomes more accessible to bioarchaeologists.

Relatedness refers to the criteria for distinguishing individuals as either relatives or non-relatives. Relatedness criteria may involve blood, genes, commensality, co-residence, experiences, adoption, and marriage (Carsten, 2000; Sahlins, 2011; Johnson, 2019). The cultural logic which determines the meaning behind family may vary within and between cultures and could be based on social, biological, legal, and emotional ties (Ensor et al., 2017; Cohen, 2018; Johnson, 2019). Therefore, attempts to reconstruct kinship and social organisation in past populations require a lens with the potential to capture these different ties to readily observable in modern populations.

Table 1.1 Definitions of kinship terms

Term	Definition
Kinship (or Kinship System)	The cultural logic used to organise individuals into familial relationships through relatedness (i.e. shared between individuals who consider themselves related); may vary within and between cultures and may be based on social, biological, legal, and emotional ties (Ensor et al., 2017; Cohen, 2018; Johnson, 2019)
Relatedness	The criteria for distinguishing individuals as relatives or non-relatives; may involve blood, genes, commensality, co-residence, experiences, adoption, and marriage (Carsten, 2000; Sahlins, 2011; Johnson, 2019)

1.2 Eurocentrism in Bioarchaeological Kinship

Current anthropological perspective opposes the idea that genealogy inherently defines family relationships (Levi-Strauss, 1983; Carsten, 2004; Bamford & Leach, 2009; Sahlins, 2011; Johnson, 2019). However, bioarchaeological research often continues to conflate genetic relationships with kinship ties, frequently appearing to overlook the social processes often involved in forming these ties. Kinship analysis based on archaeological material focuses on identifying close genetic relatives, assuming the primacy of these relationships for the culture under study (e.g. Haak et al., 2008; Baca et al., 2012), reflecting the culturally biased ideas of kinship that dominated anthropological kinship studies prior to the 1960s (Malinowski, 1930; Fortes, 1949; Murdock; 1949; Davis & Lloyd Warner, 1968).

These ideas have been criticised as ethnocentric, with anthropologists presenting their Euro-American cultural logic as a universal social reality (Franklin & McKinnon, 2000). Western cultural norms, including the kinship system in which blood ties are essential for determining relatedness, are presented as natural facts (Schneider, 1984; Sahlins, 2011). Within this paradigm, the nuclear family, with a married heterosexual couple and their children, has often been considered a universal social unit (Murdock, 1949; Parsons & Bales, 1955).

Hence, the shift away from assuming genealogy as the fundamental basis for kinship occurred within a more extensive change within anthropology as a discipline (Franklin & McKinnon, 2000). During this time, many universal facts were being questioned, such as male dominance and gender roles, within the larger context of the nature-culture dichotomy (Collier & Yanagisako, 1987). Thus, biology as a fundamental unit of kinship was no longer removed from culture, sexual reproduction did not automatically mean a family relationship, and kinship was increasingly understood as a social process (e.g. Levi-Strauss, 1983; Carsten, 2000; Carsten, 2004; Franklin & McKinnon, 2000). Nevertheless, this shift from Eurocentric biogenetic determinism has yet to occur within bioarchaeology since the primary goal of kinship analysis still appears to be the reconstruction of genealogical relationships.

Archaeology does not appear to have engaged with or contributed significantly to any shifts in anthropological concepts of kinship. However, kinship analysis remains a significant part of understanding any past society (Ensor, 2013; Ensor et al., 2017; Johnson & Paul, 2016). Most reconstructions of inter-individual kinship predominantly use phenetic biodistance or DNA analyses, sometimes along with some form of ethnographic analogy to supplement interpretations of kinship practices (Meyer et al., 2012; Ensor, 2016). These methods alone can only capture genetic relationships, constrained by the limited data provided in mortuary contexts: poorly preserved, fragmented, small, and unrepresentative samples (Larsen, 2002).

Combined with the default Euro-American logic for interpreting possible kin relationships, the post-natal social processes potentially forming relationships between genetically related and unrelated individuals may be overlooked, favouring biogenetic and procreative ties (Johnson, 2019; Bruck, 2021). Even if a direct historical analogy is used, ethnographic data are a synchronic, potentially biased glimpse of a population (Ensor, 2016). There is no guarantee that the data are relevant, especially because cultures are variable and

change over time (Ensor, 2016). Ethnohistoric data are often based on archival records collected during colonial occupation, thereby reflecting biased worldviews and kinship practices, at the very least, altered by colonialism (Stoler, 2002; TallBear, 2016). Thus, in ethnographically observed systems, such as assumed universal nuclear family units, unsuitable contexts may create concerns about the validity of any conclusions formed (Ingold, 2000).

In Western societies, nuclear families emerged because of the social, cultural, and economic circumstances of the 19th century, where industrialisation, decreased infant mortality, and the gendered division of labour appears to have transformed the extended family into small, nuclear units (Sabean & Teuscher, 2007; Sear, 2016). Therefore, it is unexpected for such a family model to be broadly applicable, especially in prehistoric populations. Hence, steps must be taken to fully incorporate bioarchaeological methods to capture non-genetic forms of relatedness to realise kinship's flexible and culturally constructed nature (Pilloud & Larsen, 2011; Gregoricka, 2013). Then, ethnographic data can be used to form hypotheses, guiding tentative interpretations of kinship based on corresponding patterns in the bioarchaeological data (Ensor, 2016).

The initial analysis of a triple burial at the Los Tolmos site in the Iberian Peninsula exemplifies the tendency for bioarchaeological interpretations to assume genetic, procreative relationships (Esparza, 1990). The burial was attributed to the Cogotas I archaeological culture from the Iberian Bronze Age which has poorly understood mortuary practices. Two adults lying on their left and right sides and a perinatal infant were buried together (Esparza et al., 2017). On the basis of morphological sex assessments, researchers first identified the adults as a male and female (Esparza, 1990). As a result, the triple burial was interpreted as a heteronormative nuclear family.

Later palaeogenetic analyses established that both adults were female (Esparza et al., 2017). Kinship analyses reveals the high likelihood of a mother-child relationship between one female and the perinatal infant. The other female was maternally unrelated and inconclusive paternal ties. Hence, the original assumption of a heteronormative nuclear family was incorrect, demonstrating the dangers of applying modern Eurocentric systems of kinship to the past.

A genealogical paradigm based on the nuclear family applied inappropriately can make it difficult to perceive other types of family and kin relationships. Subsequently

misdirecting interpretations of relationships in the past. For bioarchaeological studies of kinship in past societies to be meaningful and comprehensive, they must incorporate concepts of kinship and relatedness that acknowledge the variable and socially constructed nature of kin relations (Johnson & Paul, 2016; Johnson, 2019). Care must be taken not to impose culturally biased constructs that favour genetic relationships on interpretations of archaeological populations.

1.3 Contemporary Perspectives of Kinship in Bioarchaeology

Kinship is not a given by birth, and current anthropological theory understands that any procreative, marital, or descent-based relationship may also be constructed socially (Bamford & Leach, 2009; Sahlins, 2011). Conversely, kinship is not a foregone conclusion if individuals share a procreative relationship or a common ancestor. The kin relationship may also be socially constructed through the practice of extended nurture, living alongside each other, and the reciprocal exchange of affectionate acts (Vilaça, 2002).

For the Amazonian Jivaro, forming a father-son relationship begins with the father's contribution to procreation, then the provision of food during pregnancy. It is finally acquired by the child's nourishment throughout their lifetime (Taylor, 2000). Furthermore, the cultural logic determining post-natal relationships does not necessarily have an equivalent consanguineal designation since many different constructed forms of relatedness have been identified in ethnographic investigations (Sahlins, 2011).

For the Greenland Inuit, a name-sharing kinship (addressing each other as *atiitsara,* or 'name-sake') may be formed based on shared life experiences, such as enduring a dangerous winter hunting trip together (Nuttal, 2000). Nevertheless, there are no living informants and frequently no written records to explain cultural practices and behaviours to bioarchaeologists (Meyer et al., 2012). Hence, a method incorporating a more inclusive kinship model and engaging all relevant information provided in mortuary contexts could play a key role in moving bioarchaeological kinship analysis beyond the Western genealogical model. Indeed, skeletal material and mortuary contexts can contain a significant amount of information about past individuals' lives, such as diet, age, sex, status, health, activity, and mobility (Larsen, 2002), with the potential to be informative about their relationships beyond genealogy. Therefore, incorporating non-genetic bioarchaeological methods to investigate patterns in

these data might help uncover motional, social, and legal kinship ties acquired based on commensality, co-residence, lived experiences, adoption, and marriage (Johnson, 2019).

Some bioarchaeological kinship analyses incorporate alternative lines of evidence and non-biological types of relatedness (e.g. Haak et al., 2008; Pilloud & Larsen, 2011; Gregoricka, 2013; O'Sullivan et al., 2018). However, these studies are still in the minority, and the other data are frequently not fully incorporated in their interpretations of relatedness, with genetic data prioritised when interpreting kinship (Johnson & Paul, 2016). Thus, non-genealogical relationships are potentially identifiable through patterns of similarity between individuals found using genetic and non-genetic methods (Johnson & Paul, 2016; Johnson, 2019).

For example, O'Sullivan et al. (2018) is a recent multidisciplinary study that utilised multiple methods, including genome-wide analysis, strontium and oxygen isotope data, burial patterns, and grave goods. This study investigated 13 individuals in an Early Mediaeval (7th century CE) Alemannic graveyard in Germany. Notably, close burial proximity, locality-detecting strontium and oxygen isotopes, and genetic relatedness were combined to suggest that fellowship or adoption were potentially equal to genetic relatedness in the *familia*-based kinship structure (O'Sullivan et al., 2018). The use of multiple lines of evidence instead of mere genetic data added an extra depth of understanding for this community.

In the absence of any archaeological data, the conclusions regarding the kinship structure would have been restricted to a potential two-generation biological family. However, there was still the relative prioritisation of genetic relationships as indicative of the relative closeness of individuals. Nevertheless, it could have been potentially due to knowledge of the *familia* structure during this historical period. Indeed, the study represents a step in the right direction – how applying multiple types of evidence collectively can produce a more inclusive method of kinship analysis with the potential to capture a broader range of possible relationships, providing a more thorough framework to examine social organisation.

1.4 Ethical Consideration in Bioarchaeological Kinship Analysis

A fundamental aspect of bioarchaeological kinship research is that it involves investigating and interpreting the relationships and lives of previously living humans. These

methods provide bioarchaeologists unprecedented insight into past genetic relationships and are accompanied by serious ethical responsibilities in performing these destructive analyses, storing the genetic information of ancient humans, and presenting the findings (Kaestle & Horsburgh, 2002; DeWitte, 2015). The destructive DNA extraction methods especially require the consideration of ethical obligations prior to undertaking such methods since skeletal remains are irreplaceable and potentially culturally significant (Kaestle, 2010). Although informed consent cannot be obtained from the deceased, researchers have a responsibility to consider the wishes and rights of the descendants and those culturally affiliated with the individuals being studied. These groups may have concerns for their ancestors who prioritise religious and spiritual sanctity over scientific value (Claw et al., 2017)

Consulting with native communities is particularly important when working with indigenous and marginalised communities, as a failure to do so may perpetuate colonial dynamics, dispossessing marginalised indigenous communities of their cultural heritage (Endere, 2014; Claw et al., 2017). Early anthropological conceptualisations of kinship were disseminated as part of a broader colonial ideology where Western cultural norms, centred on heteronormative marriage and procreation, facilitated the transmission of property and maintenance of racial purity (Stoler, 2002; TallBear, 2018). Labelling any deviation from the natural kin relations as immoral helped justify colonial violence and transform indigenous groups into subjects of the colonial power. This description is simply one example of the anthropological discipline being involved in propagating Euro-American ideals to maintain colonial supremacy in the past. Adjusting studies of prehistoric kinship to be more inclusive of the variable and socially constructed nature of kin relations would allow traditional indigenous knowledge to be incorporated into kinship studies while marking a paradigm shift away from the previous colonial ideology that assumes the superiority of Euro-American norms.

1.5 Aims and Objectives

This thesis explores the methods used for kinship analysis in bioarchaeological contexts. There is a strong need for a shift beyond Eurocentric concepts of kinship, as seen in sociocultural understandings and ethnographical studies of kin relationships. Kinship is central in society but is a complicated concept to study. Yet, archaeological research often

defaults to exploring genetic relationships. This research aims to draw attention to the conceptual bias in bioarchaeological kinship research and propose how future research might apply more inclusive models of kinship. Therefore, I am going to:

1. Conduct an extensive literature review of the methods for determining kin relations in past populations, focusing on their limitations;
2. Conduct a systematic review of bioarchaeological research using ancient DNA analysis to quantify the gap between current sociocultural models of kinship and models applied in bioarchaeological studies, identify how genetic data is used compared to other bioarchaeological data, and form suggestions about how future research might utilise bioarchaeological data;
3. Conduct a simple social network analysis of a past population based on the systematic review findings to demonstrate how multiple data types might be interpreted simultaneously.

1.6 Thesis Overview

The context of this thesis appears in Chapter 1, including a discussion about the importance, past sociocultural concepts, and current sociocultural understandings of kinship.

As archaeology often defaults to genetic relationships, a review of the methods used for intra-site kinship analysis will be presented in Chapter 2. The different methods and their limitations for determining kin relations are discussed.

In Chapter 3, I present an in-depth systematic review of kinship analysis using ancient DNA, looking into the data, sequencing methods, and conclusions. This is intended to quantify the gap between current sociocultural understandings of kinship, and archaeological investigations, the limitations of genetic kinship analysis.

Chapter 4 presents a discussion of the data that has previously been used to identify non-genetic forms of kinship in the archaeological record. It also produces suggestions of the alternative types of data that might be used in future research.

Chapter 5 uses the results in Chapter 3 to perform a basic social network analysis of secondary archaeological data. It demonstrates how an unbiased, multidisciplinary approach

might be used to produce more nuanced reconstructions of kin relationships in a bioarchaeological context.

Chapter 2. Kinship Analysis in Bioarchaeology

2.1 Introduction

As a key aspect of human societies, bioarchaeologists are often interested in reconstruction the kinship in past populations. The continued application of modern, Eurocentric assumptions of kinship in bioarchaeology are implicitly enacted by the methods used for detecting kin (Crellin & Harris, 2020). Phenotypic trait analysis based on skeletal and dental trait values, and more recently DNA analysis based on genetic data, are the primary methods used by bioarchaeologists. The main aims of these studies are to attempt to quantify the degree of genetic relatedness in and between populations (Frieman & Brück, 2021). The prioritisation of these methods in bioarchaeological kinship analysis inherently limits the types of kinship that can be captured (as discussed in Chapter 1), as kinship is not a direct reflection of genetic relationships. Also, the data itself and the methods must be interrogated to understand what these methods can truly tell us about kinship in the bioarchaeological record.

This chapter presents a literature review of the two main methods used in bioarchaeological kinship studies: skeletal biodistance analysis and ancient DNA analysis. It describes the methods, the data available, and their limitations. Thereby, exploring their often-unacknowledged conceptual problems, such as the persistence of a biological perspective, and assumptions of objectivity and accuracy (Ensor *et. al.*, 2017).

2.2 Kinship Analysis Using Skeletal Biodistance

Kin relations are considered a central aspect of society. Hence, they are frequently reconstructed as an essential aspect of past populations (Ensor et al., 2017). Biological distance, or biodistance, analysis was one of the early methods used to identify kinship ties between individuals in bioarchaeology (Pietrusewsky, 2018).

Biodistance is generally understood as a measure of biological relatedness determined by applying multivariate statistical analysis to observable features of skeletal remains (Buikstra et al., 1990; Hefner et al., 2016). The relatively steady popularity of biodistance analysis in bioarchaeological kinship studies is tied to a relatively precise, easily recorded,

and non-destructive data collection method that is also inexpensive (Stojanowski & Schillaci, 2006; Pietrusewsky, 2018; Nikita, 2020; Fig. 2.1).

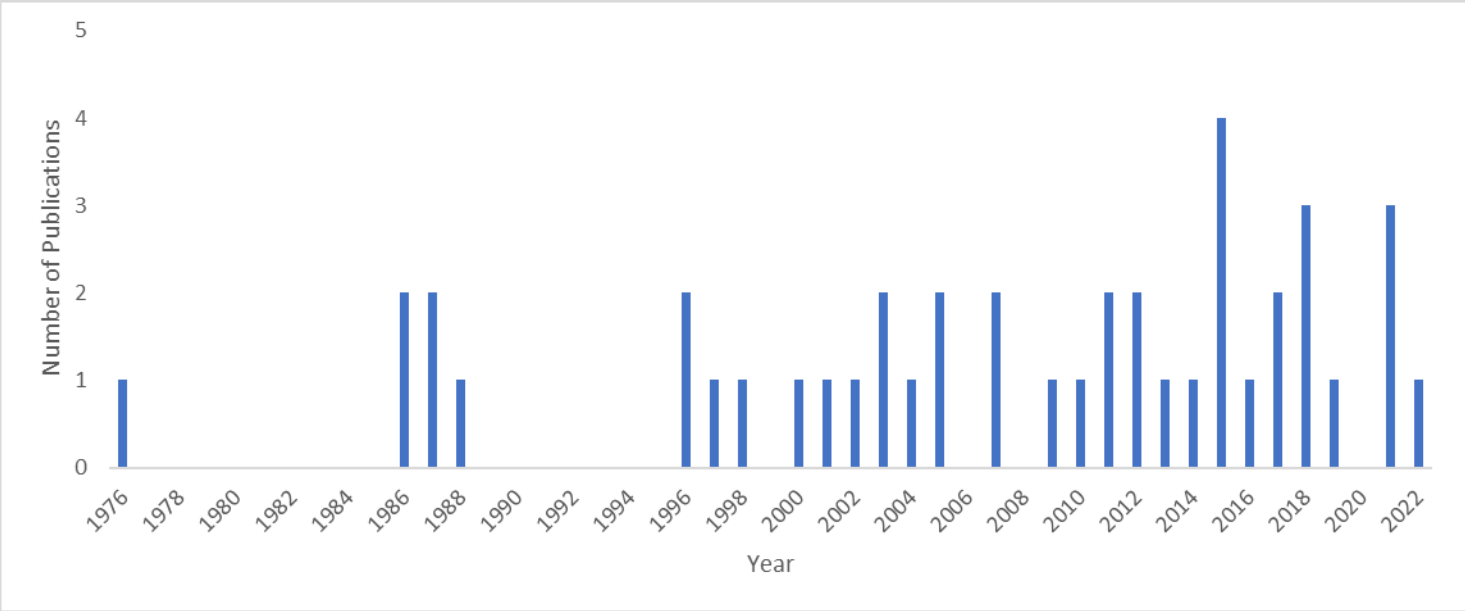

Figure 2.1. Number of publications including attempts to identify intra-site biological relatedness using skeletal biodistance. Based on a search from Web of Science, Jan. 2022.

The main aims of biodistance studies are to quantify the degree of genetic relatedness within and between populations (Buikstra et al., 1990; Stojanowski & Schillaci, 2006; Larsen, 2015). Most biodistance research focuses on answering large-scale questions about population origins, patterns of gene flow, and migration between populations (Buikstra et al., 1990; Stojanowski & Schillaci, 2006). A search of the literature found a respectable number of small-scale biodistance studies that aimed to reconstruct and interpret biological relationships within archaeological populations.

Biodistance studies are founded on the microevolutionary premise that the degree of similarity between groups reflects common ancestry and gene flow (Harpending, 1973; Knudson & Stojanowski, 2008; Relethford, 2016). Close biological relatives are more likely to express phenotypic similarity because of shared genes inherited from a common ancestor instead of the stochastic expression of the identical alleles in unrelated individuals sharing no recent ancestors (Konigsberg, 2009). Therefore, the phenotypes used in biodistance studies represent a proxy for patterns of genetic similarity (Relethford, 2016).

Biological relationships are estimated in these studies based on phenotypic (i.e. metric) or morphological (i.e. nonmetric) dental, cranial, or postcranial variables (Larsen, 2015; Table 2.1). The most appropriate traits for kinship analysis are those with an expression predominantly under genetic control and independent of age and sex (Česnys & Tutkuvienė, 2007; Alt et al., 2013). The genetic influences on a trait are generally estimated based on heritability studies to help substantiate the link between genotype and phenotype.

In heritability studies (e.g. twin studies, family studies, population studies), morphological data is compared with DNA evidence and genealogical information to determine the narrow-sense heritability (h^2) of a trait (Falconer & Mackay, 1983; Mayhew & Meyre, 2017; Table 2.1); that is, the amount of phenotypic variation due to the additive genetic influences. Most bioarchaeological studies emphasise the importance of high trait heritability for biodistance analysis, despite its limitations as a model for establishing the genetic determination of a trait (Visscher et al., 2008). Instead, narrow-sense heritability estimates the ratio of additive genetic variance, which contributes to the phenotypic variance in a defined population at a specific point in time, making extrapolation for prehistoric populations with unknown genetic structures and environments problematic (Stojanowski & Schillaci, 2006). It also does not consider interactions at the same locus, such as dominance effects and shared environmental factors, which might result in shared within-family phenotypes influencing the interpretation of biodistance (Stojanowski & Schillaci, 2006; Mayhew & Meyre, 2017).

However, high heritability is a primary factor in trait choice because it provides an indication of high additive genetic variability compared to environmental variability, making phenotype a relatively good indicator of genotype (but not vice versa). Nevertheless, traits with low heritability do not necessarily have a low genetic influence. Instead, a small proportion of the observed phenotypic variation is due to variation in genotypes. It is difficult to determine whether the low heritability results from high environmental variance, low additive genetic variance, natural selection, inbreeding, or genetic drift. Valuable traits for kinship analysis are variable, allowing skeletal populations to be tested for non-randomness concerning their phenotypic genetic proxies as potential indicators of family relationships.

Table 2.1 A Summary of Data Collection Methods and Narrow-Sense Heritabilities for Traits Commonly Used in Intra-Cemetery Skeletal Biodistance Studies

Data Type	Description	Data Collection Methods	Mode of Inheritance	Trait Heritability (h^2)
Metric	Continuous morphological variables of the skull, dentition, and post-crania	Linear measurements or indices to characterise the size and shape of the skeletal element - Traditionally measured using callipers - 3D digitisers of skeletal elements are used to produce 3D images and graphic representations of the feature	Polygenic inheritance	**Cranium** - Facial dimensions: 0.22 – 0.71 - Basicranial dimensions:0.2–0.4 - Cranial height/breadth:0.1–0.73 **Dentition** - Mesiodistal crown diameter: 0.59 – 0.91 - Buccolingual crown diameter: 0.61 – 0.91
Nonmetric	Observations of discrete morphological variables of cranium and dentition	Features observed using the bare eye - Recorded based on presence/absence - Scored based on the level of expression of feature	Polygenic inheritance with threshold expression	**Cranium** - Traits described by Berry & Berry (1967), Buikstra & Ubelaker (1994): 0–0.95 **Dentition** - ASUDAS: 0.4–0.8 - Carabelli's trait: 0.07–0.91* - Carabelli's trait: 0.8–0.93**
Anomalies	Nonmetric data referring to rare or uncommon heritable skeletal anomalies	Presence observed using the bare eye	Autosomal dominance[X]	

Note: Trait heritability ranges represent merged results from family, pedigree, and twin studies.

Metric cranium (Stefan & Chapman, 2003; Sherwood et al., 2008; Martínez-Abadías et al., 2009); dental (Dempsey et al., 1995; Dempsey & Townsend, 2001; Townsend et al., 2009; Stojanowski et al., 2017)

Nonmetric cranium (Sjovold, 1984; Carson, 2006b); dental (Paul et al., 2020)

***Carabelli's trait estimates in early literature (Biggerstaff, 1973; Berry, 1978; Scott & Potter, 1984; Skrinjaric et al., 1985).**

**** Carabelli's trait estimates in recent literature (Higgins et al., 2009; Hughes et al., 2015)**

[X]**Enlarged parietal foramina (Hoffman, 1976); brachydactyly (Cybulski, 1988); congenital calcaneonavicular coalition (Villotte et al., 2011)**

Understanding and interpreting biodistance data is complex due to the multiple potential influences on phenotype expression, intra- and inter-observer errors in data collection, and preservation issues that can lead to missing or sparse data (Larsen, 2015). Potential biological kinship is generally detected using three main approaches (Stojanowski & Schillaci, 2006; Česnys & Tutkuvienė, 2007). The first compares trait frequencies of archaeologically defined groups, such as multiple graves and households, to determine any potentially meaningful differences that might confirm familial spatial segregation (Pilloud, 2009; Pilloud & Larsen, 2011). The second involves pairwise comparison between

individuals, usually based on small subgroups of a population or small graves, to determine the genetic affinity of individuals compared to known pedigree ranges or the probability of features co-occurring at randomly given trait population frequencies. The third attempts to group individuals based on their similarity without any archaeological subgroupings, usually applied when there is no apparent spatial organisation in the burial site.

In most contexts, these approaches require the simultaneous consideration of multiple traits via multivariate statistical methods that test the null hypothesis: *the study population exhibits no phenotypic variation suggesting a genetic homogeneity that is uninformative about potential biological relationships.* Finding meaningful patterns requires the selection of traits that are relevant, available, and systematically quantifiable, as well as the appropriate methodological and statistical approach based on available data and burial context.

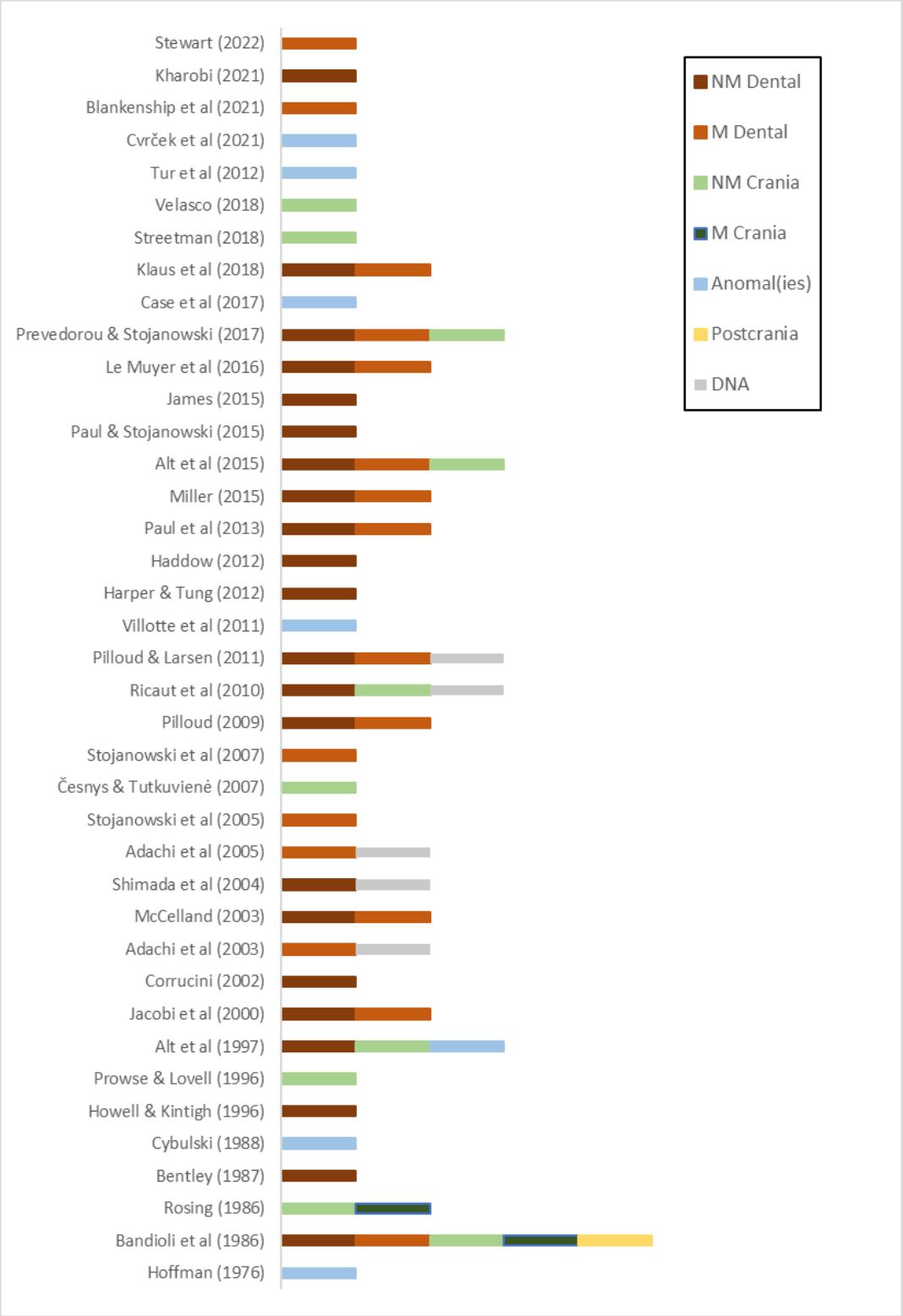

Figure 2.2 The combinations of biodistance data used studies of intra-site biological relatedness using skeletal biodistance. Based on a search from Web of Science, Jan. 2022.

