Reconstructing human population structure and history from dental phenotypes: Theory, methods and application to the ancient Greek colonization of southern Italy

Dissertation
der Mathematisch-Naturwissenschaftlichen Fakultät
der Eberhard Karls Universität Tübingen
zur Erlangung des Grades eines
Doktors der Naturwissenschaften
(Dr. rer. nat.)

vorgelegt von
Hannes Rathmann
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Gedruckt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Eberhard Karls Universität Tübingen.

Tag der mündlichen Qualifikation: 30.10.2018
Dekan: Prof. Dr. Wolfgang Rosenstiel
1. Berichterstatterin: Prof. Dr. Katerina Harvati
2. Berichterstatter: Prof. Dr. Richard Posamentir

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Acknowledgements

First and foremost, I would like to express my highest gratitude to my two supervisors, Prof. Dr. Katerina Harvati (Paleoanthropology, Tübingen University, Germany) and Prof. Dr. Richard Posamentir (Classical Archaeology, Tübingen University, Germany), whose support in the entire process of this PhD project has proven invaluable. I am deeply grateful for your trust in my scientific abilities, for your intellectual and personal support, for bringing me in contact with other researchers, and for providing the necessary space to think and work independently. I am also honored to have Prof. Dr. Joachim Wahl (Paleoanthropology, Tübingen University, Germany) and Prof. Dr. Nicholas Conard (Prehistory, Tübingen University, Germany) in my doctoral committee. While Joachim Wahl trained me a wide range of osteological techniques, Nick Conard taught me how archaeologists think.

Further thanks go to the various collaborators who I have been fortunate to work with throughout this PhD project. First of all, I thank Dr. Giulia Saltini Semerari (Classical Archaeology, Tübingen University, Germany) for the wonderful time we had together during our fieldwork in southern Italy. Without your social network and valuable contacts to local authorities – and countless hours of checking excavation diaries and museum storage lists (!) – it would have been impossible to locate and access the archaeological skeletal collections used in this dissertation. Thank you also for the numerous discussions and exchanges of ideas about the archaeology of the Greek colonization of southern Italy. Further thanks go to a group of excellent co-authors, including Dr. Hugo Reyes-Centeno (Paleoanthropology, Tübingen University, Germany); Dr. Silvia Ghirotto (Life Sciences and Biotechnologies, Ferrara University, Italy); Dr. Nicole Creanza (Biological Sciences, Vanderbilt University, USA); and Prof. Dr. Tsunehiko Hanihara (Anatomy, Kitasato University, Japan).

Moreover, I thank the following persons and institutions graciously provided access to the human skeletal collections in their care: Dr. De Siena, Dr. Cinquantaquattro and Dr. Bianco (Soprintendenza di Basilicata), and Dr. La Rocca, Dr. Schojer and Dr. Masiello (Soprintendenza di Apulia). I also thank the friendly staff of the national archaeological museums in Policoro, Metaponto and Taranto for their assistance in finding long-forgotten boxes full of ancient bones. Thanks also go to the several undergraduate students from Tübingen and Leiden that did their fieldwork internships in our project and greatly assisted in sorting, washing, and labeling

ancient skeletons prior to the osteological data collection. I want to particularly thank Anouk Vos and Ilona Gold. Without you, fieldwork would only have been half as productive and half as fun.

This PhD project wouldn't have been possible without generous financial support. I am deeply thankful to have the Gerda Henkel Foundation backing me up with a doctoral dissertation scholarship for three years. The Gerda Henkel Foundation also provided substantial funds for the numerous fieldwork campaigns in southern Italy. Further funding was provided by the State Ministry for Science, Research and Arts of Baden-Württemberg, Germany, (Project "Interdisciplinary Analysis of Culture Contacts in Ancient Peripheries") granted to Richard Posamentir, and by the German Research Foundation (Project "Words, Bones, Genes, Tools: Tracking Linguistic, Cultural and Biological Trajectories of the Human Past") granted to Katerina Harvati.

Further thanks go to a clique of loving friends. Many of my closest buddies are archaeologists, too, and I met some of them almost a decade ago when we started our BA studies at the castle of Tübingen. Thank you so much for being there and for the wonderful time we had in this city: Aytac Cakar, Chris Britsch, Conny Schmidt, Frank Brodbeck, Gregor Bader, Henrik Junius, Ilona Gold, Julian Weigle, Klaus Klein, Madita Kairies, Manuel Will, Rapha Schwörer, Recha Seitz, Sanne Jahn, Shumon Hussain, Thomas Mernik, Viola Schmid and many more. I have to thank Henrik Junius once more, as he was the one that first told me about 'dental biodistance analysis' after he returned from a semester abroad at Brown University, USA, in 2012. For me, back then, this sounded very odd but, due to whatever reason, I became interested in this research field. So thank you very much for awakening my academic interest in this topic, otherwise this PhD thesis would probably not exist. Further thanks go to all my Tübingen colleagues at the Institute for Archaeological Sciences (INA), many of whom I also consider good friends. I want to particularly thank my office mates Domenico Giusti and Tommaso Mori (the 509ers) for the pleasant working environment, your reassuring words in stressful moments and cold beverages in the evenings. Special thanks also go to Monika Doll for being the best secretary in the world and to Sara Rhodes for checking my English spelling in this dissertation. Finally, I thank my girlfriend Judith Beier. I consider myself terribly lucky to found someone like you! You greatly enrich my life!

Last but not least, I want to thank my parents Hannelore and Volker Rathmann. You have always been there for me with all your love, patience and financial support. I remember well when you read to me Homer's *Odyssey* when I was a child and I am sure that without your care and upbringing I would have never ended up studying archaeology. My doctoral odyssey is over now. I dedicate this dissertation to you!

Abstract

A central objective in many archaeological and anthropological studies is the reconstruction of past human population structure and population history. Biological distance (biodistance) analysis is a powerful tool to infer genetic relationships across samples of human skeletal remains using cranial and dental phenotypes. The underlying assumption of biodistance analyses is that skeletal samples which share a set of phenotypic features are presumed to be genetically more closely related, while those that differ in their phenotypes are presumed to be genetically less similar. Teeth have become a favored dataset for biodistance analyses primarily because dental size and shape are assumed to be highly heritable and selectively neutral. Moreover, dental form remains unchanged after full formation and teeth are generally best-preserved in the archaeological record, even when associated skeletal and DNA preservation is poor.

This cumulative dissertation has two primary objectives: (1) to quantify the utility of dental phenotypes as a reliable proxy for neutral genomic data in a population and quantitative genetic framework; and (2) to apply a dental biodistance analysis to an archaeological case study: the Greek colonization of southern Italy.

To address the first objective, I compare existing large phenotypic and genomic datasets sampled from worldwide modern human populations. Specifically, I generate biodistance estimates from two commonly employed dental phenotypic data types (metric and nonmetric traits) and two neutral genetic marker types (SNPs and STRs) using the R-matrix model, and explore their relationship using Mantel tests. Results show that biodistances based on dental phenotypes are significantly correlated with those based on neutral genetic data (on average $r = 0.574$, $p < 0.001$), validating tooth form as an efficient proxy for nuclear DNA data. Dental metric and nonmetric traits give concordant but varying results, indicating that combining both data types increases performance compared to using the features separately. Future work seeking to quantify the association between dental phenotypic and neutral genomic variation has great potential to identify dental data combinations that are most useful for tracking human population structure and history.

To address the second objective, I collected a new dataset comprising 481 human skeletons with well-preserved dentitions from six archaeological sites along the coastal area of the Gulf of Taranto, southern Italy, dating to precolonial (900-700 y BC) and postcolonial time periods (700-200 y BC). For both periods, I infer population structure using adonis, betadisper and isolation-by-distance models based on inter-individual Gower distance coefficients using a mixture of dental metric and nonmetric traits. For the postcolonial period, I furthermore determine individual ancestries using naïve Bayesian classification based on dental nonmetric traits. Results indicate that precolonial southern Italy was characterized by moderate levels of population stratification. During postcolonial times, the area became a place of high mobility hosting ~ 10 % Greek newcomers and their descendants. Interestingly, individuals of Greek ancestry were equally distributed across Greek colonies and indigenous settlements. These findings support a gradual colonization model with substantial involvement of local populations and contradict the theory that Greek colonies were homogenous enclaves within conquered territories. Future work expanding the study area to Calabria and Sicily has great potential to generate a detailed picture of the colonial history of whole Magna Graecia. The case study in this dissertation provides a conceptual template for this and the provided raw data allow for repeatability.

This dissertation bridges questions and analytical approaches of physical anthropology, population genetics and classical archaeology. Thereby it promotes multidisciplinary synergy between these research fields and highlights productive areas for future research.

Zusammenfassung

Ein zentrales Anliegen archäologischer und anthropologischer Forschung ist die Rekonstruktion von Populationsstruktur und Populationsgeschichte vergangener menschlicher Bevölkerungen. Die biologische Distanzanalyse (Biodistanzanalyse) fungiert dabei als ein leistungsstarkes Instrumentarium, denn sie erlaubt anhand phänotypischer Merkmale an Knochen auf genetische Beziehungen zwischen Gruppen zu schließen. Die zugrundeliegende Annahme von Biodistanzanalysen ist, dass Gruppen, die ähnliche phänotypische Merkmale besitzen, genetisch enger verwandt sind, wohingegen jene, die sich in ihren Phänotypen unterscheiden, als genetisch weniger nah verwandt gelten. Zähne eignen sich im besonderen Maße für Biodistanzanalysen, da davon ausgegangen wird, dass Zahngröße und -form vornehmlich erblich bedingt und selektiv neutral sind. Hinzu kommt, dass die Zahnform, einmal vollständig ausgebildet, unveränderlich ist, und Zähne im archäologischen Kontext meist am besten überliefert sind, selbst bei generell schlechter Knochen- und DNS-Erhaltung.

Die vorliegende kumulative Doktorarbeit hat zwei primäre Zielsetzungen: (1) Im Rahmen der Populationsgenetik und Quantitativen Genetik soll untersucht werden, inwiefern Zahnmorphologie als verlässlicher Proxy für genomische Daten verwendet werden kann. (2) In einer archäologischen Fallstudie zur Griechischen Kolonisation Süditaliens soll eine Biodistanzanalyse anhand von Zahndaten Anwendung finden.