2.2.1 Phenotypic Biodistance Data

Cranium

The fundamental roots of current kinship analysis using biodistance appear in 18th- and 19th-century cranial studies, which used typological "race" classifications to examine human diversity and answer large-scale questions about humankind and form typologies (e.g. Morton, 1839; Morton, 1844; Broca, 1863; Hefner et al., 2016; Pietrusewsky et al., 2018). The level of variation exhibited in the human cranial shape has made it a central element for identifying and interpreting relatedness in a wide variety of scales and settings (e.g. Jantz, 1973; Heathcote, 1986; Hemphill & Mallory, 2004; Schillaci & Stojanowski, 2005; Pietrusewsky, 2008; Pietrusewsky, 2010). There is a long history of biological anthropologists using the cranium to analyse human populations. In contrast, more recent intra-site studies have focused on forming conclusions about potential kin relationships between individuals based on patterns of cranial similarity.

Metric Cranial Data

Metric cranial data, or craniometric data, refer to measurements of various cranial features to examine human variation and biological relationships within and between populations (Dudzik & Kolatoriwitz, 2016). Many of these features are measured based on specific cranial landmarks and sites that act as anchoring points for measuring (e.g. Buikstra & Ublelaker, 1994). Early anthropologists defined many of these landmarks; subsequent anthropologists have included additional measuring points and transformed them into indices for analysis (Hrdlcka, 1952, Olivier, 1969; Bass, 1971; Howells, 1973; Buikstra & Ubelaker, 1994).

Craniometric data has been used in limited studies of intra-cemetery kinship analysis (Fig. 2.3). It was only used in the earliest studies with other data types to form conclusions about kinship (Bandioli et al., 1986; Rosing, 1986). This limited use is potentially due to the environment's role on cranium trait expression, combined with the high sexual dimorphism exhibited in skull dimensions, with males generally significantly larger (Česnys, & Tutkuvienė, 2007; Alt et al., 2013; Amores-Ampuero, 2017). Craniometric traits exhibit continuous variation that can be quantitatively measured and compared, resulting from the

combined influences of many genes and the environment (Konigsberg, 2000). The polygenic (multiple loci influencing phenotype expression) nature of the expression of these traits makes assessing the variance exhibited complex due to inheritance versus environment (Relethford, 2007). Cranial measurements are believed to reflect complex interactions between genetic, environmental, and developmental factors. Heritability studies provide varying results regarding how cranial phenotypes reflect the underlying genotypes but generally suggest a low to moderate genetic influence (Stefan & Chapman, 2006; Sherwood et al., 2008; Martínez-Abadías et al., 2009). Environmental influence, particularly mechanical loading, due to the jaw's masticatory function, influences the size and robusticity of the cranium, particularly at muscle attachment sites (von Cramon-Taubadel, 2009). The craniofacial robusticity of animals fed soft food tends to be lower than those fed hard food (Lieberman et al., 2004). The level of environmental influence and the continuous variation of traits means that interpreting patterns of similarity may be difficult, and discriminating between degrees of relatives is likely impossible with craniometric data (Rosing, 1986). Hence, the lack of craniometric data for inter-individual analysis is likely a good indicator of its unsuitability as a genetic proxy, especially alone, for kinship analysis (Fig. 2).

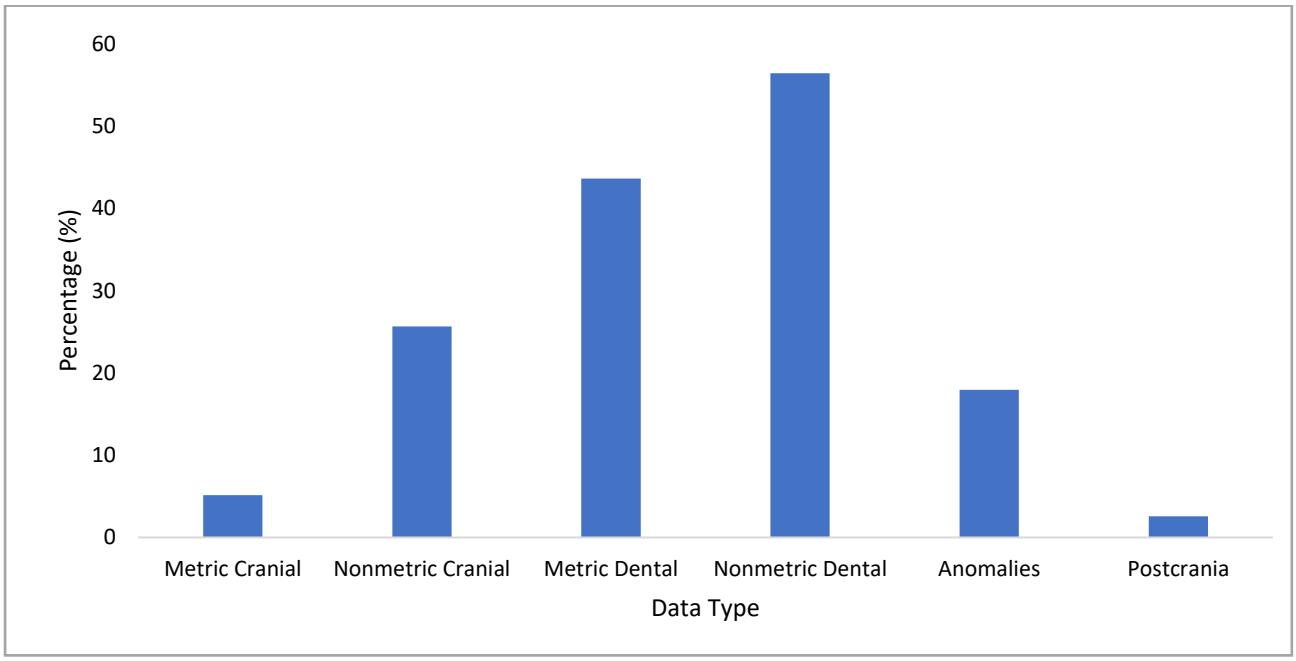

Figure 2.3 The percentage of skeletal biodistance studies using each data type to determine relative kinship within cemeteries. Based on a search from Web of Science, Jan. 2022.

Nonmetric Cranial Data

Nonmetric cranial data are favoured over craniometric traits for intra-cemetery kinship analysis in bioarchaeology (Pink et al., 2016; Fig. 2.3). The preference for nonmetric cranial traits is likely due primarily to the number of available nonmetric traits that allow data to be collected from fragmented assemblages and the ease of collection. Nonmetric cranial traits used in biodistance analysis are cranial features with morphological variants recorded on an ordinal scale based on the degree of expression, presence, or absence (Hauser & DeStefano, 1989). There are currently over 200 standardised nonmetric traits used in biological anthropology today (Berry & Berry, 1967; Hauser & De Stefano, 1989; Buikstra & Ubelaker, 1994). They can be divided into four primary types: ossicles (small bones) within cranial sutures, hyperstotic (excess bone formation), hypostotic (ossification deficiencies), and variations in the form and number of foramina.

Despite their popularity in biodistance studies, the heritability of nonmetric cranial processes is not well-understood. As with metric cranial traits, nonmetric trait expression is due to the action of multiple genes and environmental pressures, which result in the quasi-continuous expression of the traits (Cheverud & Buikstra, 1981). The use of these traits in biodistance studies involves the implicit assumption that they have a genetic basis, largely due to the non-mechanical functionality of many cranial traits (Carson, 2006a). Nevertheless, the few studies that estimate heritability show only low to moderate levels in most nonmetric cranial traits; only four traits displayed heritability estimates above 0.5 (Sjovold, 1984; Carson, 2006a; Table 2.1). Therefore, environmental factors likely play a role in variation expressed in nonmetric traits. Notably, these estimates had high standard errors (> 0.1).

Hence, although individuals are likely to experience the same environmental pressures, it is not easy to parse out environmental versus genetic influences (Visscher et al., 2008). A similar physical and nutritional environment could potentially homogenize phenotypic variation in trait expression and obscure genetic variation due to different ancestral backgrounds. However, relatively constant environmental influences due to the study area's regional scale could allow the genetic variance to be expressed in differences in between-group phenotype frequencies (Buikstra et al., 1990; Konigsberg, 2006).

It is difficult to determine the degree to which phenotypic variation reflects genetic differences without knowledge about the effect that environmental variables have on the direction and magnitude of trait expression. In previous studies, dentition was often analysed

with nonmetric cranial traits to help overcome the limitations of cranial data, which may be variably expressed depending on phenotypic plasticity, cultural cranial modification, and the relatively variable preservation of cranial remains (Paul et al., 2013).

Dentition

Dental traits are the most frequently used datatype for kinship reconstructions in skeletal populations (Fig. 2.3). The teeth have some of the best preservation in human remains, and they are also under stronger genetic control than other skeletal regions (Tyrrell, 2000*;* Harper & Tung, 2012; Khudaverdyan, 2014). The enamel and dentine components of teeth have a chemical structure that protects against the degrading effects of soil erosion, water, heat, and time (Bell et al., 1991). Teeth are also less susceptible to environmental and cultural modifications as they do not remodel, largely remaining the same size and shape once formed (Hillson, 2005). Furthermore, although studies have shown that males tend to have larger teeth than females, there is still relatively low sexual dimorphism (Zorba et al., 2011). The relative availability of teeth, combined with the reduced effects of the environment, age, and sex, allows bioarchaeological researchers to obtain useful biological data about individuals and their potential relationships (Hughes & Townsend, 2015).

Metric Dental Data

Metric dental traits are tooth dimensions that are quantified to perform biodistance analysis to investigate relationships between populations (e.g. Stojanowski, 2004; Pilloud, 2009; Irish et al., 2016), to investigate post-marital residence patterns (e.g. Schillaci and Stojanowski, 2003; Cook & Aubry, 2014) and compare individuals to test hypotheses of kinship (e.g., Jacobi, 1996; Adachi et al., 2005; Pilloud, 2009; Thompson et al., 2015). These studies have frequently assessed the size and shape of permanent teeth based on the crown's buccolingual and mesiodistal dimensions (Adachi et al., 2003; Stojanowksi, 2005; Pilloud & Larsen, 2011; Harper & Tung, 2012; Stewart, 2022). They have also used mesiodistal and buccolingual dimensions of the cervix at the cement-enamel junction (Pilloud & Larsen, 2011; Harper & Tung, 2012).

Measurements of tooth size and shape are a preferred data set for biodistance analysis because teeth are relatively well-preserved, lack remodelling, and possess fewer non-genetic influences than other skeletal features. The relative ease and standard convention of collecting metric data and the low reported mean of intra-observer (-0.06 and 0.06mm) and inter-observer (0.05–0.16mm) error likely plays a role in their technical value (Pilloud & Kenyhercz, 2016). Furthermore, although studies have shown that males tend to have larger teeth than females, the difference is still relatively low (Zorba et al., 2011). For tooth crown dimensions, a 3%–7% difference has been observed between males and females (Harris & Crouch, 2006). The level of dimorphism varies depending on the measurement and tooth types, which can influence the appropriateness of biodistance analysis.

For example, canines exhibit the most dimorphism and may be excluded due to a preference for less dimorphic teeth. While the genetics of tooth dimensions are not well-understood, various pedigree and twin studies have reported that although variation in tooth size depends on the specific tooth and trait, buccolingual and mesiodistal crown dimensions are reported to have at least moderate to high genetic control (Dempsey et al., 1995; Dempsey & Townsend, 2001; Townsend et al., 2009; Stojanowski et al., 2017; Table 2.1). The relatively high trait heritability suggests that the phenotypes are better genotype predictors than cranial data and likely more suitable for biodistance analysis than other data types (Visscher, 2008). Therefore, potential familial relationships may be established by identifying patterns of similar tooth dimensions between individuals (Pilloud & Larsen, 2011; Stewart, 2022).

One method compares tooth size-shape dimensions between archaeologically defined groups, such as households, to detect any differences representing biological affinity (Pilloud & Larsen, 2011). An alternative method groups individuals according to their similarity to identify potentially related individuals (Stewart, 2022). The repeated and continued use of metric dental data reflects the benefits of teeth as a readily available, potentially meaningful, and reliable data source for estimating biological relationships (Fig. 2.2).

Nonmetric Dental Data

Nonmetric dental traits are discrete anatomical variants generally observed macroscopically to detect potential genetic relationships in burial sites (Alt et al., 1997). They

include the anatomical variants of tooth crowns and roots, the different shapes, sizes, numbers, structures, and positions of the teeth, and are often considered nonmetric traits of the cranium and jaw. Like metric dental traits, the shape and form of teeth are a frequently utilised dataset to answer research questions in archaeology (e.g. Hanihara, 2008), palaeoanthropology (e.g. Guatelli-Steinberg & Irish, 2005; Gómez-Robles et al., 2013), and bioarchaeological kinship (e.g. Bentley, 1987; Currocini & Shimada, 2002; Alt et al., 2015).

Over 100 nonmetric traits have been identified in modern humans suitable for inter- and intra-population analyses (Alt & Vach, 1995). However, depending on the heritability, preservation, independence, and rarity in the general population, different traits are more likely to be used. The Arizona University dental anthropology system (ASUDAS; Turner, 1991) is a standardised reference system for collecting data on tooth and crown variants, which is widely implemented in biodistance studies using nonmetric dental data, including intra-cemetery analyses (e.g. Haddow, 2012; Harper & Tung, 2012; Klaus et al., 2018; Kharobi et al., 2021).

Nonmetric dental traits are favoured over cranial data for intra-cemetery kinship analysis for similar reasons to metric dental traits (Fig. 2.3). The traits described in the ASUDAS system are popular in biodistance analysis because of their preservation, ease of recording, replicability, lack of sexual dimorphism, and apparent high genetic component in their phenotypic expression (Turner, 1991; Irish et al., 2020). The standardised scoring criteria for data collection means that recorded observations are generally reliable, as suggested by calculations of intra- and inter-observer agreement, which are generally high when recording on a dichotomous scale, with only a few problematic traits (Marado, 2017; Paul et al., 2020).

Importantly, studies have suggested that nonmetric dental traits are generally under high genetic control (Berry, 1978; Alt & Vach, 1995; Scott & Turner, 1997; Table 2.1). Like nonmetric cranial traits, they show quasi-continuous expression resulting from the complex interactions of multiple genes and the environment (Harris, 1977). Although the genes contributing to dental morphology have not been identified, the inheritance of some nonmetric traits has been frequently studied.

Dental crown morphology recorded using the ASUDAS standard has also shown at least moderate estimates of narrow-sense heritability ranging from 0.4–0.8 (Paul et al., 2020; Table 2.1). Carabelli's cusp is the most extensively studied biodistance data variable

(Biggerstaff, 1973; Berry, 1978; Scott & Potter, 1984; Skrinjaric et al., 1985; Higgins et al., 2009; Hughes et al., 2015). Early studies produced estimates at extreme ends, suggesting accounts for almost no to almost all phenotypic variance (Aoyagi, 1967; Biggerstaff, 1973; Berry, 1978; Scott & Potter, 1984; Skrinjaric et al., 1985). More recent studies have identified more consistently high heritability rates (Higgins et al., 2009; Hughes et al., 2015). This discrepancy highlights the limitations of heritability estimates as predictors of genetic inheritance.

Heritability estimates are specific estimates at a certain point in time for a population, which have unique characteristics of gene flow and environmental variance that may influence heritability estimates. Isolated, endogamous populations with little gene flow and increased environmental variance may produce lower heritability estimates that may not reflect broader patterns among other populations (Stojanowski et al., 2019; Paul et al., 2020). Later heritability estimates use variance components analysis that is more statistically robust than earlier statistical approaches, supporting the recent estimates of ASUDAS traits as a reliable and reproducible proxy for genetic data (Stojanowski et al., 2019).

Skeletal Anomalies

Developmental skeletal anomalies are an alternative form of nonmetric data used to identify biological relatives in archaeological sites (e.g. Hoffman, 1976; Case et al., 2017; Tur et al., 2019; Cvrček et al., 2021). Skeletal anomalies in bioarchaeological studies generally refer to congenital structural skeletal variants, excluding skeletal dysplasia, outside previously defined standard morphological ranges (Barnes, 2012). They reflect disruptions to critical threshold events during embryonic development when new cells replicate, migrate, or differentiate, usually caused by genetic mutations acting on susceptible genetic baselines. Nevertheless, other epigenetic factors (e.g. maternal exposure to infection, environmental contaminants or drugs, nutritional disorders) have had similar effects on embryonic development (Barnes, 2012).

Several studies have found the use of skeletal anomalies to identify biological relatives in archaeological sites (e.g. Hoffman, 1976; Cybulski, 1988; Alt et al., 1997; Villotte et al., 2011; Case et al., 2017; Tur et al., 2019; Cvrček et al., 2021; Fig. 2.2). However, these studies are uncommon in the literature because anomalies are rarely present

in modern populations. Thus, they are unlikely to be present in poorly preserved archaeological populations and can easily be misinterpreted as trauma, infection, and degenerative joint disease (Offenbecker & Case, 2012).

However, the frequencies of anomalies in a population can help detect potential biological relatives. Identifying these relationships often involves rare or uncommon heritable anomalies discovered in multiple individuals from the same site (e.g. Hoffman, 1976; Villotte et al., 2011; Case et al., 2017). The heredity of the traits used in these past studies is generally well-established in pedigree studies with higher incidence among families.

In contrast to more complex polygenic traits, several anomalies in previous studies have had an autosomal dominant mode of inheritance (e.g. Hoffman, 1976; Cybulski, 1988; Villotte et al., 2011; Case et al., 2017; Table 2.1). These anomalies are potentially exhibited when an individual inherits at least one copy of the altered gene. However, shared anomalies do not conclusively signify genetic relatives; unrelated lineages may possess the same gene, or non-genetic factors may result in the expression of different anomalies. The conclusions regarding genetic relationships are supported by evidence of close burial proximity or context between affected individuals, modern or contemporaneous population anomaly frequencies, or the presence of more than one uncommon trait (Case et al., 2017).

Post-Crania

This literature review only found one study that used metric and nonmetric post-cranial data for kinship analysis (i.e. Bandioli et al., 1986; Fig. 2.2). The post-cranial elements were collected in conjunction with dental and cranial traits for large datasets of over 100 traits to test hypotheses of non-random distribution of individuals as potentially indicative of family groups (Bandioli et al., 1986). Their inherent utility as a proxy for genetic similarity between groups and individuals was limited because of their poor preservation compared to the cranium and teeth and their reduced validity due to the level of phenotypic plasticity observed in postcranial traits (Cvrček et al., 2018).

Non-genetic factors play a significant role in expressing metric and nonmetric postcranial traits. Clinical studies have shown that mechanical loading and activity patterns play a significant role in shaping bone morphology, where adults, especially males, who

exercise regularly have higher bone mass and size than more sedentary adults (Lorentzon et al., 2005). Limb lengths collected by Bandioli et al. (1986) were influenced by an individual's nutritional environment and exposure to infection during development, age and sex. Hence, they likely did not signify an appropriate proxy for genetic relatedness (Perola et al., 2007). However, it is notable that the study used post-cranial morphological data from most body parts and was one of the few studies to use morphological data from more than one part of the body. The value of this approach has been demonstrated to provide a more nuanced reflection of biological similarity not restricted by the selective pressures on a single body system (Ricaut, 2010).

2.2.2 Beyond Skeletal Biodistance Analysis

The inherent limitations of phenetic biodistance analysis justify bioarchaeologists shifting away from it as the predominant method of reconstructing kin relations among past populations. The preferred traits are variable, with high genetic control and low influence by age and sex (Stojanowski & Schillaci, 2006). The inconsistent heritability estimates for the traits used in biodistance analysis highlight the insufficient understanding of the exact processes that drive trait expression.

Nevertheless, studies using more robust statistical methods to determine heritability may increasingly provide insight into the underlying biology of the biodistance traits (Carson, 2006a; Hughes et al., 2000; Hughes & Townsend, 2013; Stojanowski et al., 2019). Many interacting genetic and environmental factors influence skeletal and dental traits to largely unknown extents (Larsen, 2015). The suitability of these phenotypes as proxies for differences in genotypes to perform biodistance analysis is challenging to confirm, particularly because the estimates of the genetic component of traits come from heritability studies of gene frequencies of particular populations at a single point in time that have unique characteristics of gene flow and environmental variance that affect heritability estimates.

Furthermore, biodistance analysis requires the analysis of multiple traits. For skeletal traits especially, the level of preservation required for comparison between numerous individuals may be infeasible in mortuary contexts with incomplete or damaged remains. A

primary advantage of skeletal biodistance analysis is that the data collection is relatively straightforward but non-destructive, allowing kinship analysis to occur according to the wishes of culturally affiliated groups, as will be discussed in Section 2.3.

Minimally, biodistance analysis can test hypotheses of random distributions of traits to find potentially meaningful patterns that indicate a higher likelihood of similarity of individuals or groups to each other than others (Ricaut, 2010). Whether these patterns are likely to indirectly reflect genetic similarity is difficult to verify and is insufficient to distinguish between different degrees of relatedness (Stojanowski & Schillaci, 2006). Genetic methods to perform intra-cemetery kinship analysis are increasingly replacing skeletal biodistance studies (Fig. 2.4). Unlike skeletal and dental traits, DNA markers correspond directly to an individual's genome rather than acting as a proxy and are more able to perform higher resolution kinship analysis.

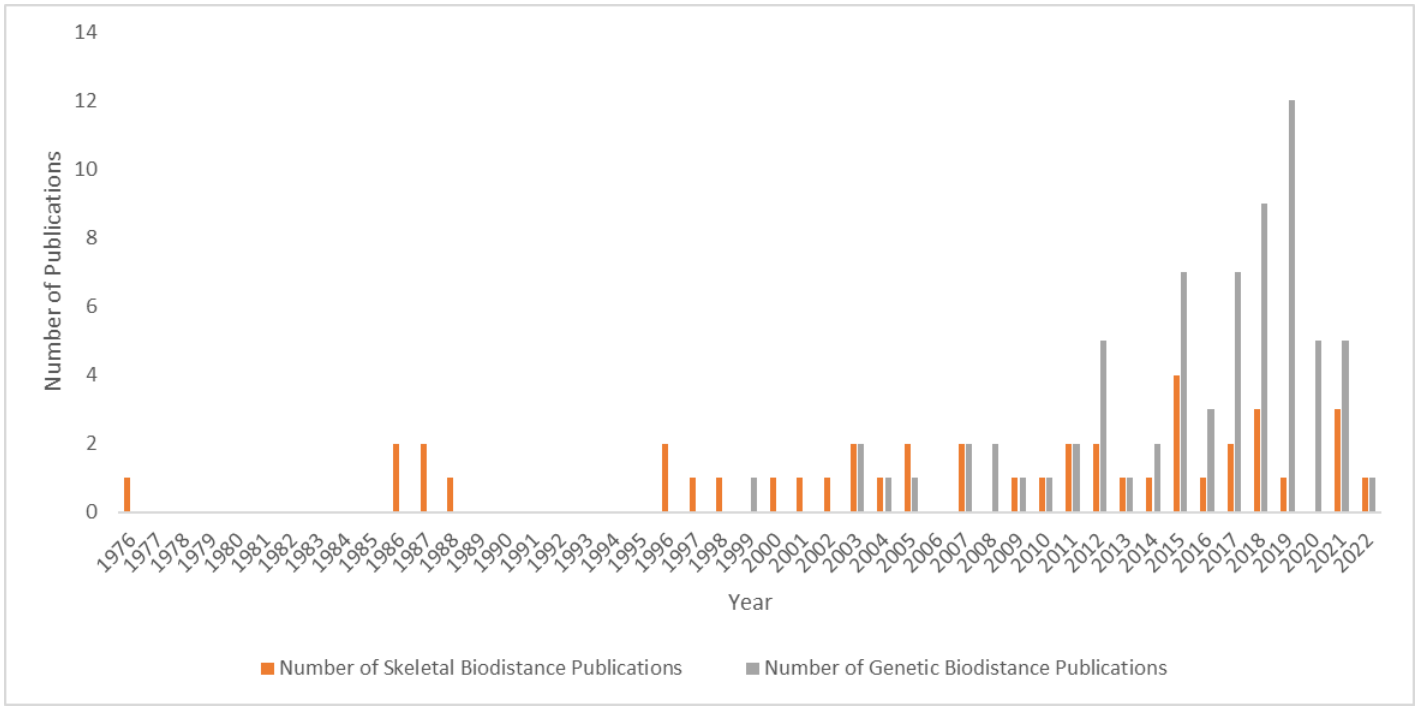

Figure 2.4 Comparison of the number of skeletal biodistance vs genetic biodistance publications that analysed intra-site biological relatedness. Based on a search from Web of Science, Jan. 2022.

2.3 Kinship Analysis Using Ancient DNA

Ancient DNA (aDNA) analysis, or palaeogenomics, can be considered a third, more direct form of phenotypic biodistance analysis as it does not rely on a proxy to determine genetic relationships between individuals (Pietrusewsky et al., 2018). Since the first extraction and sequencing of aDNA from a 150-year-old museum specimen in the 1980s, the use of DNA has become a central part of understanding biological relationships in various studies (Higuchi et al., 1984). As with skeletal biodistance, palaeogenomics is used to address a range of anthropological questions, including interpreting large-scale patterns of evolution, migration patterns, local kinship analysis, and the identification of specific individuals in forensic contexts (Bolnick & Smith, 2007; Butler et al., 2007; Kaestle, 2010; Nieves-Colón & Stone, 2018; Lan & Lindqvist, 2018).

aDNA sequencing has increasingly become the favoured method of small-scale kinship analysis, primarily due to advancing techniques that allow bioarchaeologists to access genetic information previously unobtainable due to the fragmentary nature of skeletal remains (Fig. 2.4). Genetic data has been demonstrated to have significantly higher efficacy than phenotypic data in identifying genetically related individuals (Ricaut, 2010). In a study of the Egyin Gol Necropolis in Mongolia, only 50% of the pairs of genetically related individuals identified through ancient DNA analysis were identified with the biodistance analysis of 63 nonmetric cranial, mandibular, dental, and infra-cranial traits (Ricaut et al., 2010). However, potentially related individuals were detected using trait analysis for whom no genetic data was available (Ricaut et al., 2010), reflecting the rapid degradation of DNA after death. Hence, genetic data is not guaranteed for all individuals, and techniques used in living individuals are not always suitable for ancient remains (Pääbo et al., 2004; Kaestle, 2010). Several challenges impede meaningful genetic kinship analysis using ancient DNA, requiring specialised techniques to surmount while also accepting the inherent technical limitation of working in the fragmentary archaeological record.

2.3.1 Sequencing Ancient DNA

Sanger sequencing, based on the polymerase chain reaction (PCR) and more recent next-generation sequencing (NGS) techniques, extracts remaining genetic information from ancient skeletons that usually have a low concentration of damaged and highly fragmented

DNA. The technology amplifies small amounts of DNA, which can then be analysed in more detail (Doran et al., 1986; Pääbo, 1985). The successful recovery of DNA at a given site depends on the conditions of preservation and the limits of the sequencing technique used. The validity and specificity of the subsequent genetic kinship analysis depend on the type of genetic information extracted, which can then be used to estimate biological similarity and calculate genetic relatedness.

DNA Preservation in the Archaeological Record

A fundamental step in genetic kinship analysis is extracting genetic information from ancient individuals. To retrieve aDNA from skeletal material, adequate amounts of viable DNA must be present in the archaeological record (Kaestle, 2010). The likelihood of success is highly contingent on a sample's chronological age, taphonomic environment, and exposure to contamination because of the role of these processes in DNA preservation.

After death, DNA strands accumulate damage as host repair mechanisms no longer function (Pääbo et al., 2004; Fulton & Shapiro, 2019). DNA molecules are damaged and broken down by UV radiation, enzymes, bacteria, fungi, and insects, often resulting in ancient remains with DNA strands fewer than 100 base pairs (bp) in length and chemically modified bases. Therefore, the sample's age plays a role in the DNA yields of a skeleton, although it is secondary to the influence of depositional conditions (Sawyer et al., 2012).

Cold, dry, stable conditions tend to result in longer, higher quality DNA fragments than warm, humid conditions because of the decreased reaction rate of the chemical processes involved in DNA degradation (Lindahl, 1993; Hofreiter et al., 2001). In addition to degradation processes, ancient samples are surrounded by environmental sources of DNA, such as plants, microbes, fungi, and present-day human DNA (Kaestle & Horsburgh, 2002; Lan & Lindqvist, 2018). The relatively high concentrations of unfragmented exogenous DNA may result in them being preferentially targeted for sequencing. Specialised protocols can be implemented to reduce the risk of contamination during the handling, storage, and extraction to produce authentic results (Poinar & Cooper, 2000; Kaestle, 2010; Dabney et al.,2013; Rohland et al., 2015). Negative controls and screening against researcher DNA and reference genomes can also be used to test for the presence of contaminants in the extracted samples.

Different portions of skeletal remains are uniquely affected by these processes, depending on the protective qualities of their cellular matrix, with denser elements producing a higher quality and quantity of DNA (Adler et al., 2011; Stray & Shewale, 2013). The petrous part of the temporal bone and the cementum layer of the tooth root are the best protected against DNA degradation and contamination by their high cell density, producing the highest yields of endogenous DNA. Effective alternative sources include the femur, tibia, pulp, inner dentine, and teeth (Leney, 2006; Damgaard et al., 2015).

Despite the apparent preference for skeletal elements with high DNA concentrations, the source of DNA extraction is restricted to what is available in the archaeological record. The ability to retrieve genetic information from bioarchaeological material is challenged by aDNA's degraded and contaminated nature. These limitations must be considered to apply specific techniques to capitalise on the remaining data effectively.

Sanger Sequencing

Sanger sequencing was introduced in the 1970s as the first molecular method used to reconstruct the genetic sequences of ancient individuals (Sanger et al., 1977; Tipu & Shabbir, 2015; Vai et al., 2020). It remains a common approach for sequencing ancient samples. Based on a standard PCR, it amplifies a single target sequence to produce millions of copies (Kaestle & Horsburgh, 2002; Alonso et al., 2004; Kaestle, 2010; Nieves-Colón & Stone, 2019). Unlike PCR which aims to amplify an entire DNA sequence, Sanger sequencing functions to produce every possible fragment length of a target DNA strand to identify the nucleotide sequence (Fig. 2.5).

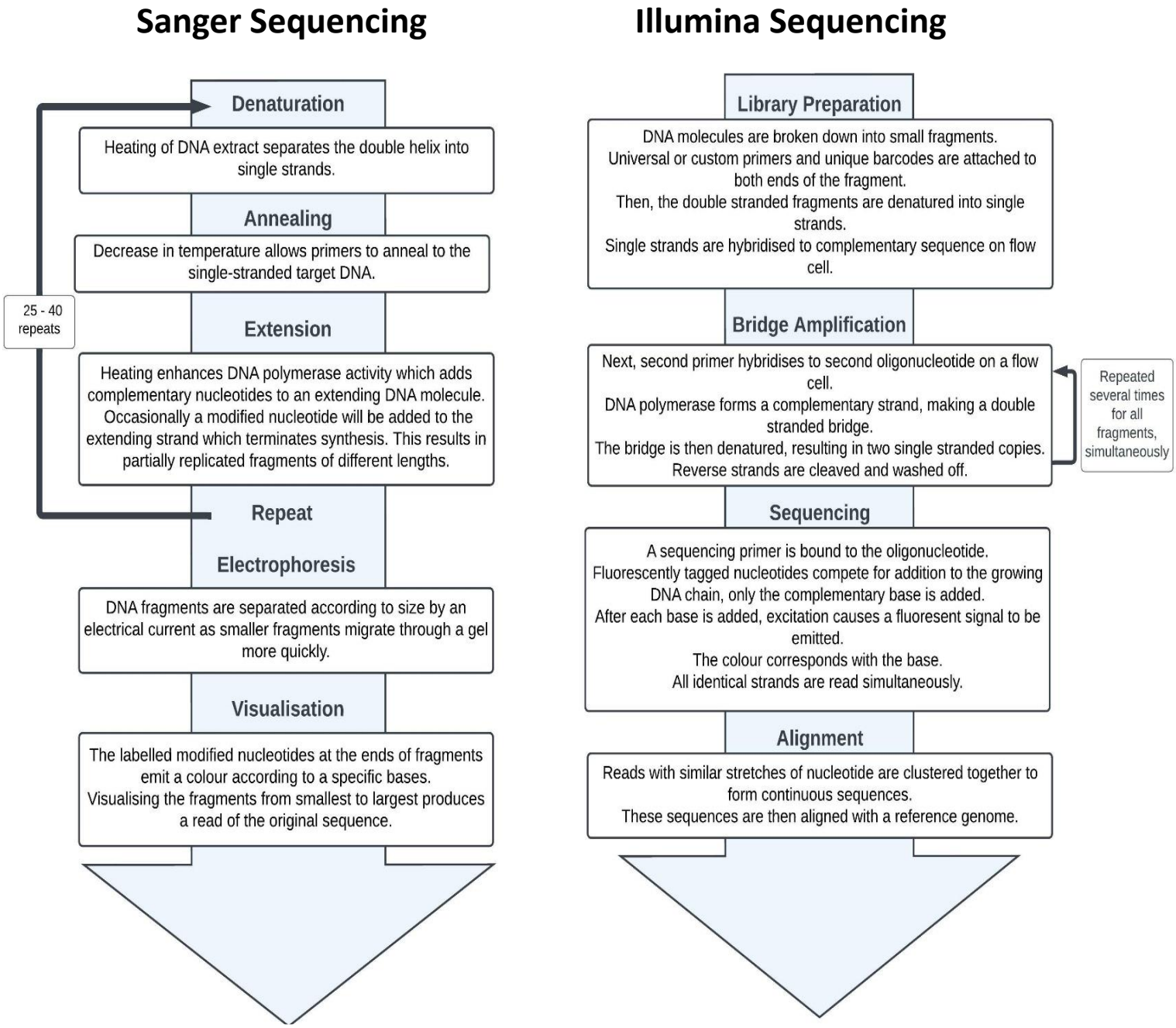

Figure 1.5 Simplified flowcharts of the Sanger and Illumina sequencing methods.

Sanger sequencing uses PCR starting materials and a cyclical heating and cooling process to amplify a specific DNA fragment. As in PCR, the amplification occurs through the repeated process of denaturation, annealing, and extension (Alonso et al., 2004). Sanger sequencing similarly requires pre-designed PCR primers, short sequences of nucleotides that

bind to the target DNA to provide a starting point for the DNA polymerase (typically, Taq polymerase) enzyme to begin synthesising copies of the template DNA.

In contrast to traditional PCR, small amounts of the nucleotides added to the PCR mixture are modified during the extension phase, chain-terminating dideoxynucleotides (ddNTPs) with fluorescent or radioactive labels unique to each nucleotide (A, T, C, or G). When ddNTPs are added to the elongating DNA strand, the DNA synthesis is terminated, resulting in partially replicated fragments of different lengths. Each of these differently sized fragments has a terminal ddNTP representing a specific nucleotide in the original sequence. After electrophoresis organises them from the smallest to largest, visualising their labels produces a read of the original DNA strand. The technique has inherent technical requirements that aDNA may not fulfil in practice due to DNA damage and fragmentation, altering how effectively this technique can be applied for extraction and analysis.

Next-Generation Sequencing

Although Sanger sequencing is still routinely used for aDNA analysis, the recent advent of NGS has made large-scale palaeogenomic studies more feasible (Balasubramanian, 2011). The massively parallel sequencing of many DNA fragments can produce much higher data outputs, millions to billions of reads, in a short period (Knapp & Hofreiter, 2010; Slatko et al., 2018; Lan & Lindqvist, 2019). In some cases, entire ancient genomes can be sequenced. Although there are several types of NGS, the most widely applied and relevant to aDNA analysis is Illumina sequencing (Rohnagi et al., 1996; Balasubramanian, 2011; Rothberg et al., 2011). NGS shares basic underlying principles with Sanger sequencing as bases are detected by the emission of signals from fluorescently labelled nucleotides, but the process can sequence many DNA fragments in parallel (Fig. 2.5).

Unlike PCR-based methods, the default NGS protocol does not rely on pre-designed primers (Fig. 2.5). Instead, sequencing libraries are prepared by attaching universal oligonucleotides, synthesised short sequence of nucleotides, to both ends of all fragmented DNA (Knapp & Hofreiter, 2010; Tipu & Shabbir, 2015; Verma et al., 2017). Each individual is also tagged with a unique barcode sequence, allowing multiple libraries to be amplified simultaneously by bridge amplification PCR once hybridised into a flow cell. This process produces approximately 1,000 copies of each DNA fragment.

DNA molecules are exposed to mixtures of all four nucleotides with reversible fluorescent blockers for sequencing. Thus, only the base complementary to the target DNA is synthesised. After synthesis, the modified nucleotides are excited to emit a signal to capture the base. The fluorescent label and blocker are then chemically removed, allowing for another sequencing cycle until the entire DNA fragment is sequenced. The subsequent fragment sequences are searched for overlapping areas to reassemble the entire sample using a reference genome efficiently. The recent advancements in DNA extraction and sequencing have moved the limits of aDNA kinship analysis, with increased access to endogenous DNA from degraded and damaged samples.

Sanger vs NGS

Sanger sequencing remains a common technique for intra-cemetery kinship analysis, despite the relatively low data output stemming from targeting a single fragment at a time (Heather & Chain, 2016; Loog & Larson, 2020). It has a narrow range of practical applications in fragmentary archaeological contexts because of the relatively high costs associated with producing many target sequences, the sequence length required, and the presence of PCR inhibitors (Kaestle & Horsburgh, 2002; Alonso et al., 2004; Kaestle, 2010; Nieves-Colón & Stone, 2019; Fig. 2.). At approximately $500 for Mb (a million bps), targeting many fragments in multiple individuals can become relatively expensive (Kircher & Kelso, 2010). A human genome would hypothetically cost $1.5 million.

However, Sanger sequencing is biased towards longer DNA molecules of at least 30 bps to allow for primer annealing, which is not always possible depending on the extent of degradation (Pääbo et al., 2004). Because of these limitations, mitochondrial DNA (mtDNA) is often the target of PCR over other genetic markers because it has a higher likelihood of amplification success due to its availability in degraded samples and sequence length, as discussed previously in this section. In well-preserved samples, nuclear short tandem repeats (STRs) have also been extracted via PCR.

However, nuclear DNA is often only present in low concentrations and highly degraded, producing either no results or incomplete profiles that provide less data for comparison with other samples. The validity of these comparisons can also be compromised by allelic dropout where an annealing and subsequent amplification failure occurs, resulting in the autosomal loci expressing false homozygous profiles (Palomo-Dìez et al., 2017).

Nuclear DNA is generally too fragmented, with levels too low for capture in most bioarchaeological contexts through Sanger sequencing. Instead, this PCR-based technique is best suited for the medium- to low- throughput retrieval of mtDNA, although the genetic profiles produced are often limited to low-resolution estimates of kin relations.

The introduction of NGS transformed the identification of potential genetic relatives in the archaeological record. Now, high-resolution kinship analysis is possible in damaged archaeological samples. Many limitations of PCR-based methods have overcome the increased cost-effectiveness of sequencing fragmentary samples with many short DNA molecules and low endogenous DNA. At approximately $0.10 per Mb, the entire genome can be sequenced for ~$300 (Kircher & Kelso, 2010; Glenn, 2011).

Sequencing a high number of DNA molecules is more cost-effective because of the simultaneous application and sequencing of multiple libraries, efficiently reassembling the entire sample quickly (Knapp & Hofreiter, 2010; Slatko et al., 2018; Lan & Lindqvist, 2018). Most significantly for bioarchaeological work, an early step in the NGS approach is the fragmentation of the target sequences, making it possible to recover very short molecules (< 50 bps) that PCR cannot capture (Nieves-Colón & Stone, 2019; Vai et al., 2020). In relatively well-preserved samples with high levels of endogenous DNA, the default shotgun sequencing method can perform whole-genome sequencing (WGS), which can reassemble whole genomes for analysis.

Moreover, for circumstances with low endogenous DNA, specialised NGS target-enrichment protocols involving custom-designed oligonucleotides and probes can efficiently capture numerous regions of interest for comparison between individuals (Maricic et al., 2010; Lan & Lindqvist, 2019). The entire mitochondrial genome can be captured using a single probe, whereas millions of informative target nuclear single nucleotide polymorphisms (SNPs) captured by custom probes can be used for high-resolution kinship analysis. Higher-throughput genetic sequencing projects are more feasible with NGS since both strategies increase the number of loci and individuals, which can be successfully sampled and genotyped for higher resolution estimates of biological relatedness up to the fourth degree (Vai et al., 2020). However, the resolution of the relatedness estimates depends on the types of DNA data available for analysis.

2.3.2 Genetic Data Types

Mitochondrial DNA

Mitochondrial DNA (mtDNA) is a small genome found in a cell's mitochondria rather than the nucleus used in most palaeogenomic kinship studies (Anderson et al., 1981; Sykes, 2001). Because of the high copy number (200–2,000 per human cell), mtDNA is more likely to be preserved over time, making it possible for traditional PCR-based amplification and sequencing techniques to be applied successfully, even in degraded remains (Alonso et al., 2004). In combination with the abundance of mtDNA, hypervariable regions (~ 400 bp) sequence lengths fit within the limits of successful PCR amplification in archaeological populations, long enough to anneal to PCR primers and short enough to be cost-effective (Pääbo et al., 2004). Specifically designed probes also allow mtDNA to be captured by NGS (Maricic et al., 2010; Lan & Lindqvist, 2018)

The mitochondrial genome is generally only passed from the mother to the offspring (in the ovum's cytoplasm), and recombination occurs within the mitochondrion with copies of itself. Therefore, the inherited sequence is usually identical across generations, making it helpful in tracing maternal ancestry (Sykes, 2001). This process typically involves sequencing the first hypervariable region (HVS-1) in the control region of the mitochondrial genome and then comparing it to reference sequences for assigning haplogroups (Butler, 2005; Behar et al., 2007).

Nevertheless, the increasingly available complete genome sequences, the second hypervariable region (HVS-2), and other sites are also used to determine haplogroup accurately. A haplogroup is defined as combinations of SNPs in mtDNA (haplotypes) inherited from a common ancestor (de Knijff, 2000). Therefore, haplogroups represent the successive accumulation of mutations through maternal lineages.

The control region is the non-coding portion of the mitochondrial genome, with a five to ten times higher mutation rate than nuclear DNA. It has a high level of diversity that facilitates genetic discrimination between individuals (Holland & Parsons, 1999; Table 2.2). Interpretations of shared mtDNA must recognise that, as some haplotypes are frequently occurring, unrelated individuals can carry identical haplotypes (Just et al., 2009). Population haplogroup frequencies may be used to predict the likelihood that individuals share a maternal lineage. However, the relative closeness of maternal relatives cannot be

distinguished (Butler, 2005). The additional consideration of alternative markers of relatedness and more genetic markers may help confirm the significance of any mtDNA similarity.

Table 2.2 The Strengths and Limitations of DNA Markers for Determining Genetic Kinship in Bioarchaeological Contexts

DNA Type	Strengths	Limitations
mtDNA	A high copy number means it is likely to be preserved over time Can be captured by both PCR-based and NGS amplification Can test hypotheses of maternal kinship	Can only determine potential maternal lineage May be shared between unrelated individuals Requires appropriate population haplogroup frequencies to predict the likelihood of relatedness
Nuclear DNA		
STRs	Increased discriminatory power because of high levels of polymorphism Fewer markers required for accurate kinship determination Can determine degree of genetic relatedness	Loci generally too long (~100–400 bps) to be recovered in degraded samples Requires appropriate population genotype frequencies to predict likelihood of relatedness
SNPs	Short (< 50 bps) enough to be recovered from degraded samples Can determine degree of genetic relatedness	Low levels of polymorphism Many SNP markers required for kinship discrimination Requires appropriate population genotype frequencies to predict likelihood of relatedness
Y-DNA	STRs long enough to be captured by PCR-based amplification SNPs can be captured by NGS in poorly preserved samples Can test hypotheses of paternal kinship	Can only determine potential paternal lineage between males Low haplogroup diversity Requires appropriate population haplogroup frequencies to predict likelihood of relatedness

Nuclear DNA

The nuclear DNA (nDNA) sequence encodes an individual's unique genetic profile in a genome over 3 billion bps long, with an estimated 30,000 genes (Sykes, 2001; Butler, 2005). Found as two copies per nucleus, it is less likely that nDNA will be sufficiently preserved for extraction and analysis. Unlike mtDNA, the nuclear genome contains thousands of independent genetic lineages, which can be used to recreate a more comprehensive picture of common ancestry between individuals (Reich, 2018; Table 2.2). nDNA, consisting of 22

pairs of autosomal chromosomes and one pair of sex chromosomes, is inherited biparentally; one chromosome of each pair is passed down from each parent (Butler, 2005; Nieves-Colón & Stone, 2019).

Combined with the recombination of chromosomes in each generation, an individual's genetic sequence will represent a mosaic of ancestral DNA from potentially thousands of ancestors (Sykes, 2001; Reich, 2018). Therefore, the kinship analysis can provide higher resolution kinship analysis not restricted to shared ancestral lineage as with mtDNA, but the degree of biological kinship (Weir et al., 2006). Two types of genetic markers are used to analyse biological relatedness: STRs and SNPs (Table 2.2).

Autosomal STRs vs SNPs

Both STRs and SNPs are polymorphic, bi-parentally inherited markers used for genetic analysis (Butler, 2005). The level of discriminatory power a marker exhibits is determined by its level of polymorphism; individuals are less likely to share a highly polymorphic variable by chance. For STRs, which are mutations where short segments (2–6 bps) of DNA have been repeated, populations exhibit high variation in the number of repeats (Schultes et al., 1997). Forensic kinship analysis predominantly uses 13–15 unlinked, highly polymorphic STRs loci to reduce the probability that unrelated individuals will randomly match (Butler, 2005). However, nuclear DNA is rarely intact in high enough concentrations to retrieve this many STR sequences in archaeological contexts.

The main advantage of SNPs is their short length, which allows them to be recovered in degraded samples by NGS, while STR loci are too long (~100–400 bps) for effective capture (Butler, 2005; Knapp & Hofreiter, 2010; Tipu & Shabbir, 2015; Verma et al., 2017). SNPs are less polymorphic than STRs. Therefore, they have less discriminatory power than STRs.

Notably, 50–80 SNPs are required to reach the same discriminatory power as 12–16 STRs in forensic contexts (Gill, 2001; Ayres, 2005). While this number is infeasible using PCR-based analysis, NGS can simultaneously sequence more than 80 SNPs, and it can sequence entire genomes. Therefore, even estimates of 50,000 SNPs being able to reduce false-positive and negative relationship classifications to below 5% are well within the range of possibility (Monroy Kuhn et al., 2018).

Y-Chromosomal DNA

Y-chromosomal DNA is typically found in the Y sex chromosome in the nucleus of male individuals (Noordam & Repping, 2006). With only one copy per cell, it is unlikely to be well-preserved in archaeological populations (Sykes, 2001; Table 2.2). Y-chromosomal DNA (Y-DNA) shares many properties with mtDNA, making it a similarly useful tool for kinship analysis (Cafer, 2010). Although Y-chromosomes do recombine with X-chromosomes, this only occurs at the tips of the Y-chromosomes (Sykes, 2001). Less than 10% of the chromosome recombines, leaving a long genetic sequence inherited paternally across generations. Therefore, Y-DNA can be used to trace paternal lineage.

While Y-DNA is not as informative as autosomal matches, the potential shared paternal lineage can be detected by analysing the mutations in the non-recombining region of the chromosome (Kayser, 2017). Commonly occurring mutations are STRs, making PCR-based analysis possible in well-preserved samples. Often, concentrations of endogenous nuclear DNA are too low for capture or only produce partial profiles, compromising the accuracy and specificity of paternal lineage identification, which increases with the number of Y-STRs considered or identifying specific sets of polymorphic Y-STRs (Kayser, 2017).

Recently, NGS techniques have been used to identify Y haplogroups as part of genome-wide analyses, allowing sequence capture from samples with low concentrations of endogenous DNA (e.g. Furtwängler et al., 2020). These methods capture thousands of SNPs from unique regions of the Y-chromosome using target-enrichment methods to more confidently map sequences to reference genomes (Kivisild, 2017; Martiniano et al., 2022). The Y-DNA does not mutate as rapidly as mtDNA because of the repair mechanisms during replication, decreasing haplogroup diversity with relatively less discriminatory power (Sykes, 2001). The diversity of Y haplogroups can show high levels of population specificity and low diversity. Hence, in some modern populations, the same haplogroup is found in every male individual (Jobling et al., 1998).

High haplogroup frequency makes discriminating between related and unrelated individuals impossible by Y-DNA alone. Haplogroup diversity varies between populations, so specific sets of Y-STRs are not equally suitable across populations for differentiating paternal lineage, which can be problematic in ancient populations where the only available

reference frequencies come from modern data. Additional relatedness markers, including other genetic markers, can help direct interpretations of paternal relatedness based on Y-DNA. Y-DNA can alternatively be used to test hypotheses of paternal relatedness in combination with autosomal or mtDNA analysis to narrow potential types of genetic kinship (Kivisild, 2017).

2.3.3 Estimating Genetic Relatedness

Individuals whose alleles are identical-by-descent (IBD) are considered genetically related. This term refers to haplogroups inherited from common maternal or paternal ancestors for uniparental markers. For biparentally inherited markers, STRs and SNPs, it refers to the inherited alleles representing thousands of ancestors. The more recent the shared ancestors and the number of shared ancestors provide the expected degree of relatedness between individuals. Related individuals share more autosomal markers at each locus because they are more likely to be IBD than unrelated individuals (Weir et al., 2006). Relatedness (*r*) may be understood as the proportion of genes shared IBD between two individuals. Because it is impossible to observe IBD directly, genetic sequencing allows the observation of the alleles at a locus to identify whether individuals share the same alleles (i.e. the alleles are IBS; Blouin, 2003; Weir et al., 2006).

The methods used to infer biological relatedness can be separated into two groups. The first method estimates the degree of relatedness between a pair of individuals based on probabilities of IBD (Queller & Goodnight, 1989; Li et al., 1993; Ritland, 1996; Lynch & Ritland, 1999; Wang, 2002). The estimation of IBD from IBS requires the population allele frequencies, which can be used to produce probabilities of two individuals sharing alleles at a given locus based on Hardy-Weinberg principles of a diploid, outbred population. Also required are the probabilities of individuals sharing two (k_2), one (k_1) or no (k_0) alleles IBD. The *k* values reflect the expected fraction of zero, one, or two alleles IBD in two individuals' genomes following Mendelian segregation (i.e. less related individuals will show higher k_0 values; Table 2.3). Therefore, the relatedness (*r*) between pairs of individuals can be estimated using these different values of *k* by using calculations derived from the function $r = \frac{k1}{2} + k_2$ (Queller & Goodnight, 1989; Li et al., 1993; Lynch & Ritland, 1999; Wang,

2002). Overall estimates of relatedness combine *r* from multiple, genetically independent loci by applying loci-specific weights based on their sampling variances that result from the different genotype frequencies and the number of alleles.

Table 2.3 Identity-By-Descent Coefficients (k_0 k_1, k_2) and Relatedness Coefficient (r) for Common Relationship Categories (R)

Relationship (R)	k_0	k_1	k_2	*r*
Monozygotic twins	0	0	1	1
Parent-child	0	1	0	0.5
Full siblings	0.25	0.5	0.25	0.5
Half-siblings	0.5	0.5	0	0.25
Avuncular	0.5	0.5	0	0.25
Grandparent-grandchild	0.5	0.5	0	0.25
Unrelated	1	0	0	0

Based on Hamilton (1964)

The second method determines the likelihood that individuals share specific genetic relationships based on the available genetic data (Milligan, 2003; Anderson & Weir, 2007; Wang, 2007). Genotype likelihoods determine which relationship is most probable by testing the observed genotype frequencies under different hypotheses of relationships (Blouin, 2003; Weir et al., 2006). The likelihood estimates also require knowledge of population allele frequencies with assumed Hardy-Weinberg equilibrium to calculate the probability that the pair of individuals share 0, 1, or 2 alleles (P_1, P_2, P_3). These *P* probabilities are used to estimate the likelihood that a pair shares a particular pair of genotypes *(G)* given a specific relationship *(R)* based on the IBD coefficients for that relationship: $P(G|R) = k_0P_0 + k_1P_1 + k_2P_2$. Final likelihoods are obtained by multiplying the probabilities from all unlinked loci.

Calculating Genetic Relatedness in Archaeological Populations

Bioarchaeological kinship analysis typically uses computer software tools tailored to the limitations of the palaeogenomic data. Relatedness estimates are KING and READ (Manichaikul et al., 2010; Monroy Kuhn et al., 2018), whereas genotype likelihoods are calculated by ML-Relate, NgsRelate, and lcMLkin (Kalinwoski et al., 2006; Korneliussen & Moltke, 2015; Lipatov et al., 2015). Low yields of endogenous DNA can result in low coverage depths that make calling diploid genotypes difficult (Vai et al., 2020). Therefore,

analyses may use a pseudo-haploid genome based on one allele being randomly sampled at each locus.

However, it only allows kinship estimates up to the second degree (Monroy Kuhn et al., 2018). Genotype likelihoods also help overcome this by incorporating all the genotype calls to calculate the most likely relationship. For example, the lcMLkin software sums all the probabilities of IBD for all the genotypes weighted by their likelihoods based on their sequence reads (Lipatov et al., 2015).

The other major issue is the unknown population allele frequencies for ancient samples. In marker-based kinship analysis, the relatedness value is calculated relative to a reference population with known allele frequencies (Wang, 2014). Datasets of ancient allele frequencies are limited. Moreover, sampling bias in burial practices may present inaccurate representations of allele frequencies.

Modern frequencies might be used when no ancient population reference is available, although it is not always clear which dataset is most appropriate. The general rule is that if there are no evident historical connections, geographically similar populations should be used while accounting for populational differences (Esparza et al., 2017; Palomo-Dìez et al., 2019). If no appropriate reference populations are available, then simple pairwise comparison to test for specific relationship hypotheses might be used (Egeland et al., 2000; Brustad et al., 2021).

For example, a parental link is considered possible if individuals share an allele at all the tested loci. Proposed kin relations are generally considered supported if there are thousands of matching loci in a relatively isolated population and if there other bioarchaeological connections between individuals (Vai et al., 2020). It is important to note that relationship categories with the same relatedness coefficient, such as parent-child and full siblings, cannot be distinguished (Weir et al., 2006). Likewise, different relationship categories, such as avuncular and half-siblings, have similar IBD coefficients when interpreting likelihood estimates. Additional genetic information, such as paternal and maternal lineages and personal information like age differences, can help discern more plausible relationships (Fig. 2.6).

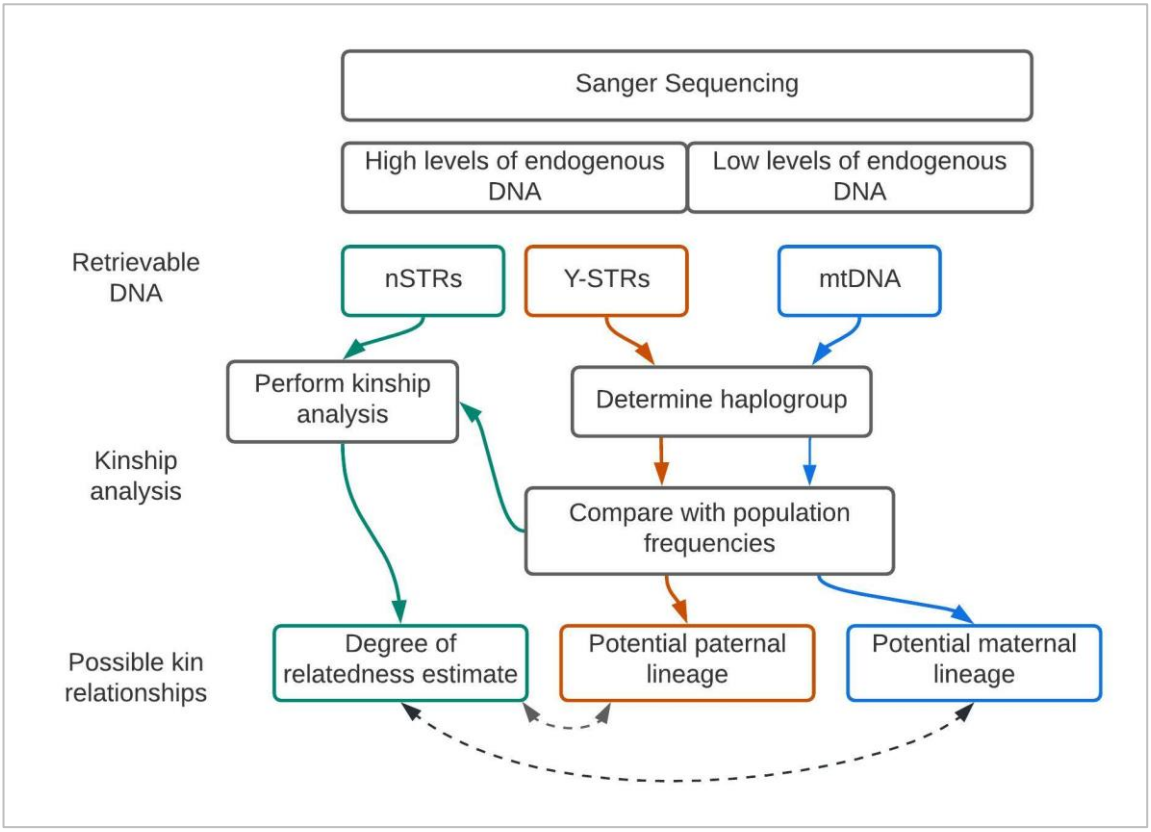

Figure 2.6 Flowchart of process of how relatedness is estimated possible for given types of DNA data available in an archaeological population.

2.4 Summary

Archaeologists often focus on the genetic relationships between individuals in past societies. Originally this involved using phenetic biodistance as a proxy for these genetic relationships. Increasingly, DNA analysis is increasingly becoming the favoured method for bioarchaeological kinship analysis as technical advances reduce costs, allow its application to degraded remains, and perform high-resolution calculations of relatedness (Kaestle, 2010; Kircher & Kelso, 2010; Vai et al., 2020). Forming conclusions about genetic relationships requires an understanding of the limitations of these techniques and the genetic data used. As the default method through which bioarchaeologists study kinship, ignoring these limitations could potentially lead to misleading confidence in proposed kinship reconstructions. With the knowledge of the flexibility of kinship and the limitations of ancient DNA analysis, a systematic review of the data, sequencing methods, and conclusions in past bioarchaeological kinship studies was conducted.