Im Hinblick auf die erste Zielsetzung werden umfangreiche dentalmorphologische und genomische Datensätze von rezenten Populationen aus aller Welt verglichen. Die Messung der Biodistanz erfolgt mittels des R-Matrix Modells anhand zweier häufig verwendeter dentalmorphologischer Datentypen (metrische und nicht-metrische Merkmale) sowie anhand zweier genetischer Markertypen (SNPs und STRs). Die Beziehung zwischen den Biodistanzmessungen wird mithilfe von Mantel Tests untersucht. Die Ergebnisse zeigen, dass die dentalmorphologischen Biodistanzmessungen signifikant mit jenen der neutralen Genomdaten korrelieren (im Durchschnitt $r = 0.574$, $p < 0.001$). Dies belegt, dass Zahnmorphologie einen validen Proxy für nukleare DNS-Daten darstellt. Metrische und nicht-metrische Zahnmerkmale liefern dabei vergleichbare wenn auch variierende Ergebnisse; eine Kombination der beiden Datentypen ist demnach ihrer getrennten Verwendung vorzuziehen. Womöglich gelingt es zukünftiger Forschung, die die Stärke des

Zusammenhangs zwischen Zahnmorphologie und neutraler genomischer Variabilität untersucht, diejenigen Kombinationen von Zahnmerkmalen zu identifizieren, die sich am besten für die Rekonstruktion menschlicher Populationsstruktur und -geschichte eignen.

Im Hinblick auf die zweite Zielsetzung habe ich einen neuen Datensatz erhoben, der 481 menschliche Skelette mit gut überlieferten Zahnapparaten von sechs archäologischen Fundstellen entlang der Küstenregion des Golfs von Taranto in Süditalien umfasst, welche in die vorkoloniale (900-700 v. Chr.) und postkoloniale (700-200 v. Chr.) Zeit datieren. Ich verwende eine Kombination aus metrischen und nicht-metrischen Zahnmerkmalen und rekonstruiere für beide Zeiträume die Populationsstruktur anhand von adonis, betadisper und isolation-by-distance Modellen, die auf inter-individuellen Gower Distanzkoeffizienten basieren. Mithilfe nicht-metrischer Zahnmerkmale und naiver Bayes-Klassifikation ermittle ich zudem die individuelle Herkunft einzelner Individuen der postkolonialen Phase. Die Ergebnisse zeigen, dass im vorkolonialen Süditalien eine moderate Populationsstratifizierung vorherrschte. Während der postkolonialen Zeit wurde das Gebiet zu einem Ort hoher Mobilität, der ~ 10 % griechische Neuankömmlinge und deren Nachkommen beherbergte. Interessanterweise finden sich Individuen griechischer Herkunft gleichmäßig über griechische Kolonien und indigene Siedlungen verteilt. Diese Ergebnisse befürworten ein schrittweises Modell der Kolonisation unter maßgeblicher Beteiligung lokaler Bevölkerungsgruppen und widersprechen der Theorie, dass griechische Kolonien homogene Enklaven innerhalb eroberter Gebiete waren. Zukünftige Forschung, die das Untersuchungsgebiet auf Kalabrien und Sizilien ausweitet, besitzt großes Potenzial ein detailliertes Bild der kolonialen Geschichte von ganz Magna Graecia zu liefern. Die Fallstudie in dieser Doktorarbeit liefert hierzu eine konzeptionelle Vorlage und die zur Verfügung gestellten Rohdaten ermöglichen Reproduzierbarkeit.

Die vorliegende Doktorarbeit verbindet Kernfragen und analytische Ansätze der physischen Anthropologie, Populationsgenetik sowie der klassischen Archäologie. Sie fördert damit multidisziplinäre Synergien zwischen diesen Forschungsfeldern und zeigt vielversprechende Bereiche für zukünftige Forschung auf.

List of publication for cumulative dissertation

Publications submitted or in preparation in fulfillment of the requirements of this cumulative dissertation. Numbers in parentheses represent percentage of own contribution to the articles or manuscripts (scientific idea/data generation/analysis and interpretation/paper writing).

Accepted articles:

- Paper I (80/0/70/70):

Rathmann, H., Reyes-Centeno, H., Ghirotto, S., Creanza, N., Hanihara, T., Harvati, K. Reconstructing human population history from dental phenotypes. *Scientific Reports* 7, 12495 (2017).
DOI: 10.1038/s41598-017-12621-y

- Paper II (100/100/100/70):

Rathmann, H., Saltini Semerari, G., Harvati, K. Evidence for Migration Influx into the Ancient Greek Colony of Metaponto. A Population Genetics Approach Using Dental Nonmetric Traits. *International Journal of Osteoarchaeology* 27, 453-464 (2017).
DOI: 10.1002/oa.2569

Manuscript ready for submission:

- Paper III (100/100/100/70):

Rathmann, H., Kyle McIlvaine, B., Harvati, K., Posamentir, R., Saltini Semerari, G. (*To be submitted*). Population structure of southern Italy during Greek colonization inferred from dental metric and nonmetric traits. Manuscript close to submission.

1. Introduction

Reconstructing the history of mankind is an exciting and challenging endeavor involving many different interdisciplinary research disciplines. Bioarchaeology is the contextual study of the biology and culture of past human populations using skeletal remains interpreted within archaeological and historical problem orientations. The focus of this PhD research is on a particular bioarchaeological tool known as **biological distance analysis** (hereafter, biodistance).[1] Biodistance analyses use phenotypic data from the cranium or dentition to estimate genetic similarity among regional or continental populations in order to reconstruct population structure and population history. These two terms are often used interchangeably, but they can be considered somewhat separately. **Population structure** refers to patterns of genetic variation among a set of contemporaneous populations generated by the effects of gene flow and genetic drift (Relethford, 1996). **Population history** is concerned more with inferences of ancestor-descendant relationships and evolutionary histories among populations caused by historical events such as large-scale population movements and culture contact (Relethford, 1996). In general, population structure studies tend to be regionally focused, while studies on population history are more continental in scale (Knudson and Stojanowski, 2008).

But before getting into the details of this approach, it is worthwhile examining a more essential question: why are we doing this? Given the increasing wealth of ancient DNA (aDNA) data extracted from archaeological human remains, it might seem rather old-fashioned to deal with phenotypic data. Today, molecular studies are undoubtedly the most accurate tool for exploring population structure and population history, primarily because they involve hundreds of thousands of analytical markers. This contrasts with phenotypic studies, in which a given analysis rarely includes more than a few dozen characters. However, one pragmatic reason in favor of phenotypic approaches is that in many archaeological contexts skeletal and dental evidence is the only source of information available to researchers as poor preservation often precludes the extraction of endogenous DNA. Moreover, aDNA

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[1] Biological distance analysis is a tool of quantitative genetics, which is a branch of population genetics, drawing from modern evolutionary theory and concerned with the variation and evolution of phenotypes. According to Hefner et al. (2016), the term “biological distance analysis” has been used in population biology and physical anthropology since the early 1960s, whereas the origin of the abbreviation “biodistance” is not as clear but could probably be dated to the late 1970s.

degrades quickly in warmer climates, making paleogenomic approaches more feasible in some parts of the globe but less promising in others. Finally, aDNA data may never be as abundant as skeletal or dental data, limiting paleogenomic investigations to single individuals rather than entire populations. Thus, even in the 21st century, the popularity of biodistance analyses is still growing.[2]

The main aim of this cumulative dissertation is to contribute to the field of biodistance analysis through both 1) methodological and 2) applied research. **First, I tested the utility of dental phenotypic data as a proxy for nuclear DNA**. In recent years, teeth have become a favored dataset for biodistance studies because dental phenotypes are assumed to be under strong genetic control. Furthermore, teeth are generally best-preserved in the archaeological record, even when the associated cranial and postcranial skeletal preservation is poor. However, despite the popularity of biodistance analyses utilizing dental phenotypes, few studies have systematically tested the assumption of genetic neutrality of dental phenotypic features in a population and quantitative genetic framework (Sofaer et al., 1972; Brewer-Carias et al., 1976; Harris, 1977; Hubbard et al., 2015). These investigations found contradicting results and some were limited by several issues related to sample size and traits selection. To contribute to this research field, I quantified the correlation of biodistance estimates among a large set of globally distributed modern human populations, derived independently from diverse dental phenotypic features and neutral genetic markers. **Second, I applied dental biodistance analyses to an archaeological case study: the Greek colonization of southern Italy**. The Greek colonization of southern Italy was a pivotal cultural encounter in antiquity, nevertheless, little is known about the scale of demographic impact and the Greek genetic legacy. Historians, archaeologists, demographers, and geneticists have proposed different degrees of Greek contribution, with scenarios ranging from a colonization process driven by a few Greek colonists living in biologically isolated enclaves within the conquered territories, to scenarios with substantial migration from Greece to Italy and intensive interaction between newcomers and locals (e.g. Pugliese Carratelli, 1996; Osborne, 1998; Yntema, 2000; Greco, 2002; Scheidel,

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[2] The popularity of biodistance analysis is attested by the vast amounts of research manuscripts, theses, and dissertations making use of this analytical tool. A recent Google Scholar search (https://scholar.google.com) for the term “biodistance” for a 10 year interval from the year 1970 up until today returned the following results: 1970-1980: 3 hits; 1980-1990: 21 hits; 1990-2000: 80 hits; 2000-2010: 313 hits; 2010-today: 719 hits.

2003; Donnellan et al., 2016b, 2016a; Tofanelli et al., 2016). Studies using aDNA could help verifying these hypotheses, but it remains to be tested if human skeletal remains from southern Italy provide ancient collagen of high enough quality to be DNA sequenced. To address this debate, I collected a comprehensive dental phenotypic dataset from hitherto inaccessible skeletal collections and performed biodistance analyses aimed at reconstructing the population structure and history of southern Italy during the Greek colonization.

The following sections introduce the theoretical, methodological and contextual backgrounds of this PhD project. Chapter 2 (Objectives and Research Questions) presents the main research goals, the specific research questions and study designs of the articles that form this cumulative dissertation. Chapter 3 (Results and Discussion) summarizes the key results of the articles and reviews the fundamental contributions of the dissertation to the field of biodistance analysis and research on the Greek colonization of southern Italy. Finally, Chapter 4 (Concluding remarks and area for future research) highlights productive areas for future work.