Chapter 3. Systematic Review

3.1 Introduction

While kinship is frequently studied as part of an invaluable part of society in prehistory, the limitations of the methods and assumptions surrounding the analyses are often overlooked. Therefore, this chapter aims to systematically review all primary bioarchaeological studies that investigate kin relationships in past societies using ancient DNA analysis. This review is intended to quantify the gap between the modern understanding of kinship and how kinship is defined in bioarchaeology with the limitations of DNA analysis, producing suggestions for future bioarchaeological research.

A systematic review identifies, selects, and synthesises all data relevant to perform a critical evaluation of a research topic. It is suitable for this thesis' research aims because it allows the large number of relevant studies, which use various methods in many different archaeological contexts. By integrating different individual studies, the systematic review can establish overall trends in applying different concepts and methods, helping develop suggestions for future research (Siddaway et al., 2019).

This chapter presents a systematic review of all previous bioarchaeological kinship studies that use non-skeletal biodistance methods. First, the review methods are summarised, including the criteria for inclusion and exclusion in the review. Then, the data extracted and synthesised from each paper are described, and the results of the data synthesis are presented. The chapter ends with a discussion of the limitations of current bioarchaeological kinship research and its implications for future research.

3.2 Methods of the Review

Search for Relevant Literature

I first identified relevant studies published before March 2022 by searching the following electronic databases: SCOPUS, Web of Science, JSTOR, Anthropology Plus, and AnthroSource. Then, to find any additional sources, I used snowballing methods, searched reference lists of identified studies, and performed electronic citation tracking. No time or

language restriction was used in the search, but a small number of studies were excluded due to a language barrier. The following search terms were used to locate relevant articles:

('Kin' OR 'family' OR 'relatives' OR 'relation' OR 'social structure' OR 'social organisation') AND ('ancient DNA' OR 'palaeogenomic' OR 'kinship analysis') AND ('archaeology' OR 'past').

Criteria for Inclusion

The initially collected publications were assessed for relevance in two phases (Fig. 3.1). First, I screened the titles and full abstracts to remove book chapters, review articles, and articles unrelated to bioarchaeology or kinship. Second, I read the full text of the remaining articles to identify those that met the following criteria: 1) the study presented primary research, 2) the study sample included at least five individuals, 3) the methods included aDNA analysis, and 4) the research presented explicit findings of kin relationships between individuals. Studies were excluded if their methods included skeletal biodistance.

Data Extraction and Synthesis

Data collected from each paper was recorded in an Excel spreadsheet for ease of filtering for comparison between papers (Table 3.1).

Table 3.1 Types of Data Collected from Each Primary Study in the Systematic Review

Data Type	Specific Data Gathered
Reference Information	Author, year
Kinship Model	Cultural data, historical data, biological kinship, non-biological kinship
Genetic Data	Type of DNA marker, number of DNA markers, sequencing method
Bioarchaeological Data	Genetic system(s), isotopes, osteological analyses, dating, burial context, burial position, grave goods
Kinship Analysis and Conclusions	Specific relationship, matrilineal relationship, patrilineal relationship, non-biological kinship
Other	Study site, country, time-period

Kinship Model Data Synthesis

Each study was qualitatively evaluated to empirically assess the gap between the current understanding of kinship as a cultural construct and how kinship is conceptualised in bioarchaeological studies. Each paper was assessed to see if it recognised kinship as a culture-dependent construct, which could occur in two ways. First, I determined if the paper mentioned historical or cultural data to support using a specific kinship model in its analysis. Second, I evaluated if a more inclusive kinship model was included and defined using more than genealogical forms of relatedness in the methods, results, or discussion section.

Genetic Data Synthesis

To evaluate the analytical basis for genetic kin assessment, I quantitatively assessed how rigorous the criteria were for including DNA data in higher resolution kinship analyses. For papers that used nSTR or nSNP markers, the number of markers used to estimate the degree of genetic relatedness or genetic relationship between individuals was compared against recommendations from forensic analysis and tests of known relationships. The minimum number of STRs or SNPs used to estimate relatedness was calculated for each paper. Notably, for STR-based analyses, at least 13 unlinked loci are recommended to reduce the probability of unrelated individuals matching by chance (Butler, 2005), while for SNP-based estimates, 2,500 SNPs are suggested to reduce false-positive and false-negative kin classifications below 5% (Kuhn Monroy et al., 2018).

Biosocial Data Synthesis

I also ascertained the data types used in the papers, which were previously identified as using more than one form of kinship in their kinship analysis (i.e. non-genetic or constructed relationships) to form recommendations for how future research could conduct more inclusive kinship analyses.

3.3 Results

3.3.1 Results of the Search Phase

The initial search phase returned 240 potentially relevant papers, and subsequent searches through the reference lists found three additional studies (Fig. 3.1). 63 of these were relevant based on the previously mentioned criteria (Appendix A). A large proportion of the excluded papers were review articles summarising the methods of ancient DNA analysis or population studies investigating migration patterns. The search produced far more relevant studies than expected, given the relatively recent development of genetic sequencing techniques.

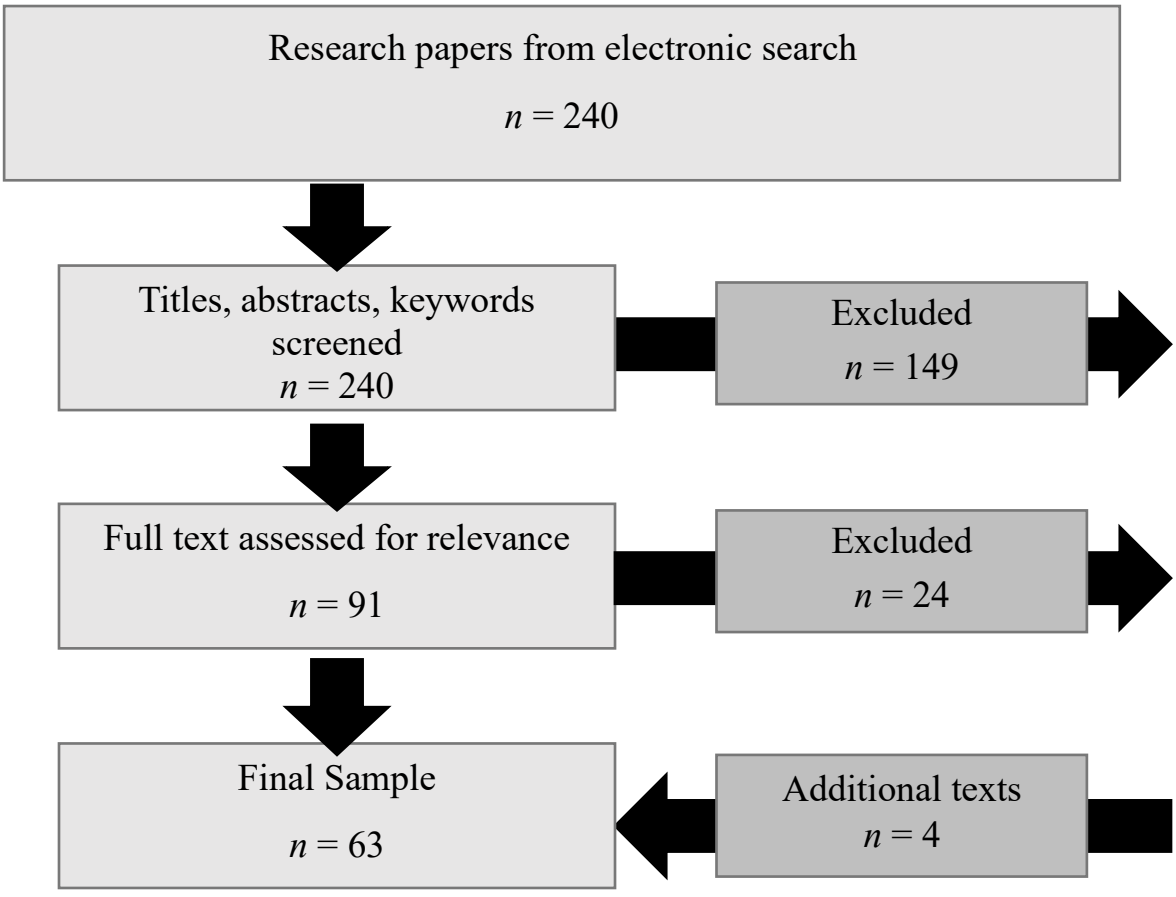

Figure 3.1 Screening process for finding relevant research articles included in the systematic review.

Characteristics of the Included Studies

The sites analysed in these papers were located primarily in Europe (Fig. 3.2): Austria (1), Czechia (1), France (3), Germany (14), Greece (1), Hungary (2), Ireland (1), Italy (2), Poland (4), Russia (4), Serbia (2), Spain (5), Sweden (2), Turkey (2), and the United Kingdom (3) (Fig. 3.2). Several sites were in Asia: China (4) and Siberia (2). A few of the sites were from North America: Greenland (1), the United States (4), and Mexico (1). Only three were from South America: Argentina (2) and Peru (1). Two sites were located in Africa: Egypt (1) and Morocco (1).

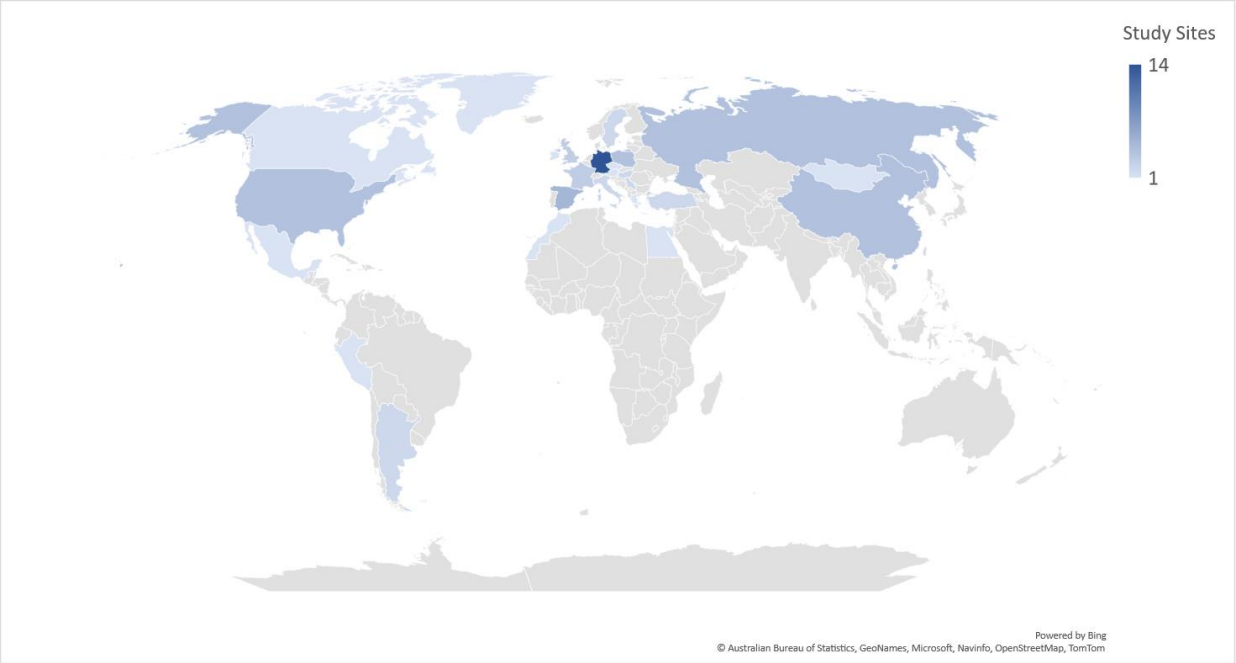

Figure 2.2 Geographic distribution of the study sites included in the systematic review.

3.3.2 Results of the Data Synthesis

Kinship Models Used in Analysis

A majority (~68%; *n* = 42) of the papers in the systematic review met at least one of the criteria to have recognised kinship as a culture-dependent construct (Fig. 3.3). Of these, ~35% (*n* = 22) met this criterion using only some form of either historical or cultural data to justify the kinship model applied to the skeletal populations under study. Only ~14% (*n* = 9) utilised a more inclusive kinship model without historical or cultural context. Moreover,

~17.5% (*n* = 11) of the papers used historical or cultural data and an inclusive kinship model to interpret the relationship between individuals in their study site. A notable portion (~33%; *n* = 21) of bioarchaeological studies did not consider the socially constructed nature at all.

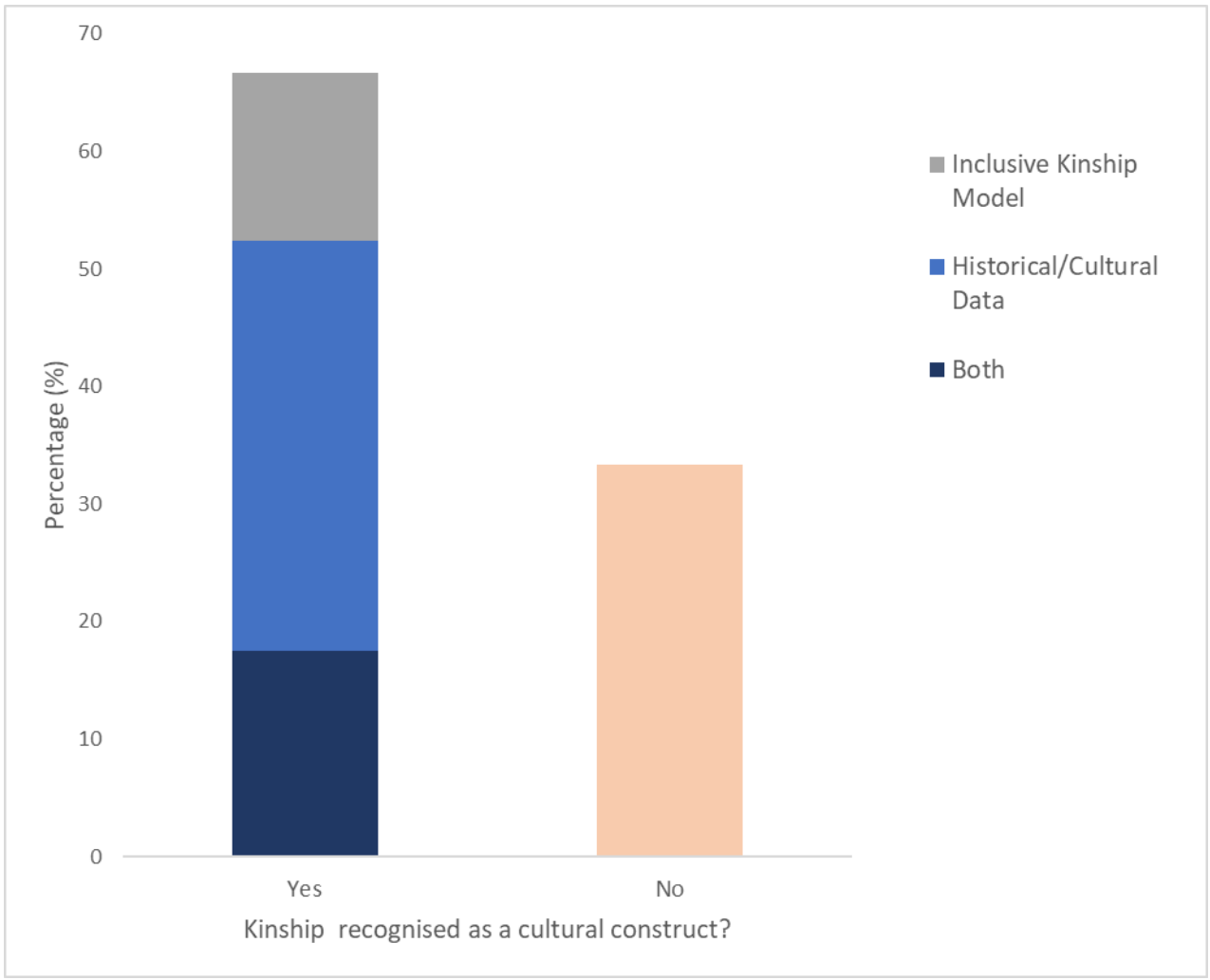

Figure 3.3 Percentage of papers in the systematic review that recognised kinship as a cultural construct.

DNA Used in Higher Resolution Kinship Analysis

Of all the papers that used nDNA markers to perform kinship analysis (*n* = 35), almost half (~43%; *n* = 15) did not meet the recommendations suggested (Fig. 3.4). For the 15 papers that used SNP markers for kinship analysis, all but one paper did not use at least

the recommended 2,500 SNPs to estimate the degree of relatedness or type of relationship between individuals in their study sample (Fig. 3.3). In contrast, a high proportion (70%; *n* = 14) of the STR-based papers used fewer markers than recommended to perform the same analyses. Of the 20 STR-based studies, only six papers (30%) used at least 13 markers.

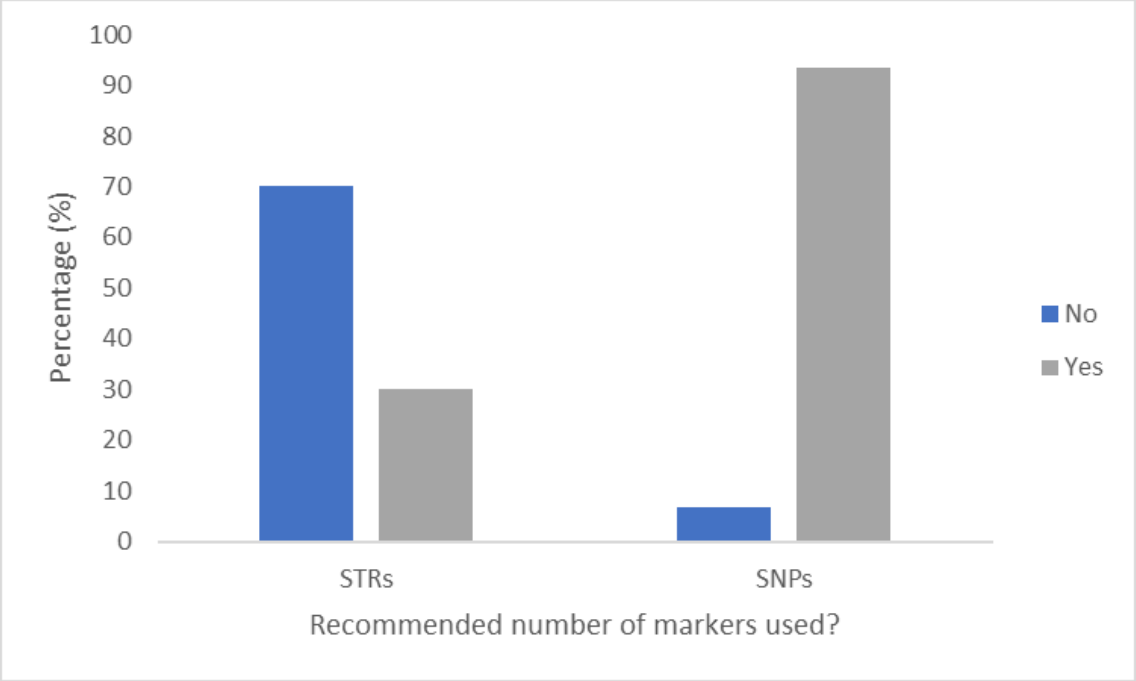

Figure 3.4 Percentage of studies that used the recommended number of DNA markers when calculating relatedness using nuclear DNA.

Data Used to Detect More Inclusive Kinship

Based on the 20 papers that identified more than one form of relatedness, the majority (*n* = 11) used shared or close burials alone to suggest that individuals might be kin (Fig. 3.5). Two papers used genetic and burial proximity between individuals to assess their relatedness. The spatial arrangement of individuals was also used in four other papers, each in conjunction with burial position, radiocarbon dates, grave goods, or an extensive pedigree. Two papers used strontium isotopes and burial locations: one in addition to grave goods and the other with oxygen isotopes. Only one paper used oxygen isotope data to reconstruct potential relationships.

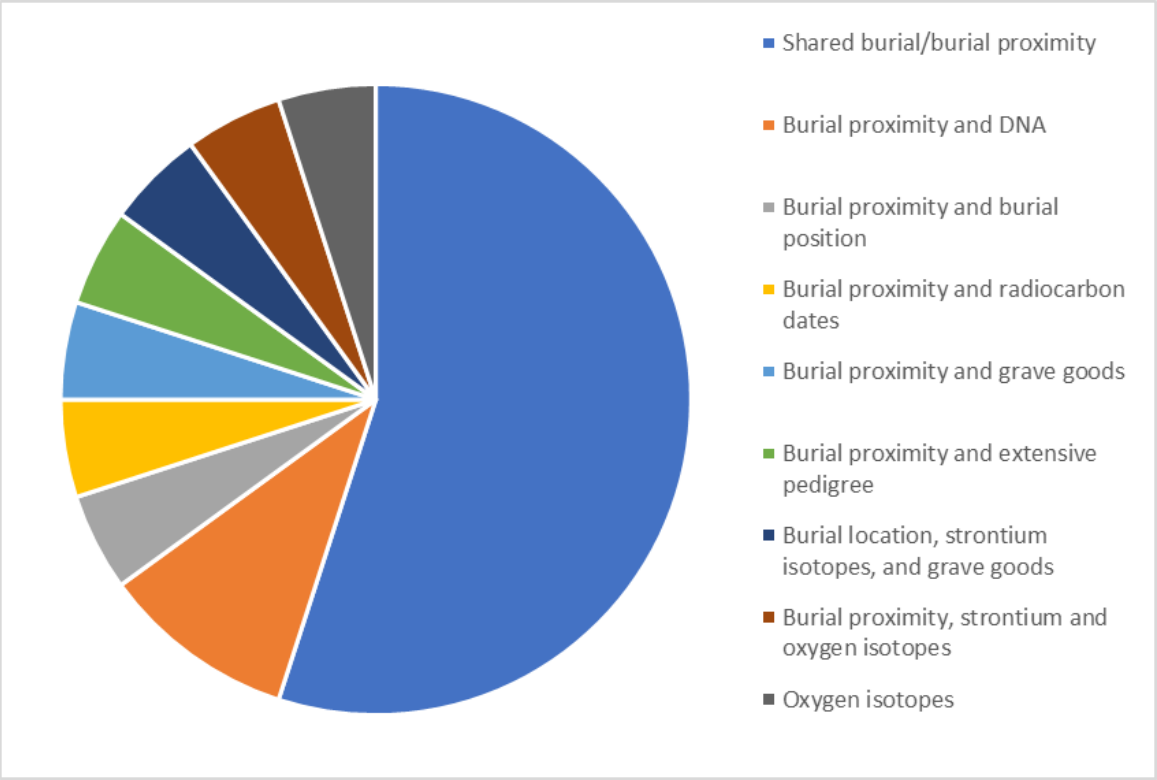

Figure 3.5 Methods used to detect inclusive forms of kinship in the archaeological record.

3.4 Discussion

3.4.1 The Gap Between the Biological and the Social

This systematic review supports the idea of a significant conceptual gap between how kinship is understood as a culturally dependent social construct and how kin relationships have previously been reconstructed in archaeological populations. The criteria formed to quantify this gap were quite lenient. Studies were only required to provide some evidence supporting the assumed cultural context of the applied kinship model. And simply mentioning social kinship was sufficient when presenting kinship as a cultural construct. Therefore, conceptual disconnect is likely present in more than the already-significant third of the papers indicated.

The applicability of the historical and cultural contexts used to shape kin interpretations in the site of interest was variable. In 10 of 33 studies using historical or cultural data to justify their kin models, records or indications of the individuals who might

be present in the site were available (e.g. Gerstenberger et al., 1999; Hawas et al., 2010; Schwarz et al., 2015; Alterauge et al., 2021). In such cases, the contextual information about potential kin relationships between individuals was more likely to lead to accurate interpretations than in sites where historical data was sparse or lacking entirely. Indirect historical accounts of family structure were also used to interpret potential family structures in several papers (e.g. Baca et al., 2012; Deguilloux et al., 2014; Russo et al., 2016; O'Sullivan et al., 2018; Rott et al., 2018; Mittnik et al., 2019).

Four of the papers used *familia*-based kinship models to reconstruct past relationships from study sites in Germany and France spanning from the late Neolithic to the mid-8th century C.E. (Deguilloux et al., 2014; O'Sullivan et al., 2018; Rott et al., 2018; Mittnik et al., 2019). Evident in ancient Roman and Greek records and the more recent legal text *Lex Baiuvariorum* from the late 7th century C.E. (Steuer, 1989; Cox, 2014), the *familia* could include close and distant relatives and friends, neighbours, and household servants. As the *familia* is an inherently inclusive kinship model, it is unsurprising that the articles using it fulfilled the second criteria and suggested the presence of non-genealogical or reproductive relationships between individuals. While some papers were vague as to *familia* ties, others suggested that adoption, fellowship, and slaves were potentially present in their populations (e.g., O'Sullivan et al., 2018; Rott et al., 2018; Mittnik et al., 2019). The combination of a relatively inclusive kinship model, with some historical evidence of its presence in these cultures, along with several bioarchaeological markers of potential kinship allowed the comprehensive reconstruction of different kin relations in these past societies.

For bioarchaeologists, comparable historical data is absent more often than not. Therefore, interpretations of cultural funerary practices regarding kinship are primarily based on archaeological material. Hence, the cultural evidence includes previous bioarchaeological kinship studies from similar eras or archaeological cultures.

Considering that in this systematic review of 63 articles, ~33% did not recognise kinship as a cultural construct as outlined in Chapter 1, potentially a third of bioarchaeological kinship studies are biased towards Western genealogical models of relatedness. This result is unlikely due to the review's restriction to aDNA analyses because, as described in Chapter 2, bioarchaeological kinship analysis methods are usually founded on genetic similarity. Therefore, use of the cultural context to interpret the relationships between individuals may be based on unrepresentative historically derived cultural norms.

For example, Haak et al. (2008) were popularly reported to have found the first nuclear family in a collective burial from 4,600 years ago and have frequently been cited in subsequent bioarchaeological kin studies (e.g. Baca et al., 2012; Schroeder et al., 2019; Sjögren et al., 2020). The conclusion that the original burial represented a nuclear family has been criticised for overlooking the culturally constructed nature of marriage and parenthood, not in the least because alternative family structures were also potentially represented at the site (Geller, 2017; Johnson, 2019). Any later studies that use similarities to these earlier sites to inform their interpretations could perpetuate the original bias towards families configured on heteronormative reproductive relationships. The conceptual gap between the sociocultural understanding of kinship is not closed by using possibly biased interpretations of a population's kinship system as a basis for the cultural context for later analyses.

Of the 20 papers that used inclusive models of kinship, 11 based their non-genetic kinship analyses on burial proximity or shared graves (e.g. Byrnes et al., 2012; Keyser et al., 2015; Juras et al., 2017; Pilipenko et al., 2017; Bus et al., 2019; Chylenski et al., 2019). Hence, social kinship was a possible alternative only in the absence of genetic kinship. The inverse was also frequently implied – that social kinship did not need to be considered in the presence of genetic kinship. This finding does not mean that burial proximity was not a potentially valid indicator of important kin relationships, as subsequently discussed in Section 3.3.3. Instead, the variability in post-natal processes that form kin relationship appeared to have been overlooked in favour of assuming a genetic kinship congruent with social kinship (e.g. Schroeder et al., 2019).

For example, Owsley et al. (2018) was one of the few papers to explore the post-natal processes involved in maintaining kinship among genetically related individuals. Moreover, it was the only one that did so in detail. This paper examined an 18th-century Maryland burial vault. It might be expected that the patriarch would be central to the relationships observed in the crypt. Instead, his wife, her adult siblings, and probable children were buried in the vault. The wife was described as performing active kin-keeping with her adult siblings, likely taking care of and supporting them to maintain nuclear family ties, potentially contributing more to their proximity in death than their genetic relationships (Owsley et al., 2018).

Although access to historical records about the individuals undoubtedly helped form such conclusions, the mortuary context, in combination with genetic data, revealed this post-natal process. This finding contrasts heavily with the papers where social kinship was

mentioned only in the final lines (e.g. Bus et al., 2019; Chylenski et al., 2019). The papers exhibiting inclusive kinship models, defined by the inclusion of kinship beyond the genealogical model, largely fulfilled this criteria using relatively superficial descriptions of social relationships between individuals buried in close proximity. Nevertheless, their relatively high presence in this systematic review suggested a hopeful future for bioarchaeological kinship analysis that incorporates multiple lines of evidence to produce more fine-scale reconstructions of kinship in past societies.

3.4.2 Challenges to the Supremacy of DNA

In modern Western populations, genetic relationships often correspond with social relationships. DNA data alone may be enough to recognise these relationships in such contexts. However, kinship is the outcome of social action in many societies that cannot be captured simply through DNA (Bruck, 2021). Separating aDNA from other forms of bioarchaeology limits understandings of the past.

DNA analysis is an undeniably powerful tool for bioarchaeologists. It directly corresponds to individual genomes, which can then be used to reveal patterns of similarity indicative of genetic relationships. DNA is broadly seen as an essential and unchangeable determination of someone's identity (Crellin & Harris, 2020). There is an implicit assumption that such an objective scientific technique produces more objective kinship reconstructions than other types of bioarchaeological data (Bruck, 2021). The nuances and limitations of genetic analyses are often overlooked, as are the complexities shaping human relationships (Crellin & Harris, 2020).