1.1. Biodistance analysis

> *"History and mathematics are not often a comfortable mix […]. That said, I must admit that biological distance analysis and paleodemography are probably the two most mathematical branches of bioarchaeology"*
> (Konigsberg, 2006: 263-264)

This chapter provides the reader with a brief introduction into the field of biodistance research. The historical development of the field, as well as recent trends in analytical approaches, have been subject to several reviews and edited books (Konigsberg, 2006; Stojanowski and Schillaci, 2006; Pilloud and Hefner, 2016) and I refer the interested reader to these excellent sources for additional detailed information. The chapter is divided into three subchapters: (1) Theoretical background; (2) Description of the data; and (3) A brief history of developments in statistical approaches.

Theoretical background

The underlying theoretical principle of biodistance analyses is relatively straightforward (Stojanowski and Schillaci, 2006): Populations which share a set of phenotypic features are presumed to be genetically more closely related, while those that differ in their phenotypes are genetically less similar. The primary assumptions of biodistance analysis are: (1) skeletal phenotypes are an adequate proxy for the underlying genotype and changes in allele frequencies result in changes in skeletal features; (2) environmental (non-genetic) effects on phenotypic variation are minimal; (3) phenotypic expressions must be independent of age and sex; (4) populations under study are accurately represented by samples of archaeological human remains with attention paid to adequate sample sizes; and (5) the archaeological samples comprise specimens aggregated over similar temporal durations.

Description of the data

Biodistance analyses utilize phenotypic observations from archaeological skeletal remains as proxies for genotypic data. **Cranial and dental observations** are preferred because they are assumed to be the most heritable features of the human skeleton. Postcranial traits are less often used because weight-bearing bones are primarily functional and, thus, subject to selective forces (Stojanowski and Schillaci, 2006). In general, there are two different families of data that can be collected for the cranium and the dentition: metric and nonmetric traits. **Metric traits** are measured along continuous scales gathered by either hand-hold sliding calipers or more advanced three-dimensional imaging techniques. Cranial metrics are most common and have the longest history in anthropological research. Data collection is performed by measuring cranial inter-landmark distances with several different data recording protocols proposed over the course of time (Martin, 1928; Howells, 1989; Buikstra and Ubelaker, 1994). Dental dimensions are another frequently used metric dataset for biodistance analysis and, as with craniometric data, many different measurement protocols have been used (Kieser, 1990; Buikstra and Ubelaker, 1994; Hillson, 1996). The most commonly used measurements are mesiodistal and buccolingual dimensions of dental crowns. Hillson et al. (2005) added to this a set of alternative dental measurements, such as diagonal crown measurements and cervical diameters at the cement-enamel junction. These alternative measurements are highly promising as they reflect similar genetic signals as traditional dental measurements but are less affected by dental wear. **Nonmetric traits** are discontinuous phenotypic expressions recorded by visual scoring on either a binary scale (i.e. ‘absent’ or ‘present’) or an ordinal scale (i.e. ‘small’, ‘medium’, ‘large’). Although the expression of nonmetric traits is discontinuous, they are assumed to have an underlying continuous polygenic mode of inheritance (Hauser and DeStefano, 1989). Many of these traits are subtle and not easy to detect; thus, nonmetric variables are somewhat more affected by observer error than metric variables. However, one decisive advantage of nonmetric traits is that they develop relatively early in the life of an individual and that their form remains unchanged after full formation. Several cranial nonmetric traits and their various expression levels have been identified, and attempts have been made to ensure standardized scoring procedures (Hauser and DeStefano, 1989). Dental nonmetric traits are another important dataset for comparative purposes gaining increasing popularity over the last few decades. Turner et al. (1991) classified a wide number of crown and root traits and established a standardized recording system which comprises a set of dental casts illustrating expression levels for various traits and specific scoring instructions to allow for repeatability (known as the Arizona State University Dental Anthropology System, or ASUDAS).

Justification for using cranial and dental phenotypes as reliable proxies for the underlying genotype almost always rests on two types of studies: heritability studies and studies seeking the correlation between genetic and phenotypic distance estimates. **Heritability** (h^2) is the statistical estimation of the relative proportion of total phenotypic variation that is due to additive genetic variation. It can be estimated by comparing phenotypic features across relatives, such as comparing parents and offspring, as well as twins. Conceptually, h^2 estimates can range from 0 % (implying that the phenotype under investigation is not genetically determined but instead heavily influenced by environmental factors) to 100 % (implying that the phenotype is purely genetically determined and not influenced by any environmental factors). Numerous heritability studies exist for cranial metrics (Sjøvold, 1984; Carson, 2006), cranial nonmetric traits (Hauser and DeStefano, 1989), dental metrics (Kieser, 1990), and dental nonmetric traits (Scott and Turner, 1997). Studies found greatly varying phenotypic heritability estimates, with the mean heritability estimate somewhere around $h^2 = 55$ % (Stojanowski and Schillaci, 2006). The heritability concept has been criticized because h^2 estimates are population-specific and do not speak to the environments of other populations (Vitzthum, 2003; Visscher et al., 2008). Studies quantifying the **correlation between genetic and phenotypic distance estimates** are an alternative way of assessing the role of genetics in phenotypic variation. These studies test the congruence of genetic and phenotypic distance estimates across a set of populations for which both genomic and phenotypic data are available. Because of the inclusion of several populations, they are better suited to account for differential large-scale environmental effects on phenotypic variation in comparison to h^2

estimates. Several researchers have applied this approach to cranial metric data (Roseman, 2004; Harvati and Weaver, 2006; Smith et al., 2007; Cramon-Taubadel, 2009; Smith, 2009; Cramon-Taubadel, 2011; Smith et al., 2013; Herrera et al., 2014; Reyes-Centeno et al., 2017), but only few have done so for cranial nonmetric traits (Herrera et al., 2014), dental metrics (Harris, 1977), or dental nonmetric traits (Sofaer et al., 1972; Brewer-Carias et al., 1976; Hubbard et al., 2015).

A brief history of developments in statistical approaches

Biodistance analysis has undergone significant methodological transformations over the last decades, primarily due to the advent of computers and automated computational power enabling researchers to perform complex and time-consuming math.

Historically, the first methods for the estimation of biodistances across groups were designed for use with **metric data**. Early multivariate statistical approaches essentially summed the mean differences in variables across groups (Pearson, 1926) but were quickly criticized primarily because they were not able to account for correlation among descriptors (Fisher, 1936). To correct for this weakness, Mahalanobis (1936) published his famous paper on the ‘generalized distance’ which is now also called the Mahalanobis distance or D^2 statistic. This distance is a special case of the Euclidean distance, being able to account for correlation among variables by incorporating a variance-covariance matrix. Although very elegant, the major drawback of Mahalanobis’ D^2 is that it assumes equal covariance structures across groups and relies on the computation of a single dispersion matrix. Statisticians therefore gained interest in developing D^2 variants allowing for unequal covariances across groups (e.g. Bhattacharyya, 1943). These approaches, however, calculate pairwise distances each in a space defined by a different norm and therefore do not allow simultaneous representation of all distance estimates in a common reference space. Mahalanobis’ D^2 therefore remained the most frequently used statistic in anthropological comparative research. Some years later, Penrose (1952) proposed his relatively straightforward ‘size and shape’ distance measurement, where ‘size’ is the square of the mean differences between groups and ‘shape’ a measure of variance. However, the primary problem of correlation among variables remained with this measure. In the early 1970s, Mahalanobis’s D^2 and Penrose’s ‘size and shape’ were fine-tuned in order to account for unequal sample sizes that are commonly employed in bioarchaeological studies (van Vark, 1970).

Techniques for the estimation of biodistances based on **nonmetric data** are more complex and therefore it was not until the 1950s and 19060s that researchers first delved into this field. Many of the early approaches are based on the comparison of frequency profiles across samples and were directly derived from the field of population genetics. Sanghvi and Balakrishan (Sanghvi, 1953; Balakrishnan and Sanghvi, 1968; Sanghvi and Balakrishnan, 1972) were probably the first to publish several Euclidean distance statistics for frequency data utilizing the chi-square statistic, with some able to account for correlation among traits. At the same time, the 'mean measure of divergence' (MMD) was devised, a fairly complex technique for measuring the average difference in frequency profiles across groups by accounting for unequal sample sizes (Grewal, 1962). The method was popularized among bioarchaeologists by Berry and Berry (1967) who were the first to apply the technique to a set of human remains. The MMD formula was further modified and fine-tuned by various researchers addressing the issue of small sample sizes, variance stabilization, and fixed trait frequencies (Sjøvold, 1973; Green and Suchey, 1976). Increasing interest in the analysis of nonmetric traits resulted in the development of new and highly sophisticated methods for the complex treatment of discontinuous traits. From the generalized D^2 distance, a Mahalanobis variant was extended for use with nominal or ordinal scaled trait data by incorporating a dispersion matrix of tetrachoric or polychoric correlations (Konigsberg, 1990; Konigsberg et al., 1993; Bedrick et al., 2000). In recent years, Nikita (2015) further modified the Mahalanobis D^2 variant for discontinuous data and refined the approach to address the issue of non-positive-semidefinite dispersion matrices and effects of unequal sample sizes.

Researchers also gained interest in **combining metric with nonmetric data** to improve biodistance performance, as compared to using the features separately. Krusińska (1987) proposed a weighted Mahalanobis D^2 distance for mixed data as the weighted sum of a Mahalanobis D^2 distance for continuous data and a Mahalanobis D^2 variant for binary data. In the same spirit, Kozintsev et al. (1999) proposed to transfer metric and nonmetric data by two separate principal component (PC) analyses into PC scores which are then used as new variables in a second 'integral PC analysis'. Although these two methods are intuitively appealing, they do not account for correlation *between* metric and nonmetric datasets. This issue was finally addressed by Bedrick et al. (2000) who developed a Mahalanobis D^2 variant for mixed ordinal and continuous data using a grouped continuous model. Shortly afterwards, Leon and Carrière (2005) developed a highly promising Mahalanobis D^2 variant for mixed binary, ordinal and continuous data by applying the Kullback-Leibler divergence to a general mixed-data model.