However, DNA is not infallible, nor does it make interpretations of archaeological remains more accurately than other bioarchaeological methods (Bruck, 2021). A single SNP-based paper did not meet the recommended 2,500 SNP markers (Monroy Kuhn et al., 2018). The 26 to 77 SNPs were, however, only used to exclude a mother-child relationship (Juras *et. al.*, 2017). Only 90 SNPs were originally targeted for capture, but these were specifically chosen for their high discriminatory power, as their high heterozygosity reduces the likelihood that individuals would share a genotype by chance (Paktis *et. al.*, 2010). In contrast, most other SNP-based studies performed shotgun or targeted enrichment that

attempted to capture up to millions of SNPs (*e.g.,* Amorim *et. al.,* 2018; Mittnik *et. al.,* 2019; Sjorgen *et. al.* 2019). The inability to successfully sequence all 90 target SNPs reflected that in poorly preserved remains, there is no guarantee that specific SNPs will survive (Juras *et. al.,* 2017). A higher number of genetic markers reduces the discriminatory power at higher degrees of relatedness and reduces the probability of genetic matching by chance (Monroy Kuhn *et. al.,* 2018; See Section 2.2).

The limitations of DNA analysis are most evident in the number of STRs used in kinship analysis: 14 of the 20 STR-based studies did not use at least 13 markers. As mentioned in Section 2.2, forensic analyses typically use at least 13 STR markers to reduce matching by chance (Butler, 2005). Fewer markers mean a higher probability of a false-positive or false-negative relationships being estimated. The minimum number of markers used per paper ranged from three to 21. The paper that used three STR markers only used them to exclude a potential mother-child relationship, while recognising the limitations using only a few markers (Deguilloux et al., 2018). However, another study utilised four STRs to estimate the presence of a potential half-sibling relationship (Rott et al., 2018). In reality, the pair in question had different Y-DNA and mtDNA lineages, which contradicted the possibility of a half-sibling relationship. These contradictions occurred between uniparental markers with low numbers of STRs, such as a father-son relationship with different Y haplotypes (Rott et al., 2018).

Even more indicative of the potential limitations of DNA data was the estimation of a parent-offspring relationship between individuals 2,000 years apart (Keyser et. al., 2015). The discrepancies observed highlight the influence of population allele frequencies on the discriminatory power of DNA markers. For the Yakut population examined by Keyser et al. (2015), the low autosomal diversity was indicative of a high level of inbreeding.

All but one of the 21 STR-based papers used at least one form of uniparental marker as well as autosomal DNA to narrow down the potential types of relationships that might be shared between individuals (Fig. 3.4). The results between the uniparental markers and autosomal DNA were not always in agreement (Rott et al., 2017; Rott et al., 2018). The allele frequencies of prehistoric populations are rarely accessible given the patchy skeletal records. Therefore, a population's history of processes that cause low genetic diversity, such as restricted gene flow, genetic drift, and small effective population size, are relatively

unknowable. Both the limitations of STR markers and the need for alternative bioarchaeological evidence to reduce inaccurate kinship classifications are clear.

Almost half of papers that used nDNA markers did not meet the suggested criteria to reduce the chance of unrelated individuals matching by chance (Butler, 2005; Monroy Kuhn et al., 2018). Although genetic kinship analyses are limited by the patchy skeletal record and unknowable population genetics, kinship estimates can be supported by using sufficient nDNA markers and uniparental markers. However, the need to critically assess DNA-based findings is clear. The assumption that genetic analyses provide more objective estimations of kin relationships than bioarchaeological data can be challenged. When the supremacy of DNA is no longer assumed, the gap between DNA and other forms of bioarchaeology might be closed leading to the capture of more inclusive forms of kinship (Crellin & Harris, 2020).

Chapter 4 Approaching an Inclusive Kinship Model

4.1 Introduction

By separating aDNA from other forms of bioarchaeology, genetic kinship often ends up acting as the framework against which other models are tested (Crellins & Harris, 2020). Putting genealogical relationships at the forefront of bioarchaeological analysis limits inferential power when performing kinship analyses (Johnson, 2019). In many contexts, genetic relationships are not salient with kin relationships. Kin relationships are instead the outcomes of social action. In such contexts, DNA data alone is not sufficient to detect the complexities of kinship (Johnson, 2019; Crellin & Harris, 2020; Bruck, 2021).

The systematic review in this paper covered 63 studies of burial sites – from ~15,000-year-old sites to those from the 19th century. Although there was a bias towards European sites, the studies covered a wide range of geographic locations. However, most of the studies were focused on the same genealogical framework for kinship. This is in sharp contrast to modern understandings of kinship practices, which are shown to be culturally and temporally variable.

A more inclusive approach is required to fully incorporate the social processes that act to form kin bonds. The papers that were identified as considering more inclusive models of kinship produced conclusions ranging from unspecific social affiliations to social parenthood and household membership. Examining the data they used provides a useful starting point for identifying bioarchaeological data that might facilitate more inclusive approaches.

4.2 Markers of Potential Relatedness

4.2.1 Burial Context

The act of burial is a meaningful and symbolic ritual that provides an opportunity to understand an individual's life, position, and membership status in a social system (Tainter, 1975, Parker-Pearson, 1999). The burial placement of the deceased may reflect intentional placement by the living, and each aspect has many potential meanings, including familial relationships (Ekengren, 2013). Depending on the cultural burial customs, therefore, where people are buried and with whom can provide evidence of important connections between

individuals (Ensor et. al., 2017). They may represent the maintenance of important kinship ties after death through burial location (Hutchinson & Aragon, 2002). For that reason, burial proximity may be a useful proxy of social, emotional, or legal ties and help to reconstruct important relationships and kin bonds.

Close burial proximity between individuals, such as shared burials, was the predominant evidence for identifying social ties that might constitute kinship (e.g. Gomes et al., 2017; O'Sullivan et al., 2018). However, these potential connections were often unspecific descriptions of social kinship (e.g. Juras et al., 2017; Pilipenko et al., 2017). Hypotheses limited to a social connection were generally based on close burial proximity in the absence of a genetic relationship. For example, Bus et al. (2019) examined the mitochondrial lineages of individuals buried in a mass grave. As no individuals shared mtDNA haplotypes, they deemed it more likely that the individuals were socially related. Similar findings were presented from a double burial consisting of a woman and child who were maternally unrelated. The burial was suggested to potentially represent social kinship ties (Juras et. al., 2017).

Specific hypotheses about potential parent-child relationships between genetically unrelated individuals were also identified through spatial proximity and individuals' relative burial positions in comparison to genetically related individuals (e.g., Haak et al., 2008; Deguilloux et al., 2018; Fowler et al., 2022). Haak et al. (2008) suggested that a maternally unrelated individual might have been the stepmother of the two children with whom she was buried. The woman and children were arranged facing away from each other, which contrasted with a nearby burial where presumed parent-child relationships were expressed through a face-to-face burial arrangement. Deguilloux et al. (2018) used a similar comparison of burial arrangements between genetically related and unrelated individuals to suggest a putative stepmother relationship between an adult woman and adolescent buried in the same grave.

Fowler et al. (2022), however, produced a comprehensive reconstruction of a five-generation pedigree of a Neolithic tomb. The adult male sons of women who reproduced with men from the pedigree lineage were also buried in this family tomb. Hence, adoption and social fatherhood were suggested to have potentially been as valuable as procreative fatherhood. Kin based on association or co-residence were also considered possible based on the incorporation of other distantly related individuals in the tomb.

4.2.2 Co-Residence

Co-residence can play an important role in the process of kinship formation (Carsten, 1995; Hill et. al., 2011; Sahlins, 2013). People living together often share experiences, eat together, and work together, which are all social processes involved in the construction of kinship. Depending on cultural burial practices, important co-residential relationships may be reflected in the spatial distribution of individuals. This was evident in two papers which used burial location as indicative of shared households (Chyleński et al., 2019; Mittnik et al., 2019). One used burial proximity and position to identify a potential *familia* connection.

The spatial patterns of burials were the predominant data used to identify social units such as families or households. The discovery of spatial associations with residential buildings was an important marker of co-residence. Chyleński et al. (2019) suggested that individuals buried under the same building were part of the same kin group, potentially based on non-genetic affinity. Mittnik et al. (2019) similarly determined that burial in cemeteries associated with farmsteads likely represented shared *familia* or *oikos* households. A burial containing a child and an elderly man facing each other was considered indicative of a shared household in a *familia* context (Rott et al., 2018).

Grave goods were used to identify the presence of groups of genetically unrelated females and lower status groups within the household (Mittnik et. al., 2019). This reflects the belief that grave goods are potentially indicative of an individual's status or position in life (Ekengren, 2013; Lopez-Costas, 2015). Grave goods, often clothing and food-related items, may have been possessions of deceased individuals, gifts to the dead, or objects for the afterlife (Parker-Pearson, 1999). As such, Mittnik et al. (2019) suggested that the sparsity of grave goods in some graves reflected the lower social status of the occupants, and potentially that they were slaves of the household.

Mittnik et al. (2019) also used strontium isotope ($^{87}Sr/^{86}Sr$) values to provide additional evidence about household dynamics. $^{87}Sr/^{86}Sr$ values in tooth enamel reflect the geochemical environment in which they were formed and, thus, may reflect migration (Price et al., 2002). By identifying high strontium isotope values and rich grave goods, Mittnik et al. argued that a grave contained a group of genetically unrelated, non-local, wealthy females from a farmstead household.

Sjorgen et al. (2020) suggested the presence of a fostered juvenile due to outlying oxygen isotope values ($\delta\ ^{18}O$). Variation in oxygen isotope $\delta\ ^{18}O$ values in terrestrial water result from differences in climate. Therefore, the isotopic composition of an individual's hard tissues, especially tooth enamel, reflects the climate conditions that existed when the tissue was forming (Fricke et al., 1995). Therefore, tooth enamel $\delta\ ^{18}O$ values can be used to determine an individual's childhood residence. The detection of outlying $\delta\ ^{18}O$ in a juvenile in comparison to his genetic father with whom he was buried suggested that this individual had been fostered elsewhere as a young child before returning to his birthplace.

Bioarchaeological kinship analysis appears to be particularly skewed towards the use of burial proximity between genetically unrelated individuals to determine unspecific social bonds. A few papers used other data such as burial positioning, grave goods, strontium isotopes, oxygen isotopes, and grave goods to produce more specific interpretations of the possible social processes involved in these bonds. Nevertheless, these papers exhibit a limited range of data and conclusions and do not recognise the extent of the embodied experiences that might be recorded in skeletal remains (Johnson, 2019).

4.3 Additional Biosocial Markers of Relatedness

The narrow scope of data and methods implemented by previous bioarchaeologists has reduced their ability to detect more inclusive types of kin (Johnson, 2019). Previous papers that produced the most detailed analysis of the kinship in past societies used multiple types of bioarchaeological data (e.g. Mittnik et al., 2019; Sjorgen et al., 2020). These included burial proximity, burial positioning, grave goods, strontium isotopes and oxygen isotopes. These data, however, do not represent the extent of data available to bioarchaeologists (Johnson, 2019).

As discussed in Chapter 1, kinship constructed through social practice of shared experiences such as commensality, shared labour, co-residence, migration, similar pathogen exposure and injuries (Sahlins, 2011; Johnson, 2019). Through the practice of forming kinship ties through these processes these relationships can become embodied (Johnson, 2019). Therefore, detectable aspects of kin relationships may leave a mark on skeletal remains. Thus, incorporating multiple types of biosocial data might capture more diverse kinship relationships in the future. Using multiple markers of familial relationships and

detecting patterns of similarity between individuals could identify different levels of affiliation without relying on genealogical frameworks. Critical to these endeavours is the simultaneous analysis of multiple lines of evidence that might serve as proxies for relatedness criteria, such as commensality, co-residence, adoption, marriage, and shared life experiences as well as genes.

4.3.1 Diet

Kinship, for many societies, is embedded in food-sharing practices (e.g. Weismantel, 1995; Carsten, 1995; Dombrowski et. al., 2013). For Malays who live on the island of Pulau Langkawi, giving and receiving food is of vital importance in social identity (Carsten, 1995). Individuals become related to each other through sharing food cooked at the same hearth. Similarly, for the Ku Waru of the Nebilyar Valley in New Guinea, *kopong*, or 'grease', is the essential matter of all living organisms and is originally sourced in the soil. It is involved in the construction of kinship through semen and breastmilk, but also through garden produce (Merlan & Rumsey, 1991).

Reconstructions of diet through specific archaeological techniques could be used as indicators of shared diets, which could act as a proxy for status, commensality, and food-sharing. The predominant method of diet reconstruction in archaeological contexts is stable isotope analysis (Schoeninger, 2010). This is possible due to the correlation between carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios in diet and their isotope ratios in human tissue, such as bone collagen, bone minerals, hair, and nails. The plants and animals in a diet leave measurable traces in an individual's bone chemistry (Larsen, 2002). Measured ratios of nitrogen and carbon isotopes can be compared to associated archaeological isotopic measurements from the environment, modern human data (O'Connell, et al., 2001), and controlled animal experiments (DeNiro & Epstein, 1978) to gain ideas of the sort of prey and food items that might be represented by an individual's isotopic ratio (Schwarcz & Schoeninger, 1991; Bocherens & Drucker, 2003). Therefore, similar isotope ratios of the same tissues of different individuals can provide a meaningful marker of a shared diet that might represent commensality, similar social status, or food-sharing, which have been identified as playing a crucial role in kin bond formation in some societies (Weismantel, 1995; Carsten, 1995; Dombrowski et. al., 2013).

4.3.2 Life-History

Life experiences are more likely to be shared by relatives than by strangers because of the similar physical, and social environments families embody (Baker, 2001). Patterns of shared life experiences may be useful general markers of relatedness and delineate between groups of kin who inhabited different physical and social spheres. Osteological data could identify shared diseases, health experiences, or work experiences (Larsen, 2002). An individual's skeleton represents a direct line of evidence regarding various aspects of an individual's life. Many types of data can be incorporated to capture a more complete picture of an individual's life experience that can then be compared between individuals. This data includes – but is not limited to – specific diseases, non-specific pathologies, trauma analysis, and musculoskeletal stress markers.

Disease

Infectious diseases such as dental caries, tuberculosis, and leprosy can be diagnosed from characteristic lesions on the skeleton (Powell, 2000; Larsen, 2002). More recently, histological analysis of bone tissue (Schultz et. al., 2001) and the extraction of the DNA from infectious organisms in skeletons have been used to identify the presence of specific diseases (Stone & Ozga, 2019). These markers are useful as indicators of shared status and shared household environments, as they may reflect sanitation, hygiene, and access to food (Patel, 2017). For example, in modern Indian populations, co-residence is associated with the risk of chronic disease even among genetically unrelated individuals (Baranwal et. al., 2014; Patel, 2017). This association of households with certain diseases is also present in studies of tuberculosis, whereby the familial contact within households is a major cause of transmission (Augustynowicz-Kopeć, 2012). Therefore, each type of infectious disease provides another opportunity to detect patterns of similarity that might indicate co-residence, which can play an important role in the formation and maintenance of kin bonds (Sahlins, 2013).

Non-Specific Markers of Stress

The disruption of growth during development resulting from an impoverished environment can also result in identifiable markers of stress in the teeth and skeleton (Lewis & Roberts, 1997; Simpson, 1999). These include markers of interrupted growth due to prolonged physiological stress, such as enamel hypoplasias in the teeth (Goodman & Rose, 1991) or Harris lines in the growth plates (Geber, 2014). Such markers may result from long-term physiological stress lasting weeks to months – for example, malnutrition or disease, or a combination of the two. Markers likely to represent nutritional deficiencies include cribra orbitalia and porotic hyperostosis on the cranium, which are believed to result from anaemia (Stuart-Macadam, 1992). In addition, the bowing of wight-bearing long bones is indicative of vitamin D deficiencies resulting in rickets and osteomalacia (Stuart-Macadam, 1992). Most of these skeletal indicators are non-specific and can be remodelled over a lifetime. Therefore, even if they were present during the lifetime of an individual, they may not be recorded in the individual's skeletal remains (Larsen, 2002). However, the presence of any of these markers in the skeletal record is an important representation of elevated stress during an individual's lifetime. Thus, commonalities between individuals might represent physico-social environments with levels of physiological stress that are more likely to be shared among family members than among strangers. For example, prevalence of anaemia in Indian children under five was linked to aspects of the household environment, such as hygiene conditions, type of house, cooking fuel, and source of drinking water (Baranwal et. al., 2014). Therefore, markers of nutritional deficiencies or physiological stress could provide useful indicators of shared households and, potentially, other types of socially constructed kinship. This is especially true if there are similar patterns of shared labour and disease as well as shared burial context and diet.

Physical Stress

Skeletons may also contain traces of physical stress resulting from violence, accidents, and habitual physical activity. These may be associated with occupational activity or labour (Larsen, 2002) that is more likely to be shared among related individuals (Johnson, 2019). Evidence of trauma, such as dislocations or fractures in the skeleton, can provide

information about an individual's interaction with their physical and socio-cultural environments (Lovell & Grauer, 2018). Differences in fracture patterns can be linked to accidents and falls due to irregular terrain (Jimenez-Brobeil et. al., 2007), subsistence activities, technologies, and occupations that might expose people to higher risks of trauma (Agnew et. al., 2015).

Activity-related markers on skeletons may also provide further information regarding individuals' lifestyles (Villotte & Knusel, 2013). Habitual physical activity due to occupations, hobbies, or locomotion can result in changes to the skeleton as the musculoskeletal system adapts to biomechanical loading. These markers include entheseal changes and degenerative joint disease (Larsen, 2002). Entheseal changes are characterised by the abnormal size or morphology of muscle attachment sites (Karakostis et. al., 2017). Therefore, the differential presence of entheseal changes can be used to distinguish the levels of physical activity between different individuals (e.g. Villotte et. al., 2010; Havelkova et. al., 2011; Henderson et. al., 2013).

Degenerative pathology of the auricular joints is considered an indicator of joint use during a person's lifetime (Larsen, 2002). The auricular surface of a joint is covered by cartilage that erodes as the joints are used over time. Pathologies can be used to detect the degree of joint use through the presence of osteophytes, porosity, and eburnation at the margins and surfaces of the joints (Rogers & Waldron, 1989). The locations of these markers may also help narrow down the type of activity, because different activities place mechanical loads on different joints (Larsen, 2002). However, the clear interpretation of these patterns often requires additional information such as biomedical correlates or historical contextual information (Villotte et. al., 2010; Havelkova et. al., 2011).

Individuals might exhibit shared physical stress patterns if their family shares an occupation, such as farming or slavery. Therefore, incorporating these interpretations of shared trauma and activity patterns as evidence of shared labour or occupations provides another line of evidence for identifying similar lifetime physico-social environments between individuals. Many types of analysis are necessary to capture a more complete picture of life experiences for effective comparison between individuals, such as the presence of non-specific pathologies, the diagnosis of specific diseases, trauma analysis, and the analysis of musculoskeletal stress markers. These patterns of shared life experience could be compared with patterns of diet, stress, and status to see if any meaningful patterns emerge.

4.4 Limitations of Biosocial Markers

There are several inherent limitations of these techniques that must be considered when attempting to extract information regarding relatedness and kinship structure. First, it cannot be assumed that individuals within the same cemetery or those buried within the same area lived at the same time. Therefore, some method of chronologically connecting individuals is necessary, such as radiocarbon dating or shared primary burial contexts.

Independently, each of the proposed techniques that can be incorporated into a multidisciplinary approach to kinship analysis may have limited abilities to reconstruct kinship, as they are frequently non-specific. Multiple avenues can produce similar outcomes on a skeleton, such as different diet types that result in the same isotope ratios or different stressors that result in the same non-specific stress markers (Larsen, 2002; Schoeninger, 2010). Pathological effects on a skeleton are usually later symptoms of diseases and, therefore, are paradoxically more likely to present in healthier individuals who survived with a disease for longer. Thus, the skeletal record is not a direct reflection of the health status of individuals in the population (Larsen, 2002). For these reasons, the simultaneous use of multiple markers of potential relatedness is crucial for establishing potentially meaningful connections between individuals.

Burial rites also change throughout history. Cultural and social changes and transitions can lead to changes in funerary practice (Ekengren, 2013; Lopez-Costas, 2015). The purpose behind differential burial treatment may reflect an individual's life, afterlife, but may also be a display for the living (Ekengren, 2013). Therefore, using aspects of burial context, such as grave goods and burial orientation, as proxies of shared status must be considered within their temporal context and may be supported by additional information such as differential health outcomes expressed by osteological markers (e.g. Peck, 2013). For example, if there is a lower frequency of individuals found in elaborate burial contexts exhibiting physiological stress markers, there is a higher likelihood they reflect an archaeological sub-group potentially differentiated by status. This is also true of other potential markers of connections: the cultural context, a large sample size, and multiple lines of evidence are invaluable tools for identifying patterns and narrowing down the types of relationships that are potentially present between individuals.

Importantly it cannot be assumed that individuals at the same site lived at the same time, as it may lead to faulty conclusions about kinship between individuals who never interacted, such as the supposed parent-child pair that lived 2,000 years apart (e.g. Keyser et. al., 2020). Therefore, alternative methods such as radiocarbon dating, chronology from grave goods, and shared primary burials might help narrow down potential relationships (Vai et al., 2020).

Furthermore, populational subgroups, such as individuals of the same age, sex, or socioeconomic status, may show more similarity for some of these markers than individuals from the same family. This may be due to divisions in terms of access to food, occupation, labour, exposure to trauma, and burial goods. Cemetery organisation may also be organised in such a way that divides individuals based on age, gender, or status, rather than familial organisations (Parker-Pearson, 1999). For example, in all Sulawesi, mortuary practices infants are placed separately and treated differently from adults (Hutchinson & Aragon, 2002). Detecting and controlling for the influence of these other variables is an important aspect of a multidisciplinary approach to kinship analysis.

The potential changes in the meaning of burial practices, the potential for multiple causes of the same marker, and the variables other than family membership influencing the distribution of proposed markers of relatedness empathises the necessity of multiple markers of potential relatedness to establish meaningful biosocial similarity between individuals. The simultaneous use of multiple markers of potential relatedness that is crucial for establishing fine-grained connections between individuals.

4.5 A Multidisciplinary Approach to Inclusive Kinship Analysis

The systematic review quantified a considerable conceptual gap of at least 33% between bioarchaeological kinship analysis and the conceptual understanding of kinship as a social process. Not only are genetic relationships not the sole form of kinship, but genetic analysis was also shown to have inherent limitations that are often overlooked when identifying kin. This brings into question the idea of DNA as a superior and objective means for identifying kin, especially in the absence of sufficient DNA markers. Therefore, the

frequent prioritisation of genetic findings over archaeological connections as a more objective analysis overlooks the limitations of ancient DNA analysis (Crellin & Harris, 2020; Bruck, 2021). It also undervalues the potential for bioarchaeological data to contribute to the identification of kin.

Implementing a multidisciplinary methodology that incorporates both genetic and social data is necessary for reconstructing the social processes involved in kinship formation. Although there were relatively few of them, the papers that utilised non-genealogical explanations of kinship demonstrated some of the types of social data that can be used to determine kinship relations. Additional markers have been suggested that might capture patterns of commensality, co-residence, status, and life experiences that if used together might produce meaningful patterns about the relationships between individuals.

Chapter 5. Trial Social Network Analysis with Secondary Data

5.1 Introduction

Understanding the processes of relationship formation and maintenance is integral to creating rich reconstructions of kinship in the past. The aim of this chapter is to demonstrate how a multidisciplinary approach that integrates genetic and non-genetic markers of kinship could capture socially constructed kinship bonds. Because human skeletons contain records of the physical and social environments that individuals experienced in life it is possible to explore more diverse forms of relatedness such as those described in Chapter 4 (Larsen, 2002).

Social network analysis is a popular technique for visualising and interpreting interactions between individuals and social group formation (Borgatti *et. al.,* 2002a). It is used in biological studies of animal behaviour and in sociology, ethnographic, and archaeological research (Flack *et. al.,* 2006; Friemel, 2007; Wei *et. al.,* 2008; Marin & Wellman, 2011; Mills, 2017). Relatively few studies have used social network analysis on bioarchaeological data (Terrell, 2010; Johnson, 2016).

Social network analysis explores the diversity of ties between entities, groups, or individuals and is flexible to a diversity of connections, thus providing a tangible representation of the interactions between individuals (Brughmans, 2013). This makes social network analysis a valuable tool for exploring the range of biosocial connections between people in the past. However, the inability to directly observe interactions and relationships in skeletal populations can make bioarchaeological social network analysis challenging. Data collected from the sparse archaeological record is all that is available (Brughmans, 2013; Coward & Knappett, 2013). Therefore, the appropriateness of using bioarchaeological data must be justified by showing that it is reflective of social relationships.

The use of proxy markers of relatedness detectable in human remains are supported by the range of modern and ethnographic evidence of individuals forming relationships that might be reflected in the skeletal record (e.g. Carsten, 1995; Weismantel, 1995; Augustynowicz-Kopeć, 2012; Dombrowski et. al., 2013). This chapter examines patterns of potential relatedness markers between 13 individuals from an early mediaeval Alemanni site in Germany to produce simple reconstructions of potential kin groups (Wahl *et. al.,* 2014; O'Sullivan *et. al.,* 2018). Using social network visualisation and analysis, I explore how

different patterns of similarity between individuals and identify potential kin relationships might be interpreted using secondary biosocial data collated from Wahl et al. (2014) and O'Sullivan et al. (2018).

5.2 Material and Methods

5.2.1 Site Context and Material

The 13 individuals used in this analysis were found in an Alemannic burial site at Niederstotzingen in southern Germany (Wahl *et. al.,* 2014; O'Sullivan et. al., 2018; Fig. 5.1. The Alemanni were a confederation of Germanic tribes that had an extensive range across Europe. They were present in modern-day France, Austria, Germany, and Switzerland. The site was first excavated in 1962 and was located at the crossing point of two ancient Roman roads (Wahl et al., 2014). Based on belt styles and inscriptions found in the burial site, it was determined to have been active during a short period between 580 and 630 C.E. (Wahl et al., 2014). This time period combined with the rich furnishings present in the graves suggest the site is a well-preserved *Adelsgrablege* – an elaborate gravesite of an Alemannic household or *familia*.

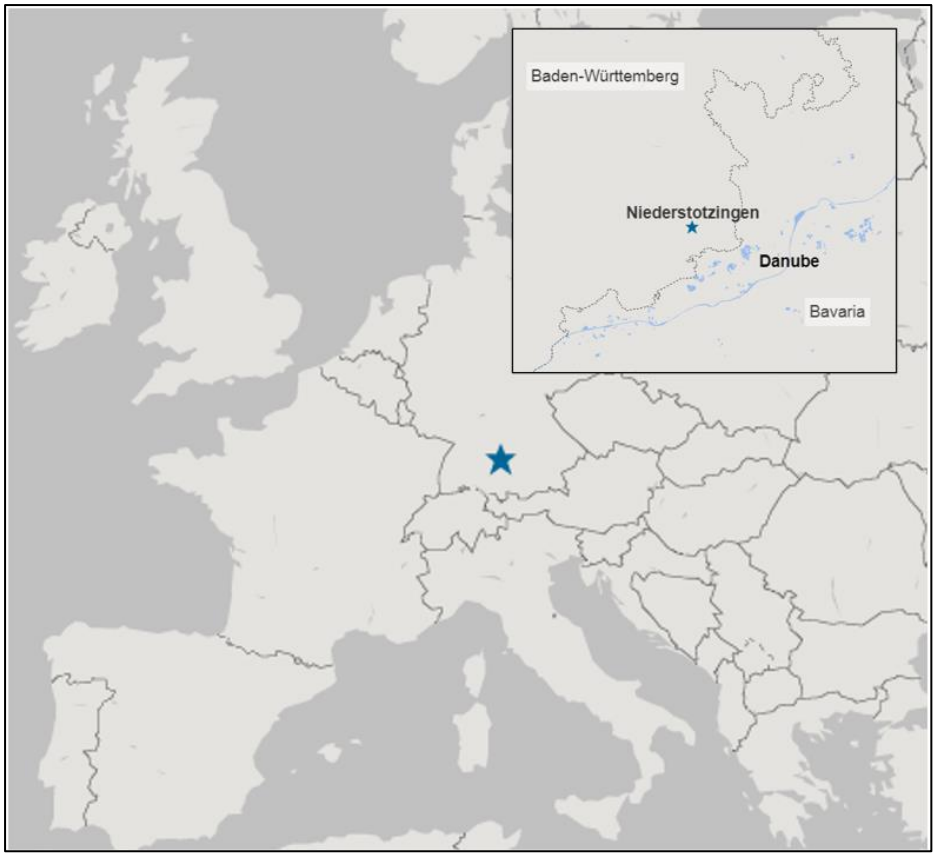

Figure 5.1 Location of Niederstotzingen burial site in southwest Germany.

Niederstotzingen contained seven individual graves and two triple burials. The occupants included nine adults and four sub-adults who are all believed to have been male (Wahl, 2014; O'Sullivan et al., 2018; Fig. 5.2) The burials contained a wide range of grave goods, including armour, weaponry, jewellery, and horse-riding gear. The artefacts originated in Byzantine, Lombardy, or Frankish tribes. Two horse graves and a plundered grave also appeared to be present.

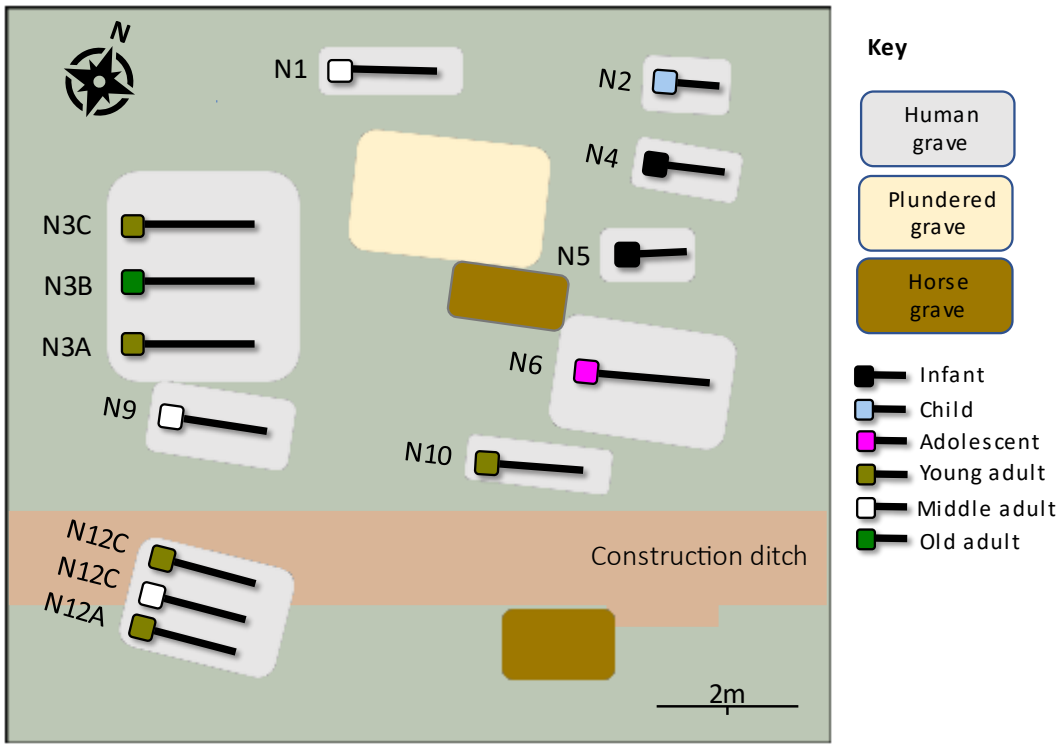

Figure 5.2 Diagram of the Niederstotzingen burial site. Based on Wahl et al. (2014).

This burial site was chosen for its relatively high level of preservation and the extensive archaeological data collected and analysed by Wahl et al. (2014) and O'Sullivan et al. (2018). Combining biosocial data collected from both studies provided comprehensive age-sex profiles for all 13 individuals as well as genetic and archaeological data that could act as markers of potential relatedness.

The age-at-death estimations were obtained from Wahl et al.'s (2014) osteological analyses. For sub-adults, estimations were based on tooth eruptions and the level of epiphyseal closures (Sicher & Tandler, 1928; Krogman, 1955). Wahl et al. (2014) also used the level of cranial suture fusion and tooth crown abrasions to assess the skeletal ages of the adult individuals (Vallois, 1937; Todd & Lyon, 1924; Nemeskéri et al., 1960). The genetic sex of the individuals was determined by O'Sullivan et al. (2018) through a genetic analysis of the Y-chromosomes.

Biosocial data were included as 'ties' in the social network if their analysis was available for most of the population sample and they represented a potentially meaningful marker of relatedness, as discussed in Section 3.3.4. From the archaeological report of Wahl et al. (2014), I was able to extract information regarding the presence of shared burials,

associated grave goods, skeletal trauma, linear enamel hypoplasia (LEH), and caries for the 13 individuals. O'Sullivan et al. (2018) determined the individuals' cultural affiliations and genetic relationships up to the second degree based on NGS and examined locality status based on oxygen and strontium isotope data derived from tooth enamel.

5.2.2 Data Preparation

Social network analysis studies the relationships among social units (Borgatti, 2002a). In this study, the social units are the 13 individuals from the burial site at Niederstotzingen. These individuals were referred to as 'nodes.' The relationships represented by lines connecting individuals are called 'ties.' Because social networks investigate the similarity between social entities, an adjacency matrix was made to show shared markers of relatedness (Appendix B).

Each of the markers was scored on a binary scale to generate an inter-individual similarity matrix and combine the quantitative and qualitative data. The presence or absence of a shared proxy of relatedness, or 'tie,' was scored as 1 or 0, respectively (Table 4.2.1). This required grave goods to be placed into five coded categories based on their related activity or purpose, which was done as follows: 1 = weaponry (arrows, saex, shields, swords, lances, and lamellar armour), 2 = horse-riding equipment (bridles), 3 = belts, and 4 = jewellery (gold rings and pearls). Binary data is also required for analyses such as cliques. Using UciNet software, I joined the different matrices to form a single multilevel matrix that combined all the relatedness markers into a visually adjustable network.

Table 5.1 Criteria Used to Determine Pairs' Binary Similarity Scores for Each Potential Marker of Relatedness

Potential marker of relatedness	Shared marker (1)	No shared marker (0)
Burial context	The individuals are interred in the same grave	The individuals are interred in separate graves
Grave goods	The individuals share the same type of grave goods	The individuals are associated with different types of grave goods
Skeletal trauma	The individuals both exhibit evidence of trauma in the skeleton	Skeletal trauma not evident in both individuals
Linear enamel hypoplasia	The individuals both exhibit LEH	LEH not evident in both individuals
Caries	The individuals both exhibit dental caries	Dental caries not evident in both individuals
Cultural affiliation	The individuals are associated with grave goods with the same cultural identifying markers, i.e., ornamentation style	Pair of individuals associated with grave goods with different or no cultural identifying markers
Genetic relationship	The individuals are close genetic relatives, i.e., genetically related to at least the second degree	The individuals are distantly related or not genetically related, i.e., have less than second degree relatedness
Locality	The individuals show strontium isotope ($^{87}Sr/^{86}Sr$) and oxygen ($\delta^{18}O$) isotope values that signal common geographic origin, i.e., local or non-local signal	The individuals show strontium isotope ($^{87}Sr/^{86}Sr$) and oxygen ($\delta^{18}O$) isotope values that signal different geographic origin, i.e., local or non-local signal

5.2.3 Data Visualisation and Analysis

Visualisation of Network

The overall structure of the network was visualised using NetDraw (Borgatti, 2002b). For clarity, the graph-theoretic layout was used as the overlap of nodes and lines was reduced (Hanneman & Riddle, 2011). To determine pairwise connectedness between individuals, ties were weighted according to the number of markers they shared. Visualisation of the relationships between individuals was also used to make informal estimations regarding the individuals more likely to be related.

Identification of Potential Kin Groups

Potential kin groups were identified using sub-group analysis and hierarchical clustering analysis, which could then be evaluated and contextualised based on the untransformed biosocial data. These data were used to test the hypothesis that genetic and bioarchaeological markers were randomly distributed across the network. The alternative model, therefore, was that some individuals shared more markers with each other than with others in the site. As social networks are sensitive to how data is dichotomised, I compared the analytical results between different cut-off points in the formation of the binary data (Johnson, 2016).

Sub-groups represent individuals who are most similar or share the most 'ties' to each other in comparison to those outside the group (Marin & Wellman, 2011). I used the clique analysis to find sub-groups that might potentially represent kin groups. Clique analysis finds clusters of individuals where every possible pair is directly connected by a 'tie' (Borgatti et al., 2002). Therefore, every individual in a clique will share a direct marker with every other clique member, making it a suitable tool for estimating groups of relatives. The calculations for cliques in UciNet use the Bron and Kerbosch (1973) algorithm to find completely connected cliques that represent potential kin groups. I used a minimum clique size of two, which can capture potential kin groups of two individuals or more. The UciNet calculations also included an analysis of patterns of overlap, which meant that individuals present in more than one clique could be identified. This allowed individuals present in more than one kin group to be recognised and patterns of connectedness between sub-groups to be considered.

Hierarchical cluster analysis was also applied to the matrix in UciNet as an additional means for identifying and visualising potential closely related individuals. UciNet implements Johnson's (1967) hierarchical clustering analysis based on a similarity matrix. This algorithm produces hierarchically nested groups of individuals according to their levels of pairwise similarity, or number of shared markers. Initially, individuals are in different clusters. The most similar pairs are then successively joined together until all individuals have been included. By defining the distance between clusters as the weighted average between pairs, clusters that are calculated to have the largest average similarity value weighted by cluster size are combined. A dendrogram was used to visualise the hierarchical relationship between clusters of individuals. This generated groups of individuals who shared the most markers of potential markers of relatedness that might be indicative of kin groups.

5.3 Results

5.3.1 Network Visualisation

Shared Burial

As evident from initial observations of Niederstotzingen, there were two multiple burials containing three individuals each (Fig. 5.3). One burial contained individuals N3A, N3B, and N3C, with N3A and N3C estimated to have been 20–30 years old at death and N3B estimated to have been 50–60 years old (Wahl et al., 2014). The other triple burial contained individuals N12A, N12B and N12C. N12A was estimated to have been 25–35 years old, N12B 30–40 years old, and N12C 20–30 years old at death.

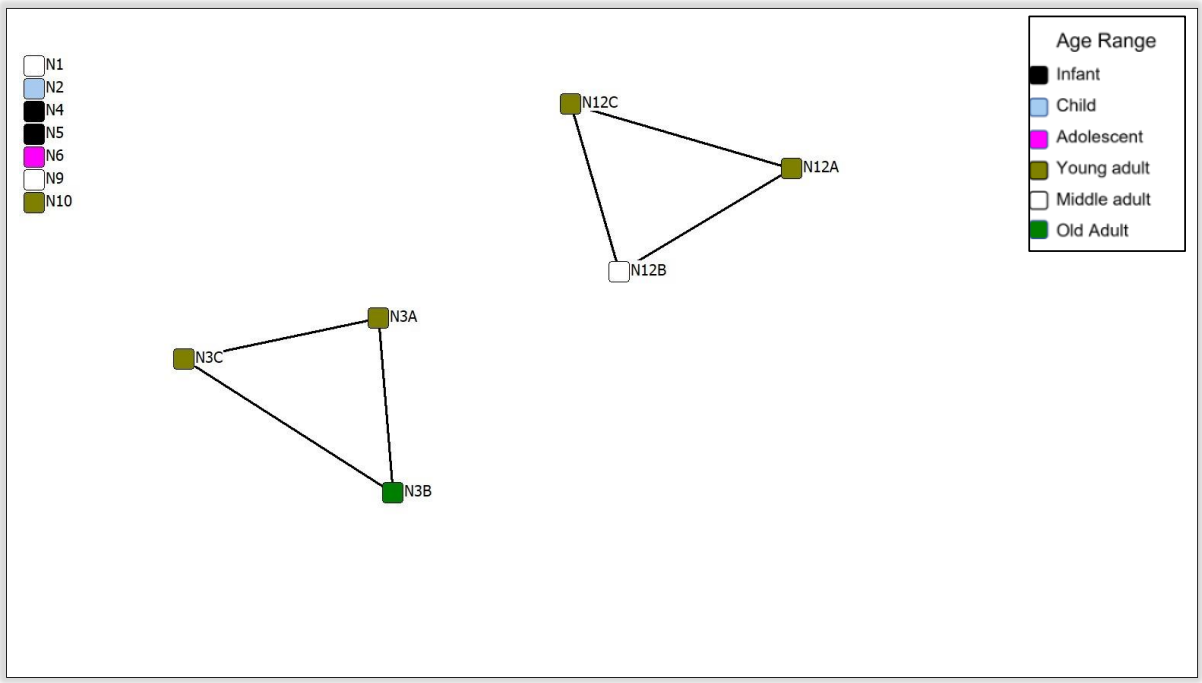

Figure 5.3 Visualisation of social network showing individuals at Niederstotzingen who were connected by a shared grave.

Grave Goods

The initial network based on grave goods showed that almost all individuals at the site were associated with at least some form of weaponry (Fig 5.4a). The only exceptions were individuals N4 and N5, who were both approximately two years old at death. They were instead both buried with belts, with infant N4 also being associated with a gold ring and pearls.

When the grave good 'warrior' category was divided into 'decorated' (three or more weapons) and 'normal' (one or two weapons) warriors, a more informative network was produced (Fig. 5.4b). Three apparent groups of individuals were produced: belts (individuals N4, N5, and N6), 'normal' warriors (individuals N2, N6, N10, and N12C), and 'decorated' warriors (individuals N1, N3A, N3B, N3C, N9, N12A, and N12B). However, subsequent clique and hierarchical clustering analysis was unaffected by the additional warrior category.

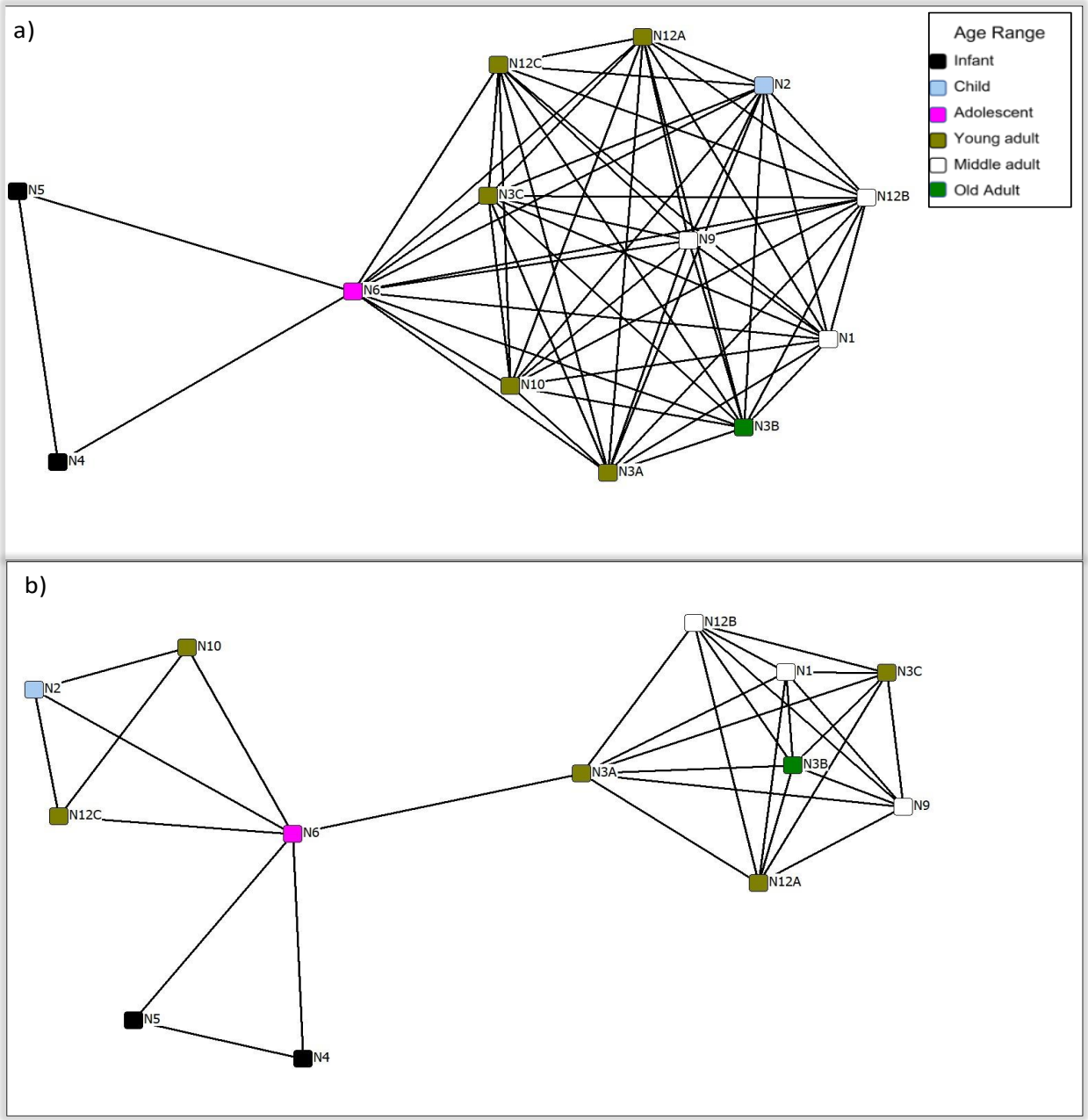

Figure 5.4. Visualisation of social network showing individuals at Niederstotzingen who were: a) connected by the same grave goods; b) connected by the same grave goods with the weaponry category divided into 'normal' and 'decorated' warriors.

Cultural Affiliation

Only three pairs of individuals shared cultural affiliations based on their associated grave goods (Fig 5.5). These pairs of individuals all shared Byzantine-style grave goods. N3A had a bridle with Byzantine-style ornamentation, whereas both N12A and N12B had Byzantine-style lamella armour.

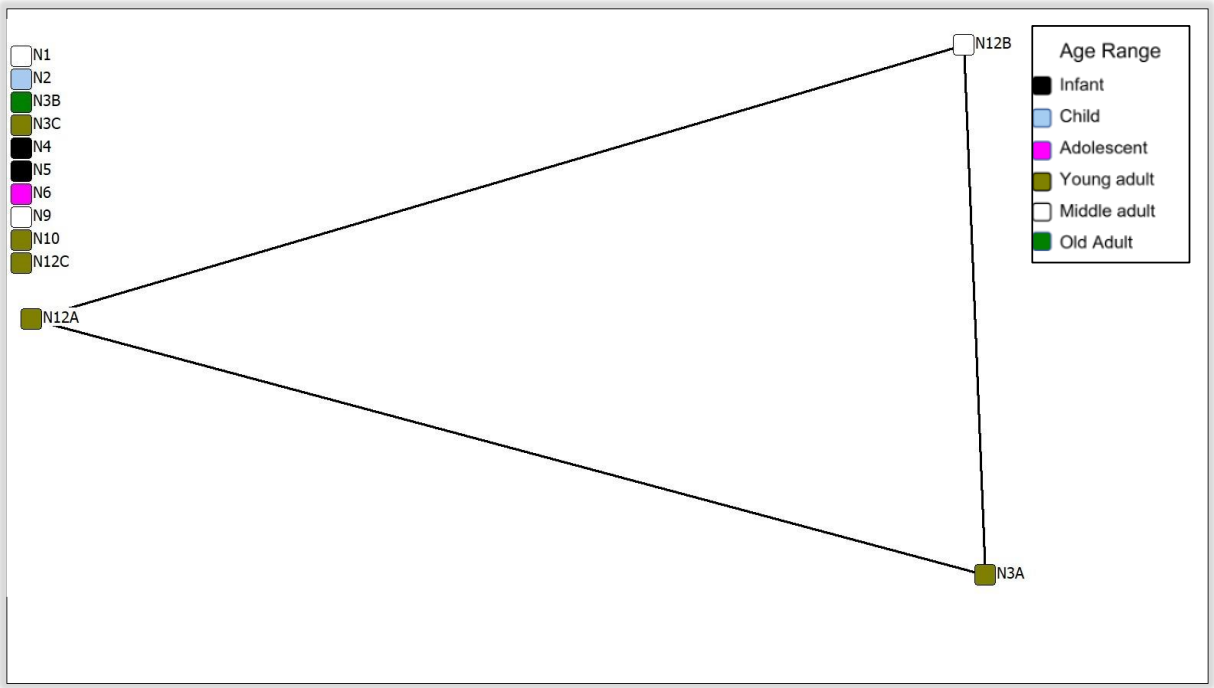

Figure 5.5 Visualisation of social network showing individuals at Niederstotzingen who were connected by the same cultural affiliation.

Dental Caries

Only three individuals exhibited dental caries (Fig. 5.6). They were of varying ages. Individual N2 was estimated to have died at 9–11 years old, individual N3A at 20–30 years old, and N3B at 50–60 years old.

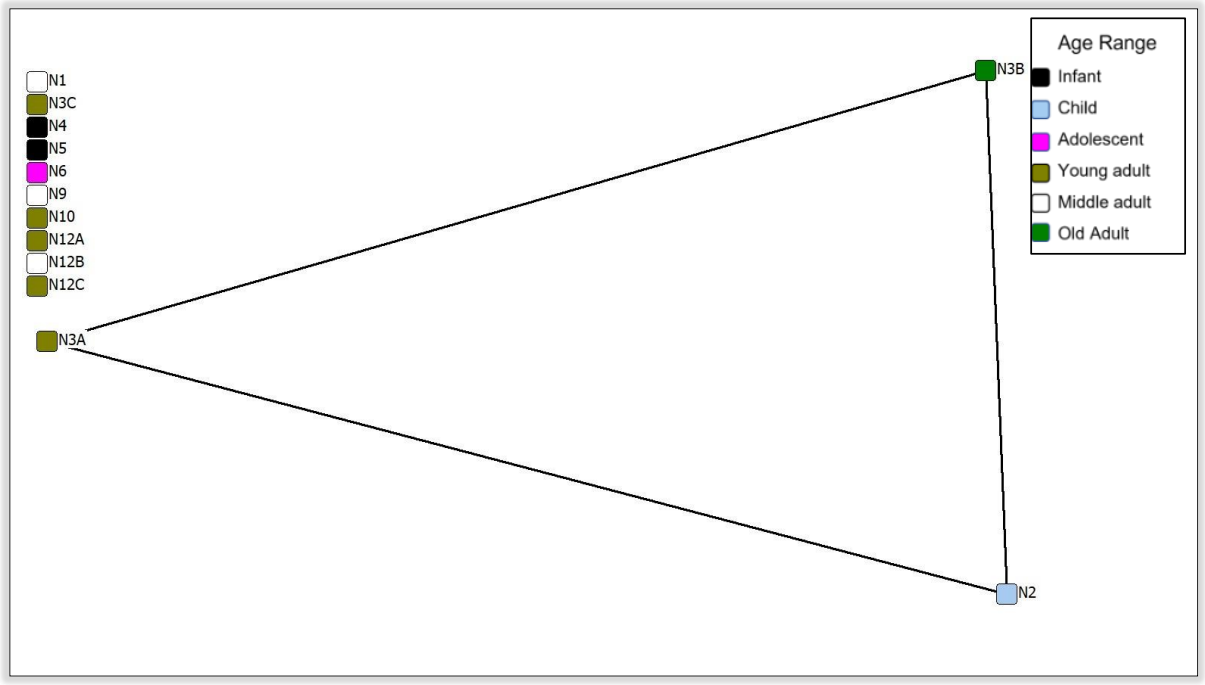

Figure 5.6 Visualisation of social network showing individuals at Niederstotzingen who exhibited dental caries.

Skeletal Trauma

Four individuals appeared to have received skeletal injuries during their lifetimes (Fig 5.7). Individuals N1 and N9 both appeared to have healed fractures on their right tibias. N3B and N6, both 14–17 years old, appeared to have suffered blows to their craniums.

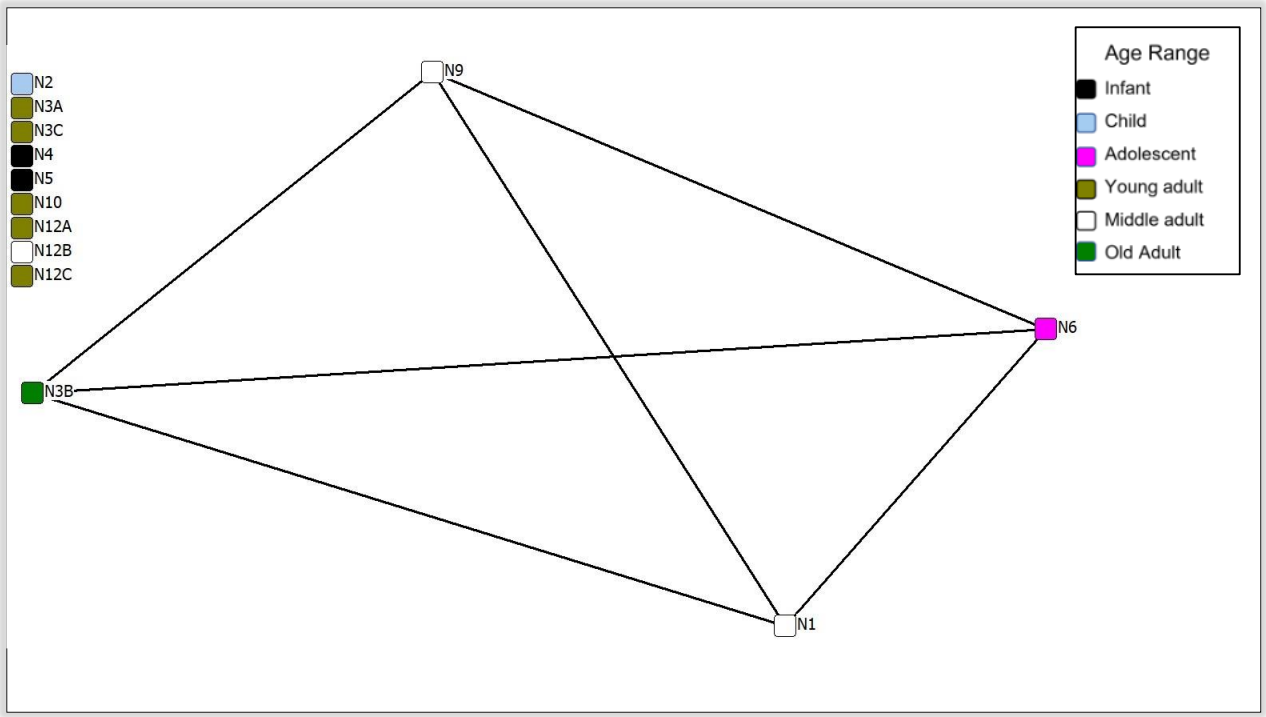

Figure 5.7 Visualisation of social network showing individuals at Niederstotzingen who experienced skeletal trauma.

Linear Enamel Hypoplasia

Seven of the 13 individuals exhibited LEH in their teeth (Fig. 5.8). All of these individuals were estimated to be at least young adults. However, individuals N1 and N9, who were both estimated to be 40–50 years old when they died, did not appear to have any LEH.

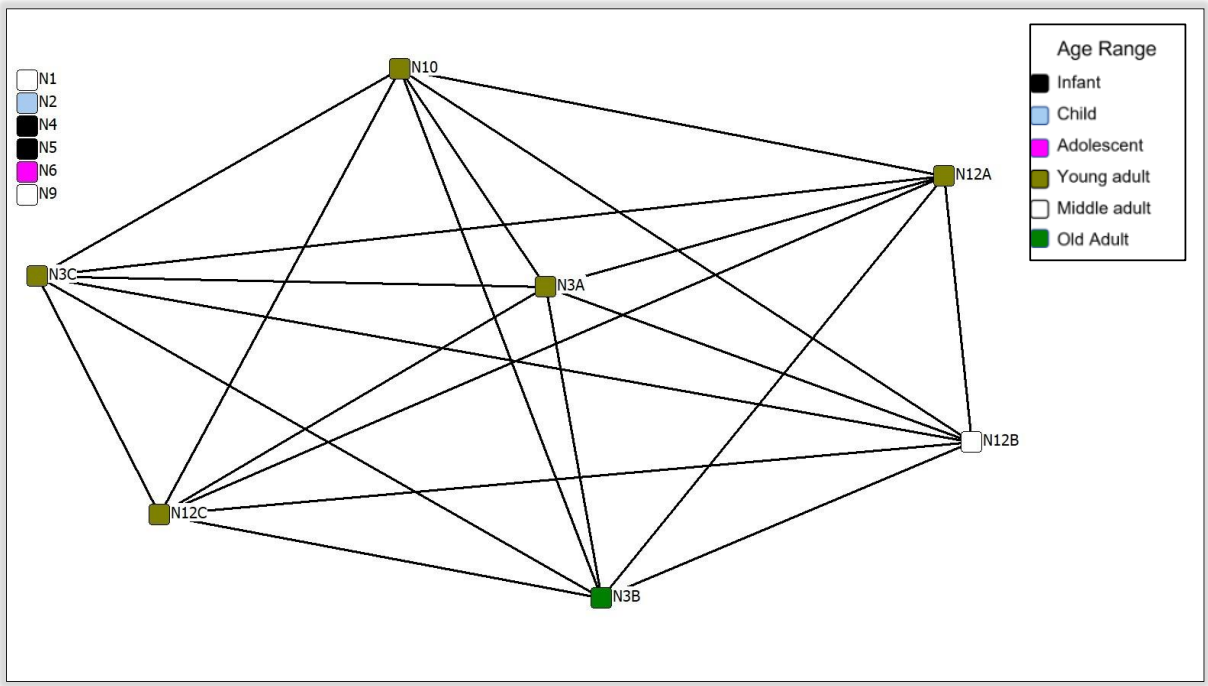

Figure 5.8 Visualisation of social network showing individuals at Niederstotzingen who exhibited linear enamel hypoplasia.

Genetic Relatedness

Nine pairs of individuals appeared to be genetic kin to at least the second degree (Fig. 5.9). Pairs N1 and N6, N1 and N3A, N1 and N9, and N9 and N12B all appeared to have first-degree genetic relationships based on autosomal SNP data as analysed by O'Sullivan et al. (2018). N1 and 12B, N3A and N6, N3A and N12B, and N6 and N9 each shared second-degree genetic relationships.