In the 1970s and 1980s, researchers started to incorporate quantitative and population genetic theory into biodistance studies. Relethford and Lees (1982) distinguished between 'model-free' and 'model-bound' approaches for studying phenotypic variation across groups. **Model-free** approaches use for example D^2 or MMD distance statistics to describe patterns of variation that can be interpreted in light of population structure and history. **Model-bound** approaches on the other hand are directly derived from population genetic theory and allow estimation of specific model parameters.

A famous example of a model-bound approach is the **R-matrix method** which was originally developed to work with allele frequency data (Harpending and Jenkins, 1973), and later modified for use with morphometrics (Williams-Blangero and Blangero, 1989; Relethford and Blangero, 1990; Relethford et al., 1997) and nonmetric traits (Konigsberg, 2006; Irish, 2010). The R-matrix method can be used to estimate a number of specific model parameters, such as F_{ST}, an index summarizing genetic differentiation among groups relative to the total amount of variation expected under no subdivision. Moreover, the R-matrix method can be used to estimate genetic distances among groups that account for the confounding effects of genetic drift. Finally, the R-matrix can be used to assess the level of extra-local gene flow experienced by the groups under study. Although promising, the R-matrix theory should be used sparingly in archaeological contexts unless the following assumptions are met: (1) the sampled groups are from the same time period and could have reasonably exchanged mates; (2) heritability estimates of the traits used are available; and (3) the effective population sizes of the groups are known or can be at least approximated. Since most archaeological samples violate the assumptions of the R-matrix theory, traditional model-free approaches continue to play a pivotal role in biodistance studies.

Another famous example of a model-bound approach is the **isolation-by-distance** model (IBD), originally proposed by Wright (1943). The model states that if mobility was low biodistances are expected to be strongly correlated with geography, whereas if mobility was high, biodistances are expected to be weakly correlated with geography. This expected relationship is usually investigated by performing Mantel tests (Mantel, 1967), a method determining the fit between a matrix summarizing biodistances across samples and another matrix summarizing geographic distances among samples. If samples are not contemporaneous researchers can use partial Mantel tests to control for the effect of a third matrix that summarizes temporal distances among samples (Legendre, 2000). Just recently, Loog et al. (2017) proposed a promising method that maximizes the correlation between a biodistance matrix and a matrix combining both spatial and temporal optimally scaled distance information (called mobility estimator S_{max}). Because IBD tests allow for samples from different time periods, they are most flexible and well-suited for analyzing mobility from spatially and temporarily sparsely sampled data.

Although the emphasis of biodistance research is on group-level approaches, comparisons are also possible among individuals. **Individual-level approaches** gain increasing popularity because they are better suited for capturing the significant amount of human genetic variation within groups. A concentration on broad group-level comparisons ignores such variation by treating the group as the unit of analysis. Probably one of the most useful inter-individual biodistance statistics is Gower's similarity coefficient (Gower, 1971) because it can incorporate multiple variable scales (i.e. nominal, ordinal, continuous), different variable weights, and missing data. Individual-level analyses within a region or cemetery furthermore allow for a wide range of investigations directly articulated with the study of past social structures, such as the identification of kinship or the inference of post-marital residence practices (for an excellent review see Stojanowski and Schillaci, 2006). Individual-level approaches also allow for the classification of individuals into predefined groups which can be useful to detect individual ancestries. Although classification techniques are conceptually not considered distance estimates, they still belong to the broad field of biodistance research (see contributions in Pilloud and Hefner, 2016). The rise of learning machines has led to an explosion of highly sophisticated classification methods such as 'neural networks', 'random forest', or 'support vector machines'. These methods overcome problems of more traditional classification techniques such as overfitting or strong assumptions about the distribution of variables. Further details and elaboration of classification concepts lies beyond the scope of this chapter but can be found elsewhere (e.g. Ousley, 2016).

Nowadays, a countless variety of different biodistance statistics are available and researchers have to choose carefully which methods best suit their specific research questions. This primarily depends on the scale of investigation (group-level vs. individual-level), the inferential framework (model-free vs. model-bound) and the nature of the dataset (metrics vs. nonmetric traits), including attention paid to sample sizes and the amount of missing data.

1.2. Archaeological case study: the Greek colonization of southern Italy

"[Metapontion] is said to have been founded by the Pylians who
sailed from Troy with Nestor; and they so prospered from farming,
it is said, that they dedicated a golden harvest at Delphi"
(Strabo's Geography: 6.1.15)

This chapter provides the reader with a brief introduction into the research field of the Greek colonization of southern Italy. The causes and modes of the Greek colonization has fascinated scholars for centuries and has spawned a huge bibliography. This chapter is by no means exhaustive but may serve as a concise review of previous research contributions in order to outline productive areas for future biodistance analyses. The chapter is divided into five subchapters: (1) Historical background; (2) Evidence from ancient written sources; (3) Archaeological evidence; (4) Present-day DNA evidence; and (5) Osteological evidence.