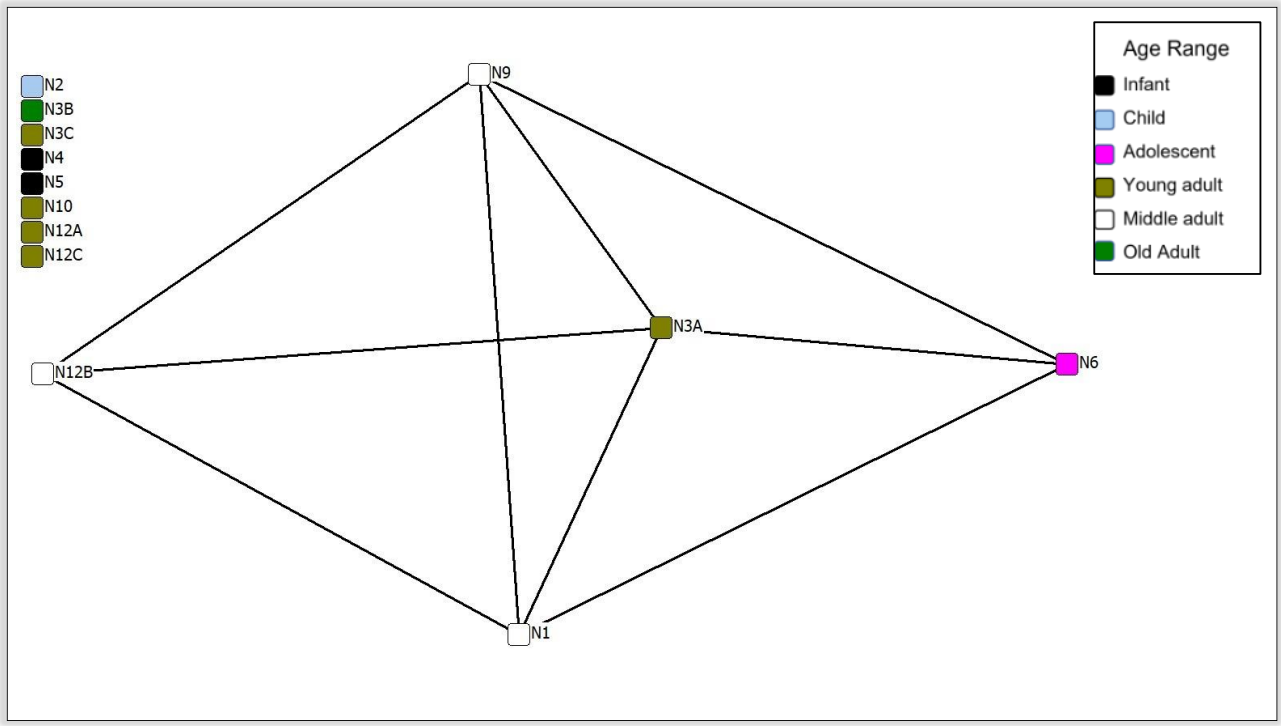

Figure 5.9 Visualisation of social network showing individuals at Niederstotzingen who genetically related to at least the second degree.

Locality

All but two of the 13 individuals at Niederstotzingen showed tooth enamel $^{87}Sr/^{86}Sr$ isotope (min = 0.7089, max = 0.7105) and $\delta^{18}O$ isotope (min = -5.5, max = -7.13) values that were consistent with a local geographic origin (Wahl et al., 2014; Fig. 5.10). Individuals N10 ($^{87}Sr/^{86}Sr$ = 0.7104; $\delta^{18}O$ = -7.37), a 20–25-year-old male, and N3B appeared to have a similar non-local origin ($^{87}Sr/^{86}Sr$ = 0.7105; $\delta^{18}O$ -6.76). Wahl et al. (2014) suggested these two non-local individuals may have originated from a higher-altitude area, such as the Swiss-German Alpine foothills.

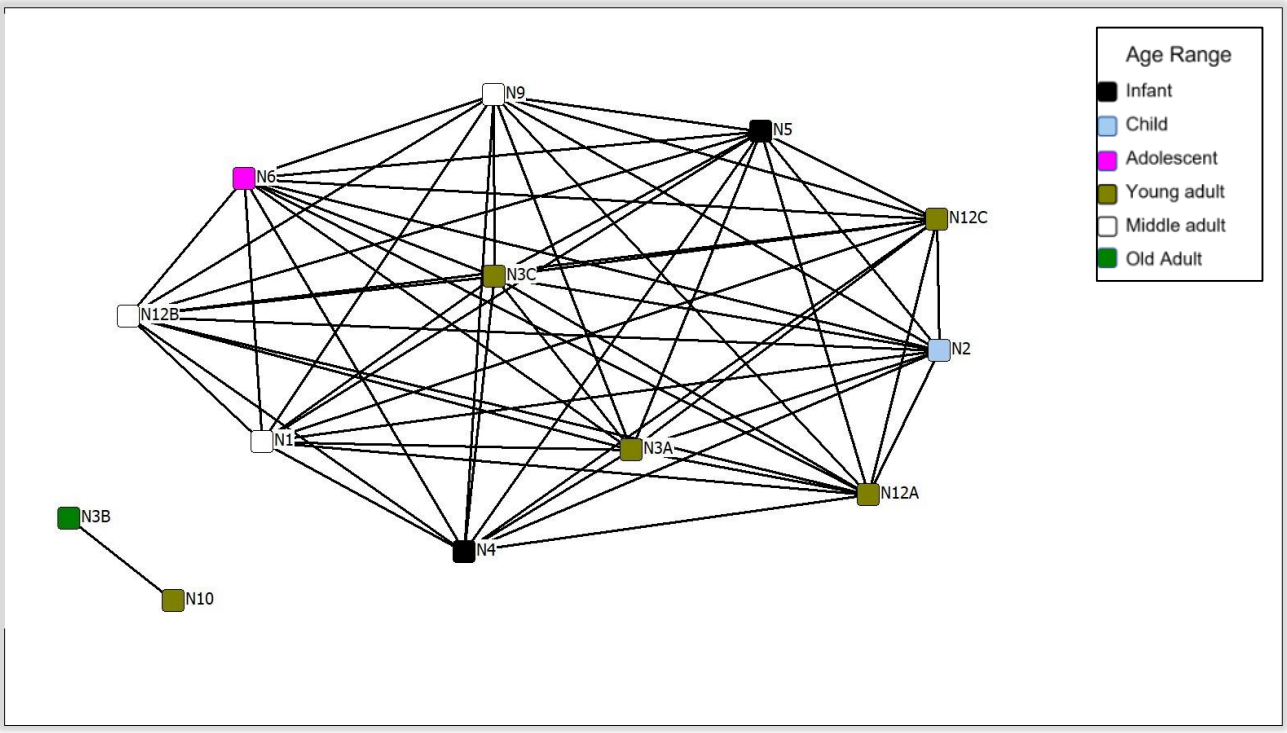

Figure 5.10 Visualisation of social network showing individuals at Niederstotzingen who were locals based on strontium and oxygen isotope analysis.

Overall Network Structure

The graph-theoretic layout of the number of ties shared between all individuals at this Alemannic burial site exhibit presents a relatively cluttered network, as all individuals shared at least one marker of relatedness (Fig. 5.11a). There did appear to be a be a core of more similar individuals – particularly N3A and N12B and N12A and N12B, who each shared five markers of relatedness.

The clutter and overall connectedness of the network was reduced when locality was not included, and notably, the infants N4 and N5 were only connected to each other and adolescent N6 through their similar associated grave goods (Fig. 5.11b). The uniformity of the locality status of most individuals, as well as the incorporation of individual N3B into a multiple burial makes it seem reasonable that the locality status of individuals was unlikely to have played a major role in kin formation. Therefore, its removal should not significantly impact the interpretive power of the potential kin relationships revealed among this population. It simply reduced the similarity between non-local individuals N10 and N3B.

The groups of individuals who shared the most markers of relatedness clearest in Figure 5.11c, which only shows the connections between individuals with at least three potential markers of relatedness. Visual observation of this network displays three groups of individuals (N1, N6, and N9; N3A, N3B, and N3C; and N3A, N12A, N12B, and N12C) in Niederstotzingen that shared the most measured markers of potential relatedness. These individuals were potentially members of the same kin groups as defined by an inclusive model of kinship.

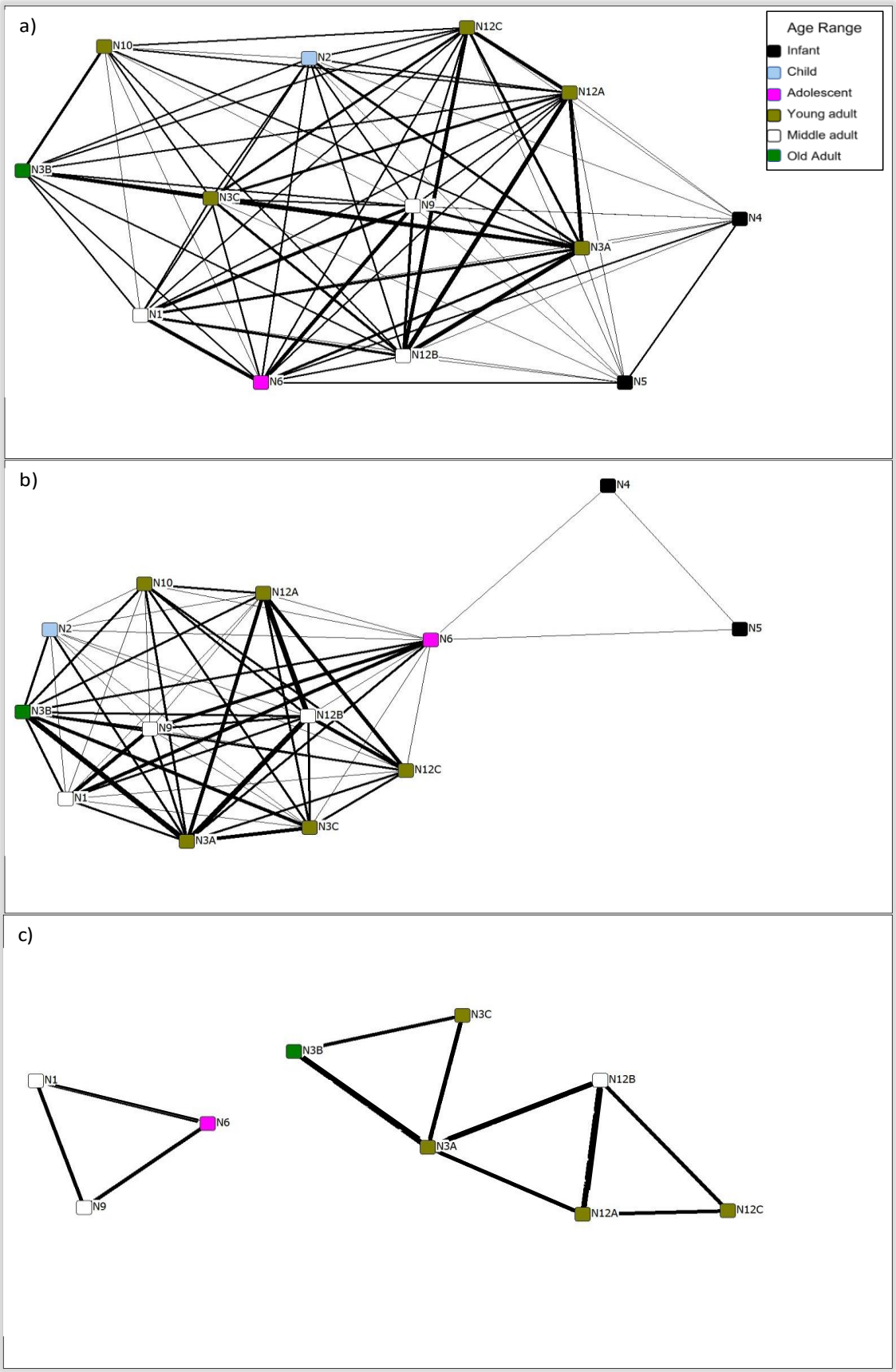

Figure 5.11 Social network displaying strength of connections (line thickness) between individuals at Niederstotzingen. a) Shows all ties between individuals; b) excludes locality as a tie; c) shows only individuals connected by at least three ties.

5.3.2 Potential Kin Groups

According to clique analysis, there was only one cluster of individuals in which all its members were more similar to each other than other members of the site population, representing a potential kin group. This group consisted of individuals N2, N3A, and N3B. All the individuals were genetically male, and their ages at death were estimated to be 9–11 years, 20–30 years, and 50–60 years old, respectively. They were genetically unrelated to individual N3B of non-local origin, and all three were associated with weaponry and had dental caries. Additionally, individuals N3A and N3B were buried in the same grave and both had LEH.

The hierarchical cluster analysis provided different potential kin groups than the clique analysis (Fig. 5.12a). The cluster analysis was largely in agreement with the informal visual analysis of the network, although individuals N3A, N3B, and N3C were not clustered together. There were two clear clusters of the most similar individuals sharing more than four ties: N1, N6, and N9 and N3A, N12B, and N12A. All individuals in these clusters appeared to be of local origin and were associated with weaponry, with only individual N6 associated with fewer than three weapons. N1, N6, and N9 were also all genetically related and appeared to have suffered traumatic skeletal injuries. N3A, N12A, and N12B all exhibited LEH and were associated with Byzantine-style grave goods. N3A and N12B were also second-degree genetic relatives, and N12B and N12A were buried together. Individual N12C might also be a member of this potential kin group as they had LEH and were buried in the same grave as individuals N12B and N12C.

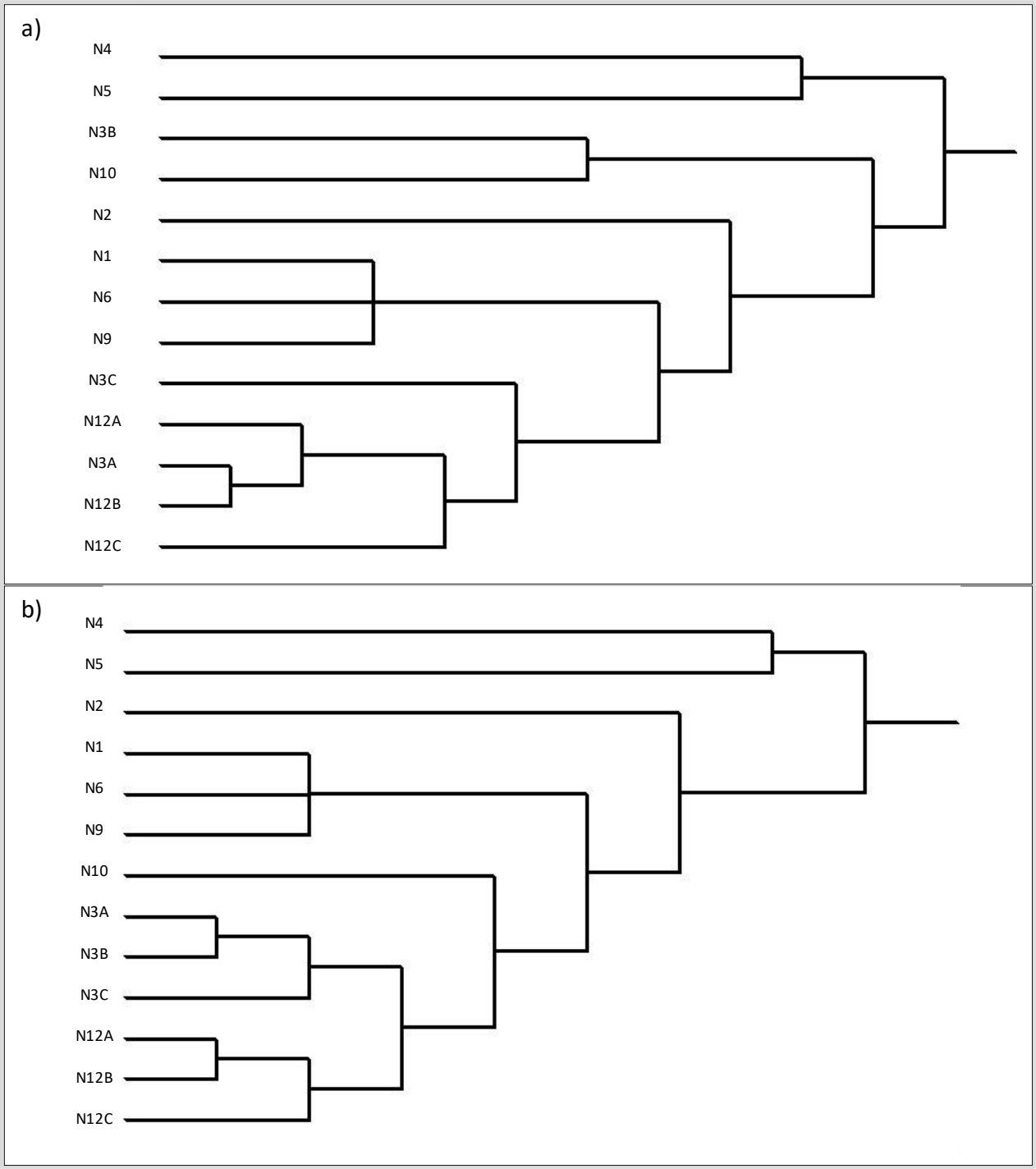

Figure 5.12 Dendrogram showing similarity between individuals at Niederstotzingen based on hierarchical cluster analysis: a) with all ties; b) with locality excluded.

Removing locality as a tie in the hierarchical cluster analysis provided slightly different potential kin groups, as the non-local individuals N10 and N3B were less similar to each other (Fig. 5.12b). The three potential kin groups based on individuals sharing at least

three ties also included a grouping of N1, N6, and N9. However, N3A, N3B and N3C and N12A, N12B, and N12C appeared to be two potential kin groups that were broadly clustered together. Both groups represented the two multiple burials observed at Niederstotzingen. All six individuals in both clusters exhibited LEH and were associated with weaponry grave goods. N3A and N3B also both had dental caries, whereas individuals N12A and N12B were associated with Byzantine-style grave goods.

5.4 Discussion

The results of the visualisation of the network, clique analysis, and cluster analysis suggest that at Niederstotzingen, proxies of potential relatedness were not distributed randomly among individuals. The patterns of similarity among the individuals also suggested that genetic relationships were not the sole means through which these people formed relationships in life. Interpreting the meaning behind these apparent connections and whether these similarities were likely indicative of kin relationships requires a more in-depth look at the specific relatedness markers, while also considering the possible kin formation processes that are likely to have existed in a mediaeval Alemanni tribe.

Previously, based on their genetic analysis and the age-at-death of these individuals, O'Sullivan et al. (2018) determined that five individuals were likely part of the same family tree (N1, N6, N9, N3A, and N12B; Fig. 5.13). Based on the degree of relatedness and age-at-death data, they suggested potential father-son relationships between N9 and N1, N9 and N3A, and N1 and N6. Potential sibling relationships were also suggested to exist between individuals N1-N3A and N9-N12B. Pairs N3A-N6, N1-N12B, N6-N9, and N3A-N12B may have been uncles or cousins. However, the inclusion of genetically unrelated individuals in multiple graves is suggested to potentially represent the equality of fellowship and genetic familial ties. It has been proposed that individuals might have been adopted as children and raised within the *familia* (Steuer, 1989). Wahl et al., (2014) mentioned several possible explanations for the multiple burials containing genetically unrelated individuals. One explanation offered for the joint burial of genetically unrelated individuals was a common geographical origin. It was also considered that they were retainers or cupbearers following their military officers into death. This was based on observations of the Viking Age ship-

burial at Hathebu, where a well-equipped individual was buried with two others (Shenk, 2002; von Carnap-Bornheim & Hilberg, 2007).

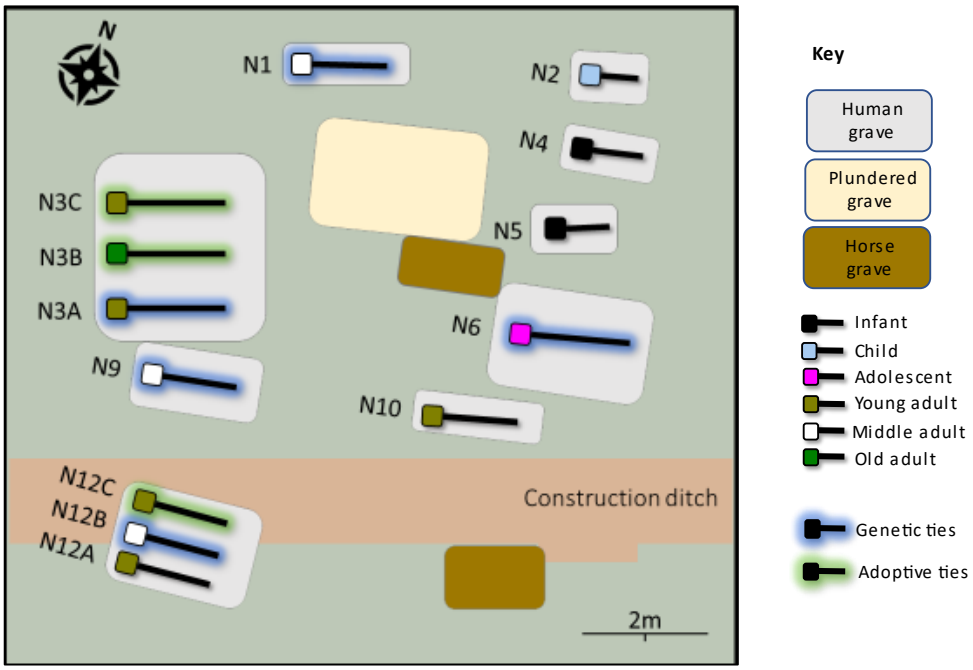

Figure 5.13 Diagram of Niederstotzingen showing kin group based on genetic analysis and burial location (O'Sullivan et al., (2018).

The potential kin groups identified in this study by incorporating biosocial data produced contrasting results compared with studies focused on genetic connections (Fig. 5.14). However, the groups also varied depending on the type of analysis and data used. Clique analysis identified one group of individuals as more connected to each other than others in the population (N2, N3A, and N3B). These individuals included a child (N2), a young adult (N3A), and a non-local older adult (N3B). They were all genetically unrelated but appeared to be connected by their associated weaponry. Although individual N2 was only associated with a saex, individuals 3A and 3B were both associated with several weapons. They were both equipped with a lance, a saex, a shield, and a double-edged sword. 3A was additionally associated with arrows and a horse bridle. Importantly, they were the only individuals who exhibited dental caries. The main drivers in the occurrence of dental caries are aspects of consumed food, such as its consistency, preparation, and carbohydrate content, although there are alternative causes (Hillson, 2008). As such, it may be an important

indicator of more similar diets for these three individuals in comparison to others at the site. Commensality is considered an important kin formation process in many societies (e.g. Weismantel, 1995; Carsten, 1995; Dombrowski et al., 2013; see Section 4.3).

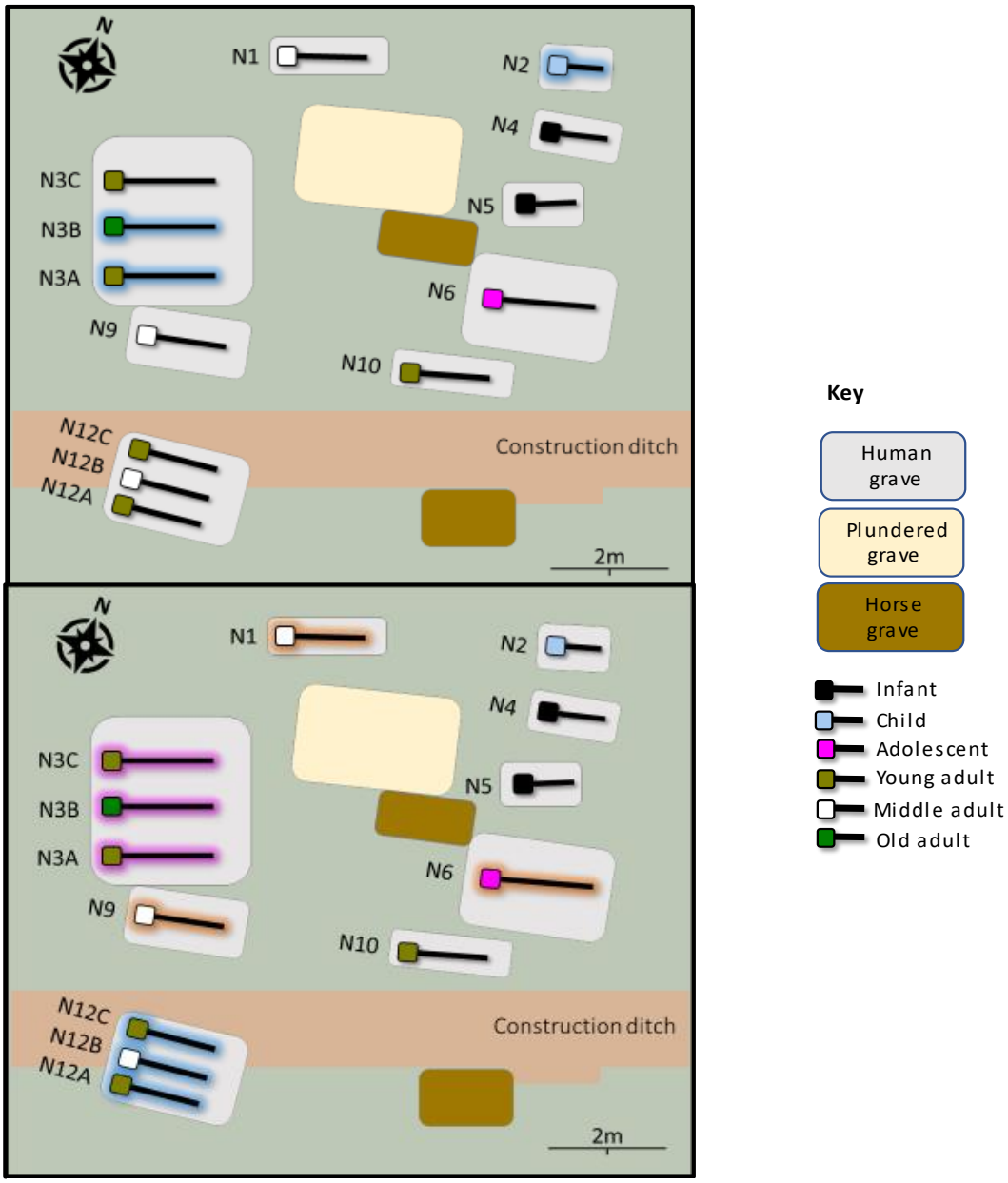

Figure 5.14 Diagram of potential kin groups at Niederstotzingen based on clique analysis (above) and cluster analysis without locality (below).

Although, commensality may not play a crucial role in Alemannic kin formation, the possible shared diet might also be indicative of co-residence between these individuals,

potentially through the adoption of genetically unrelated individuals (O'Sullivan et al., 2018). Assuming that the individuals died over a short time period, individuals N2, N3A, and N3B may represent the multi-generational incorporation of non-genetic individuals into a household or family. Consistently interacting, eating, and living together can play a role in forming important social and emotional bonds between individuals that might constitute kin (Sahlins, 2011).

Household membership, genes, adoption, brotherhood, and shared experiences may explain the clusters observed by cluster analysis when locality is removed as a 'tie.' Hierarchical cluster analysis, without locality status, provided three potential kin groups (Fig. 5.14). Those in the two multiple graves and genetically related individuals N1, N6, and N9. Each of these groups shared at least three ties which provide slightly different potential social processes through which kin relationships might have formed. The connections observed between these individuals serve to highlight how different biosocial data might be used to explore potential kin formation process that might have occurred in this Alemannic population. Individuals at this site may have considered themselves related through commensality, co-residence, genes, adoption, or shared experiences (Sahlins, 2011).

Household membership may have acted as the basis for kin formation (e.g. Carsten, 1995; Hill et. al., 2011). Modern West African adoption and fosterage practices may involve individuals leaving their natal households at birth or when weaned, or in their adolescence (Goody, 1982; Silk, 1987). In their foster households, genetically unrelated individuals are nurtured, fed, socialised, and educated together. The children are active participants in the household economy and are trained in the trade of their foster parents (Goody, 1982). Similar processes are potentially indicated by the potentially shared status and physico-social environments identified based on grave goods, skeletal trauma, dental caries, burial locations, and cultural affiliations.

All individuals in these three potential kin groups were associated with weaponry. Only individuals N6 and N12C were associated with fewer than three weapons. Grave goods may have been the possessions of the deceased, and may also have been indicators of status or position (Lopez-Costas, 2015). In the Merovingian context of Niederstotzingen, both may have been the case. As there may have been a military function to the burial site, the function of the weaponry in these burials may have been expressing the status of the warriors (Steuer,

1989). They may, therefore, have all shared the same military occupation, which fits the apparent military context of the site. The distinctions between the three groups came from their genetic relationships and potentially different physico-social environments suggested by their skeletal trauma, LEH, dental caries, burial locations, and cultural affiliations.

Potential kin group N1, N6, and N9 was made up of three locals who were genetically related: two middle-aged adults (N1 and N9) and an adolescent (N6) (Fig. 5.14). The fact that the genetic relationships were not obscured by their equivalence to archaeological data during the analysis suggests that there was some level of congruence between genetic and archaeological data. N1 was identified as being a first degree relative of both N6 and N9, with N6 and N9 showing a second-degree relationship (O'Sullivan *et. al.,* 2018). N1, N6, and N9 also showed evidence of potential skeletal trauma (Wahl *et. al.,* 2014). N1 and N9 were reported to have had healed fractures in their right tibias, with periostitis potentially associated with the right tibia. N6 had a potential slash injury to his frontal bone. This could be indicative of a shared occupation and life experience. There are several potential explanations for the shared presence of tibial fractures, such as violence, falling from a height, or repetitive force (Lovell, 1997). Several explanations fit well with the military context of the site and the presence of associated weaponry for these individuals. The obvious suggestion is a violent injury, but other military experiences such as horse-riding and marching are potential causes of tibial fractures (Lovell, 1997). For these three individuals, the three generations of potentially military men may have had similar military life experiences that were expressed in the presence of trauma and grave goods. Although these relationships were, perhaps, founded in their genetic kinship, they did not end with it. The occupation and life experiences they appeared to share speaks to social processes, such as working and potentially suffering together, maintaining kin relations (*e.g.,* Nuttal, 2000).

Although genetically related, the cluster analysis separated individuals N3A and N12B from their genetic relatives N1, N6, and N9 (Fig. 5.14). Instead, they were clustered more closely with unrelated individuals: N3A to N3B and N3C and N12B to N12A and N12C. It would appear, therefore, that the link between genetic kinship and social constructions of kinship may not be straightforward, and non-genetic kinship might play an equal part in the formation of close kin bonds.

Individuals N3A, N3B, and N3C were a group of genetically unrelated individuals who were buried together. This group included individual N3B, an old adult male who may

have originated in the Swiss-German Alpine foothills. Not only did they share similar grave goods, but they all exhibited LEH. The presence of LEH may reflect an important indicator of elevated stress in an individual's lifetime, as discussed in Section 4.3. Although not used in the analysis, individuals 3C and 3B also exhibited cribra cranii, which has been associated with anaemia. These markers could represent exposure to the same physiological stressors (Larsen, 2002). The dental caries in two of the three individuals are indicative of a shared diet. The resemblance between these individuals, therefore, might represent a shared environmental exposure to poorer conditions and meals due to living in the same household (e.g. Baranwal et al., 2014). The shared burials might support this as a significant indicator of an individual's important connections in life (Ensor *et. al.*, 2017).

Similarly, individuals N12A, N12B, and N12C might also have been members of the same household and experienced bonds based on exposure to similar physico-social environments. They all exhibited LEH, which broadly groups them with individuals N3A, N3B, and N3C. However, the separation of these two groups into two different graves might be indicative of their social or emotional bonds in life (Ensor *et. al.*, 2017). They may have also been separated due to a temporal gap between their deaths. Nevertheless, the potential social significance for individuals within each grave remains. The exposure to similar environmental stressors is supported by the fact that both individuals N12A and N12B tested positive for tuberculosis (Wahl et al., 2014). In addition, both N12A and N12B were associated with Byzantine-style armour (O'Sullivan et al., 2018). The association of grave goods with the same cultural affiliation might have any number of explanations, including a similar status or position (Lopez-Costas, 2015). In Merovingian society, it has been suggested that grave objects may have been a means to maintain family identity and status and differentiate individuals from others (Kars, 2011). Taken together with the shared grave, LEH, and tuberculosis, there is some support for the suggestion that these individuals might have experienced the same physico-social environment in life, which is more likely to be shared among households or family members. The important relationships potentially indicated by the shared graves, therefore, may have been constructed through the process of living together, eating together, and suffering together (Sahlins, 2011).

Common to all the identified potential kin was the potential for shared occupation, experiences, and potentially household membership. Both the clique and cluster analysis provided evidence of close bioarchaeological associations between genetically unrelated individuals that appear as significant as those between genetically related individuals. This

suggests that there is a flexibility in the link between genetic and realised kinship. Genealogy may not have been essential for these individuals to create familiar bonds. The *familia* household structure that is assumed to be present at the site supports such a connection (O'Sullivan *et. al.,* 2018). Unrelated and related individuals may have been closely associated and part of the same family, such as through adoption or fellowship, uniting for a common purpose. The addition of the biosocial data to the analysis supports the inclusion of non-genetic and genetic relatives in the same family unit while also providing indications of the social for the social processes that might have formed these bonds.

Adoption was widely practiced in ancient European societies (Huard, 1955). It is described to have occurred in ancient Greek states, Roman societies, and Celtic clans (Huard, 1955; Parkes, 2006; Heubner, 2013). Therefore, it would not be surprising for adoption to have occurred in this Alemannic tribe. Mediaeval adoption practices varied culturally. In the ancient Mediterranean, adoption could occur for several reasons: to provide a childless couple or man with an heir, to provide an abandoned child with a home, to secure support in an individual's old age, to maintain a household across generations, or to form connections between high-status families (Heubner, 2013). Such reasoning could explain the potential kin group (N2, N3A, N3B) which may have spanned three generations. The sub-group might, therefore, represent the adoption of genetically unrelated individuals intro a household to secure the continuation of a lineage or household.

In mediaeval Wales, freeborn children were often fostered by local lords, where they were trained and formed retinues of foster siblings (Parkes, 2006). In mediaeval Ireland, there was a long practice of fostering invaders, such as the Vikings, for assimilation. In late-antiquity Byzantium, through ritual kinship practices such as swearing a formal oath or serving in the military together, men might come to call each other 'brother' (Rapp, 2016). Alemannic individuals appear to have differentially shared physical dangers, exposure to stress, and disease, as well as a social position. In the military context of Niederstotzingen, the shared training of individuals may have resulted in sub-groups of foster-siblings. Bonds formed through the shared experience and common military goal could have also formed a similar type of brotherhood like the Byzantines. The apparent maintenance of non-genetic bonds was potentially maintained in the afterlife through shared burials is in line with such an explanation (Hutchinson & Aragon, 2002).

Although, it is unknown whether the cultural logic described in these other mediaeval populations are representative of Alemannic society, the examples help form hypotheses to explain the patterns observed in the biosocial data (Ensor, 2016). The apparent shared life conditions and social bonds, based on the non-random distribution of biosocial markers, provided more in-depth insight into the lived experiences that might have formed the potential kin-bonds beyond the previously suggested genetic and adoptive ties. Common to all of these was the potential for a shared occupation, training, and household membership that could have been indicative of adoptive bonds or brotherhood. The clique and cluster analysis provided evidence of close bioarchaeological associations between genetically unrelated individuals that appear as significant as any of those between genetically related individuals. This suggests that there is a flexibility in the link between genetic and realised kinship. In addition, it suggests that genealogy was not always essential for individuals to create familiar bonds.

5.4 Limitations of Social Network Analysis

It is important to note that the results are not intended to produce an exhaustive conclusion about the kin relations among the individuals at Niederstotzingen. To attempt to do so would be likely produce misleading results. First, the creation of the social network required all the data to be dichotomised, meaning that nuance and detail regarding the similarities and differences between individuals was lost. For example, the amount of weaponry associated with each person was lost. Therefore, it is vital to return to qualitative archaeological data when interpreting any possible kin groups based on statistical analyses. Second, the role of age and sex on the distribution of biosocial markers cannot be overlooked. If age or sex plays a role in the acquisition of certain markers, then it is possible that individuals could end up grouped by age or sex rather than potential kin relations. Third, archaeological data will always be limited by the level of preservation. Markers of potential relatedness, therefore, are likely to be missing in some individuals. Hence, interpretations may exclude individuals as potential kin due to preservation differences. Fourth, although Niederstotzingen was active from ~580–630 C.E., the individuals were assumed to have died over a relatively short period of time (O'Sullivan et al., 2018). For some individuals, such as

those in multiple graves, this is more likely. However, there is potential for generational gaps to have existed between burials. Similarities between individuals are unlikely to represent a kin relationship if they are temporally removed from each other. More specific dating techniques for each individual, such as radiocarbon dating, might reduce such concerns in future research. Ultimately, this social network was not intensively rigorous. Instead, it produced a simple reconstruction of the potential kin groups at Niederstotzingen to explore the types of conclusions that can be drawn by incorporating biosocial archaeological data.

5.5 Conclusion

The incorporation of a social network model that equalised genetic and bioarchaeological data allowed me to form hypothetical kin groups with no knowledge of which markers of relatedness were being used to suggest close associations. This meant that there was no genetic framework around which ideas were being shaped, even unconsciously. The main challenge was determining whether the dichotomisation of the data was justifiably representative of the different proxies for relatedness. Not only did the data produce non-random patterns of similarity between individuals, but the patterns were also potentially meaningful. Although the cluster analysis blindly captured a genetic kin group, the individuals at the site shared too many overlapping bonds to capture more extended bonds with the clique algorithm. The cluster analysis provided disparate but rather complementary patterns of similarity between individuals. It was able to combine the many paths of relatedness into a workable hypothesis about the social bonds in a mediaeval Alemanni tribe. The hypotheses was produced using patterns of biosocial data, which means it is possible to interpret potential kin formation processes by incorporating a wide variety of data. This study was not intended to be a thorough investigation of Alemannic culture at Niederstotzingen, and therefore the conclusions are not as rigorous as they would be in a full-scale piece of primary research. Instead, this basic statistical model was meant to demonstrate alternative hypotheses of relationships that might be developed by integrating multidisciplinary data and unbiased thinking.

Chapter 6. Conclusion

This is the first study which has presented a detailed systematic review of the data, methods, and findings of previous bioarchaeological research reconstructing kin relationships between individuals. The purpose of this study was to establish the importance of implementing a methodology that incorporates both biological and social data for unbiased kinship analysis in bioarchaeology. Therefore, this thesis conducted a systematic review of 63 primary research papers which aimed to identify kin relationships in past populations. This thesis demonstrated how biosocial data might be integrated to interpret potential kin relations by completing a simple trial social network analysis using secondary data.

First, this quantitatively determined a considerable gap between sociocultural concepts of kinship as variable and socially constructed and the models of kinship used in bioarchaeology. The implication for the identification of related individuals is serious. Prioritising genetic relatedness may result in the post-natal social processes involved in kinship formation being overlooked and potentially imposing culturally biased constructs of kinship. Second, the study reveals important limitations in genetic analyses often overlooked when detecting kinship in the past. DNA analysis is presumed to yield an inherently accurate and objective estimate of kinship, but by challenging that assumption, the gap between DNA and bioarchaeological data might be closed and integrated into future research to capture more inclusive forms of kinship. And third, this thesis identified potential markers of relatedness embodied in the archaeological record that future researchers could use to identify more inclusive kinship bonds. Despite its simplicity, the trial social network analysis of secondary data combined genetic and non-genetic markers to develop hypotheses that provided in-depth insights into lived experiences and social processes that could have contributed to the patterns observed in biosocial data. Therefore, the trial statistical model presents a workable baseline from which future studies can be developed.

Bioarchaeology remains in the past when conceptualising kinship in past societies. Most studies aiming to reconstruct kin relationships use primarily genetic ties between individuals. Though there has been some consideration of alternative forms of kinship, no studies have incorporated biosocial data without an underlying genetic framework as in this thesis's trial social network analysis. However, this type of analysis would be improved by including markers of relatedness supported by cohesion from several kinds of data. Future

research should integrate more data into this multidisciplinary approach to more closely examine kin relationships that may have existed in the past and provide rich interpretations of these relationships.

Appendix A

Data Retrieved from Primary Research for the Systematic Review

Author	Study Site Country	Kinship recognised as a cultural construct?	Justification for kinship model	Inclusive kinship model	Evidence used in inclusive kin detection	DNA markers used	Min. no. of nDNA markers
Fowler et al., 2022	United Kingdom	Yes		Yes	Burial proximity and extensive genetic pedigree	WGS	Yes
Zegarac et al., 2021	Serbia	Yes		Yes	Burial proximity	WGS	Yes
Erlikh et al., 2021	Russia	No		No		mtDNA	
Alteraug e et al., 2021	Germany	Yes	Historical evidence of family structure	No		STR; mtDNA; YDNA	No
Ning et al., 2021	China	No		No		WGS	Yes
Vai et al., 2021	Poland	Yes	Contemporaneous archaeological findings about funerary practices	No		WGS	Yes
Csaky et al., 2020	Germany	Yes	Contemporaneous archaeological findings about funerary practices	No		mtDNA; YDNA	
Verdugo et al., 2020	Belize	No		No		mtDNA	
Linderh olm et al., 2020	Poland	No		No		WGS	Yes
Mittnik et al., 2019	Germany	Yes	Historical evidence of family organisation	Yes	Burial location, strontium isotopes, and grave goods	WGS	Yes
Sanchez-Quinto et al., 2019	United Kingdom, Ireland, Sweden, Czechia	No		No		WGS	Yes
Schroed er et al., 2019	Poland	Yes		Yes	SNPs and burial proximity	WGS	Yes
Sjorgen et al., 2019	Germany	Yes	Contemporaneous archaeological findings; ethnographic analogues	Yes	Outlying Oxygen isotopes in comparison to genetic father	WGS	Yes
Mary et al., 2019	Russia	No		No		STR; mtDNA; YDNA	Yes

Drosou et al., 2019	United Kingdom	No		No		mtDNA	
Bus et al., 2019	Sweden	Yes		Yes	burial proximity (same grave)	mtDNA	
Fleskes et al., 2019	United States	Yes	Historical records of known individuals	No		mtDNA	
Olasz et al., 2019	Hungary	Yes	Historical records of known individuals	No		STR; mtDNA; YDNA	Yes
Chyleński et al., 2019	Turkey	Yes	Contemporaneous archaeological findings about funerary practices	Yes	Burial proximity	mtDNA	
Amorim et al., 2018	Hungary, Italy	Yes	Historical evidence of family organisation	No		WGS	Yes
O'Sullivan et al., 2018	Germany	Yes	Historical evidence of family organisation	Yes	Burial proximity, strontium, and oxygen isotopes	WGS	Yes
Deguilloux et al., 2018	France	Yes		Yes	Burial proximity and burial postion	STR; mtDNA	No
Rott et al., 2018	Bavaria	Yes		Yes	Burial proximity and burial postion	STR; mtDNA; YDNA	No
Serventi et al., 2018	Italy	No		No		STR; mtDNA	Yes
Mendisco et al., 2018	Argentina	Yes	Contemporaneous archaeological findings about funerary practices	No		STR; mtDNA; YDNA	Yes
Van de Loosdrecht et al., 2018	Morocco	No		No		WGS	Yes
Owsley et al., 2018	United States	Yes	Historical evidence of family structure	Yes	Shared mtDNA and shared burial vault	mtDNA	
Gomes et al., 2017	Spain	Yes		Yes	Burial proximity	mtDNA	
Juras et al., 2017	Poland	Yes	Contemporaneous archaeological findings about funerary practices	Yes	Burial location	SNPs; mtDNA	No
Kennett et al., 2017	United States	Yes	Historical evidence of family organisation; ethnographic analogues	No		WGS	Yes
Sikora et al., 2017	Russia	Yes		Yes	Burial proximity and radiocarbon dates	WGS	Yes
Pilipenko et al., 2017	Siberian Federal District	Yes		Yes	Burial proximity	STR; mtDNA; YDNA	No

Knipper et al., 2017	Germany	No		No		mtDNA	
Rott et al., 2017	Germany	Yes	Historical evidence of family structure	Yes	Shared grave	STR; mtDNA; YDNA	No
LeRoy et al., 2016	France	Yes	Contemporaneous archaeological findings	No		mtDNA	
Russo et al., 2016	Argentina	Yes	Historical evidence of family organisation	No		STR; mtDNA; YDNA	No
Hervella et al., 2016	Spain	No		No		mtDNA	
Alt et al., 2016	Spain	No		No		mtDNA	
Cui et al., 2015	China	Yes	Historical records of known individuals	No	Shared tomb	STR; mtDNA	No
Keller et al., 2015	Germany	No	Contemporaneous archaeological findings about funerary practices	No	Shared grave	STR; mtDNA; YDNA	No
Keyser et al., 2015	Russia	Yes	Contemporaneous archaeological findings about funerary practices	Yes	Burial proximity	STR; mtDNA; YDNA	Yes
Dong et al., 2015	China	Yes	Ethnographic analogues	No		mtDNA; YDNA	
Schwarz et al., 2015	Austria	Yes	Historical evidence of family structure; tombstone inscritions	No		STR; YDNA	Yes
Rothe et al., 2015	Berlin	No		No	Same burial	STR; mtDNA	Yes
Deguilloux et al., 2014	France	Yes	Historical evidence of family organisation	No		mtDNA	
Knipper et al., 2014	Germany	No		No		mtDNA	
von Grumbkow et al., 2013	Germany	No	Contemporaneous archaeological findings about funerary practices	No		STR; mtDNA; YDNA	No
Matney et al., 2012	Turkey	Yes	Contemporaneous archaeological findings; ethnographic analogues	No		mtDNA	
Baca et al., 2012	Peru	Yes	Historical evidence of family organisation	No		STR; mtDNA; YDNA	No
Byrnes et al., 2012	United States	Yes	Historical evidence of family structure	Yes	Burial proximity	mtDNA	
Overholtez, 2012	Mexico	Yes	Historical evidence of family structure	Yes	Burial proximity	mtDNA	
Gamba et al., 2011	Spain	Yes	Burial inscriptions	No		STR	No
Simon et al., 2011	Spain	Yes	Contemporaneous archaeological findings about funerary practices; ethnographic analogues	No		mtDNA	

Hawas et al., 2010	Egypt	Yes	Historical records of known individuals	No		STR; YDNA	No
Igawa et al., 2009	Canada	No		No		mtDNA	
Haak et al., 2008	Germany	Yes	Contemporaneous archaeological findings about funerary practices	Yes	Shared grave	mtDNA	
Bouwman et al., 2008	Greece	No		No		mtDNA	
Gao et al., 2007	China	No		No		mtDNA	
Gilbert et al., 2007	Greenland	No		No		mtDNA	
Mooder et al., *2005*	Siberian Federal District	No		No		mtDNA	
Keyser-Tracqui et al., 2003	Mongolia	Yes	Contemporaneous archaeological findings about funerary practices	No		STR; mtDNA; YDNA	No
Dudar & Saunders, 2003	Serbia	Yes	Historical evidence of family structure	No		mtDNA	
Gerstenberger et al., 1999	Germany	Yes	Historical records of known individuals	No		STR; YDNA	No

Appendix B

Distance Matrices for Social Network Analysis

Locality													
ID	N1	N2	N3 A	N3 B	N3 C	N4	N5	N6	N9	N10	N12 A	N12 B	N12 C
N1	0	1	1	0	1	1	1	1	1	0	1	1	1
N2	1	0	1	0	1	1	1	1	1	0	1	1	1
N3A	1	1	0	0	1	1	1	1	1	0	1	1	1
N3B	0	0	0	0	0	0	0	0	0	1	0	0	0
3C	1	1	1	0	0	1	1	1	1	0	1	1	1
N4	1	1	1	0	1	0	1	1	1	0	1	1	1
N5	1	1	1	0	1	1	0	1	1	0	1	1	1
N6	1	1	1	0	1	1	1	0	1	0	1	1	1
N9	1	1	1	0	1	1	1	1	0	0	1	1	1
N10	0	0	0	1	0	0	0	0	0	0	0	0	0
N12A	1	1	1	0	1	1	1	1	1	0	0	1	1
N12B	1	1	1	0	1	1	1	1	1	0	1	0	1
N12C	1	1	1	0	1	1	1	1	1	0	1	1	0
Same Grave													
ID	N1	N2	N3 A	N3 B	N3 C	N4	N5	N6	N9	N10	N12 A	N12 B	N12 C
N1	0	0	0	0	0	0	0	0	0	0	0	0	0
N2	0	0	0	0	0	0	0	0	0	0	0	0	0
N3A	0	0	0	1	1	0	0	0	0	0	0	0	0
N3B	0	0	1	0	1	0	0	0	0	0	0	0	0
3C	0	0	1	1	0	0	0	0	0	0	0	0	0
N4	0	0	0	0	0	0	0	0	0	0	0	0	0
N5	0	0	0	0	0	0	0	0	0	0	0	0	0
N6	0	0	0	0	0	0	0	0	0	0	0	0	0
N9	0	0	0	0	0	0	0	0	0	0	0	0	0
N10	0	0	0	0	0	0	0	0	0	0	0	0	0
N12A	0	0	0	0	0	0	0	0	0	0	0	1	0
N12B	0	0	0	0	0	0	0	0	0	0	1	0	0
N12C	0	0	0	0	0	0	0	0	0	0	1	1	0
Grave Goods													
ID	N1	N2	N3 A	N3 B	N3 C	N4	N5	N6	N9	N10	N12 A	N12 B	N12 C
N1	0	1	1	1	1	0	0	1	1	1	1	1	1
N2	1	0	1	1	1	0	0	1	1	1	1	1	1
N3A	1	1	0	1	1	0	0	1	1	1	1	1	1
N3B	1	1	1	0	1	0	0	1	1	1	1	1	1
3C	1	1	1	1	0	0	0	1	1	1	1	1	1
N4	0	0	0	0	0	0	1	1	0	0	0	0	0
N5	0	0	0	0	0	1	0	1	0	0	0	0	0

N6	1	1	1	1	1	1	1	0	1	1	1	1	1
N9	1	1	1	1	1	0	0	1	0	1	1	1	1
N10	1	1	1	1	1	0	0	1	1	0	1	1	1
N12A	1	1	1	1	1	0	0	1	1	1	0	1	1
N12B	1	1	1	1	1	0	0	1	1	1	1	0	1
N12C	1	1	1	1	1	0	0	1	1	1	1	1	0
Grave Goods with Two Warrior Categories													
ID	N1	N2	N3 A	N3 B	N3 C	N4	N5	N6	N9	N10	N12 A	N12 B	N12 C
N1	0	0	1	1	1	0	0	0	1	0	1	1	0
N2	0	0	0	0	0	0	0	1	0	1	0	0	1
N3A	1	0	0	1	1	0	0	1	1	0	1	1	0
N3B	1	0	1	0	1	0	0	0	1	0	1	1	0
3C	1	0	1	1	0	0	0	0	1	0	1	1	0
N4	0	0	0	0	0	0	1	1	0	0	0	0	0
N5	0	0	0	0	0	1	0	1	0	0	0	0	0
N6	0	1	1	0	0	1	1	0	0	1	0	0	1
N9	1	0	1	1	1	0	0	0	0	0	1	1	0
N10	0	1	0	0	0	0	0	1	0	0	0	0	1
N12A	1	0	1	1	1	0	0	0	1	0	0	1	0
N12B	1	0	1	1	1	0	0	0	1	0	1	0	0
N12C	0	1	0	0	0	0	0	1	0	1	0	0	0
Cultural Affiliation													
ID	N1	N2	N3 A	N3 B	N3 C	N4	N5	N6	N9	N10	N12 A	N12 B	N12 C
N1	0	0	0	0	0	0	0	0	0	0	0	0	0
N2	0	0	0	0	0	0	0	0	0	0	0	0	0
N3A	0	0	0	0	0	0	0	0	0	0	1	1	0
N3B	0	0	0	0	0	0	0	0	0	0	0	0	0
3C	0	0	0	0	0	0	0	0	0	0	0	0	0
N4	0	0	0	0	0	0	0	0	0	0	0	0	0
N5	0	0	0	0	0	0	0	0	0	0	0	0	0
N6	0	0	0	0	0	0	0	0	0	0	0	0	0
N9	0	0	0	0	0	0	0	0	0	0	0	0	0
N10	0	0	0	0	0	0	0	0	0	0	0	0	0
N12A	0	0	1	0	0	0	0	0	0	0	0	1	0
N12B	0	0	1	0	0	0	0	0	0	0	1	0	0
N12C	0	0	0	0	0	0	0	0	0	0	0	0	0
LEH													
ID	N1	N2	N3 A	N3 B	N3 C	N4	N5	N6	N9	N10	N12 A	N12 B	N12 C
N1	0	0	0	0	0	0	0	0	0	0	0	0	0
N2	0	0	0	0	0	0	0	0	0	0	0	0	0
N3A	0	0	0	1	1	0	0	0	0	1	1	1	1
N3B	0	0	1	0	1	0	0	0	0	1	1	1	1
3C	0	0	1	1	0	0	0	0	0	1	1	1	1

N4	0	0	0	0	0	0	0	0	0	0	0	0	0
N5	0	0	0	0	0	0	0	0	0	0	0	0	0
N6	0	0	0	0	0	0	0	0	0	0	0	0	0
N9	0	0	0	0	0	0	0	0	0	0	0	0	0
N10	0	0	1	1	1	0	0	0	0	0	1	1	1
N12A	0	0	1	1	1	0	0	0	0	1	0	1	1
N12B	0	0	1	1	1	0	0	0	0	1	1	0	1
N12C	0	0	1	1	1	0	0	0	0	1	1	1	0
Caries													
ID	N1	N2	N3 A	N3 B	N3 C	N4	N5	N6	N9	N10	N12 A	N12 B	N12 C
N1	0	0	0	0	0	0	0	0	0	0	0	0	0
N2	0	0	1	1	0	0	0	0	0	0	0	0	0
N3A	0	1	0	1	0	0	0	0	0	0	0	0	0
N3B	0	1	1	0	0	0	0	0	0	0	0	0	0
3C	0	0	0	0	0	0	0	0	0	0	0	0	0
N4	0	0	0	0	0	0	0	0	0	0	0	0	0
N5	0	0	0	0	0	0	0	0	0	0	0	0	0
N6	0	0	0	0	0	0	0	0	0	0	0	0	0
N9	0	0	0	0	0	0	0	0	0	0	0	0	0
N10	0	0	0	0	0	0	0	0	0	0	0	0	0
N12A	0	0	0	0	0	0	0	0	0	0	0	0	0
N12B	0	0	0	0	0	0	0	0	0	0	0	0	0
N12C	0	0	0	0	0	0	0	0	0	0	0	0	0
Skeletal Trauma													
ID	N1	N2	N3 A	N3 B	N3 C	N4	N5	N6	N9	N10	N12 A	N12 B	N12 C
N1	0	0	0	1	0	0	0	1	1	0	0	0	0
N2	0	0	0	0	0	0	0	0	0	0	0	0	0
N3A	0	0	0	0	0	0	0	0	0	0	0	0	0
N3B	1	0	0	0	0	0	0	1	1	0	0	0	0
3C	0	0	0	0	0	0	0	0	0	0	0	0	0
N4	0	0	0	0	0	0	0	0	0	0	0	0	0
N5	0	0	0	0	0	0	0	0	0	0	0	0	0
N6	1	0	0	1	0	0	0	0	1	0	0	0	0
N9	1	0	0	1	0	0	0	1	0	0	0	0	0
N10	0	0	0	0	0	0	0	0	0	0	0	0	0
N12A	0	0	0	0	0	0	0	0	0	0	0	0	0
N12B	0	0	0	0	0	0	0	0	0	0	0	0	0
N12C	0	0	0	0	0	0	0	0	0	0	0	0	0
Genetic Relatedness													
ID	N1	N2	N3 A	N3 B	N3 C	N4	N5	N6	N9	N10	N12 A	N12 B	N12 C
N1	0	0	1	0	0	0	0	1	1	0	0	1	0
N2	0	0	0	0	0	0	0	0	0	0	0	0	0
N3A	1	0	0	0	0	0	0	1	1	0	0	1	0

N3B	0	0	0	0	0	0	0	0	0	0	0	0	0
3C	0	0	0	0	0	0	0	0	0	0	0	0	0
N4	0	0	0	0	0	0	0	0	0	0	0	0	0
N5	0	0	0	0	0	0	0	0	0	0	0	0	0
N6	1	0	1	0	0	0	0	0	1	0	0	0	0
N9	1	0	1	0	0	0	0	1	0	0	0	1	0
N10	0	0	0	0	0	0	0	0	0	0	0	0	0
N12A	0	0	0	0	0	0	0	0	0	0	0	0	0
N12B	1	0	1	0	0	0	0	0	1	0	0	0	0
N12C	0	0	0	0	0	0	0	0	0	0	0	0	0

Sum of All Markers													
ID	N1	N2	N3 A	N3 B	N3 C	N4	N5	N6	N9	N10	N12 A	N12 B	N12 C
N1	0	2	4	2	2	1	1	5	5	1	2	3	2
N2	2	0	3	2	2	1	1	2	2	1	2	2	2
N3A	4	3	0	4	4	1	1	3	4	2	4	5	3
N3B	2	2	4	0	3	0	0	2	2	3	2	2	2
3C	2	2	4	3	0	1	1	2	2	2	3	3	3
N4	1	1	1	0	1	0	2	2	1	0	1	1	1
N5	1	1	1	0	1	2	0	2	1	0	1	1	1
N6	5	2	3	2	2	2	2	0	4	1	2	2	2
N9	5	2	4	2	2	1	1	4	0	1	2	4	2
N10	1	1	2	3	2	0	0	1	1	0	2	2	2
N12A	2	2	4	2	3	1	1	2	2	2	0	5	4
N12B	3	2	5	2	3	1	1	2	4	2	5	0	4
N12C	2	2	3	2	3	1	1	2	2	2	4	4	0

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