Historical background

During the eighth century BC, people coming from ancient Greece started to settle along the coasts of the Mediterranean. Greek seafarers founded more than 500 colonies along the shores of the Black Sea, Anatolia, southern Italy, Sicily, North Africa and along the coast of southern France and Spain (Hansen and Nielsen, 2004). Among the regions settled by the Greeks, southern Italy was one of the most densely populated areas and eventually became known as *Megálē Hellás* or *Magna Graecia* (Greater Greece). Archaeological evidence suggests that Greek colonists initially settled in small groups, sometimes embedding themselves within the local indigenous populations. Over time, Greek colonies became increasingly urbanized and culturally distinct settlements. The Greek colonization has been called "*one of the most important cultural encounters in world history*" (Angelis, 2016: 101), and its consequences in Mediterranean history were profound and long-lasting. It contributed to the creation of a Mediterranean-wide network of exchanges (Malkin, 2005), to the development of urbanization along its shores (Malkin, 1994), to the spread of the alphabet (Boardman, 2014), and to the diffusion of Greek artistic and architectural traditions (Greco, 1992).

The reasons why Greeks left their homeland and started colonization ventures are hotly debated and many opposing theories exist (e.g. overpopulation, hunger for territory, trade), each having supporters and opponents (Yntema, 2000). Overpopulation used to be a commonly accepted explanation but was gradually rejected by scholars as being void of actual evidence and plausibility (Scheidel, 2003). Hunger for territory as the decisive factor for colonization has been advocated by historians referring to ancient written sources. However, archaeological evidence showing that many of the colonized territories along the Mediterranean coast were already inhabited by large indigenous groups (e.g. Frey, 1991; Carter, 1998) suggests that this explanation is unlikely. Indeed, it might have been quite difficult for a small number of territory hungry Greek conquerors to survive and thrive in an adverse environment inhabited by hostile locals. From the 1980s onwards, trade became the prevailing explanation for the Greek diaspora, mainly because archaeological evidence proved that the exchange of goods pre-dated the arrival of Greek colonist by several decades (Yntema, 2000).

While the impact of the Greek colonization is clearly visible in the historical and cultural heritage of southern Italy (e.g. through archaeological remains, architectural legacy and Greek-speaking minorities), researchers have argued for decades about its genetic contribution. In particular, the numerical size of migration from Greece and the degree of admixture between Greek colonists and indigenous populations are debated. Historians, archaeologists, demographers, geneticists, and osteologists have proposed different degrees of Greek contribution, with scenarios ranging from a colonization process driven by few Greek colonists living in biologically isolated enclaves within the conquered territories, to scenarios with substantial migration from Greece to Italy and intensive interaction between newcomers and locals (Osborne, 1998; Yntema, 2000; Scheidel, 2003; Donnellan et al., 2016b, 2016a; Tofanelli et al., 2016). In the following, I will briefly review these scientific contributions to the debate in order to outline productive areas for biodistance analyses.

Evidence from ancient written sources

Early interpretations on the causes and modes of the Greek colonization were primarily based on survived texts from ancient Greek writers (Mitford, 1784; Grotte, 1856; Beloch, 1886; Pais, 1894). Drawing from these historical sources, it was thought that the colonies (or *apoikiai*) were centrally organized expeditions sent out by a 'mother city' under the auspices of the Delphian oracle. They were led by an official founder (the *oikistes*), who chose the location and proceeded to divide the land in regular allotments distributed to the colonists. The *oikistes* was also responsible for establishing local cults (largely derived from the mother cities) and public spaces. In this view, the role of local populations, if at all considered, was essentially passive. They were deprived of land, resources and women to fulfill the colonies' needs while being the object of gradual acculturation (or 'hellenisation') (Burgers, 2004; Shepherd, 2005). Central to those early interpretations was the assumption that Greek colonies formed culturally and biologically homogenous enclaves within the conquered foreign territories. Within this framework, archaeological evidence (being very scarce at these times) was only considered when it supported the written evidence. Wherever the two research sources did not correspond, excuses were found to discard the former (Yntema, 2000). Philological approaches are, however, somewhat problematic, since most ancient written sources postdate the Greek colonization by several centuries and portray it solely from a Greek perspective.

Ancient texts were also used to extrapolate the numerical size of Greek colonists that migrated to southern Italy. For example, Beloch (1886) reckoned that ~ 10 % of the population in southern Italy was Greek during Classical times (i.e. 400 y BC); an estimate based on approximated census counts primarily derived from historical enumerations of Greek military forces documented in ancient texts. In a more recent study, Scheidel (2003) performed demographic modeling based on reasonable population growth rates utilizing the census counts provided by Beloch (1886) and estimated that the original founding population during Archaic times (i.e. 700 y BC) was likely around 30,000 to 60,000 immigrants. The order of magnitude of such estimates, however, must be taken with caution as there is no guarantee that the reported population estimates for the Classical period are correct or represent a reliable enumeration of 'biological' Greeks.

Archaeological evidence

Over the course of time, increasing archaeological evidence from early colonial contexts (700-500 y BC) pointed to a more complex picture that contrasted the proposed settlement homogeneity of the traditional colonial narratives. Specifically, in many Greek colonies, local indigenous material culture was unearthed from the earliest phases of the sites and, conversely, some non-colonial sites revealed evidence of mixed Greek-indigenous assemblages (Siena, 1986; Berlingò, 1993; Carter, 2006; Crielaard and Burgers, 2012; Denti, 2018). Based on those findings, the colonization process was considered an interplay of actions of both colonizers and colonized, and a more balanced investigation of both Greek and indigenous roles in the colonization process has been advocated. These developments led to an interpretation of Greek colonization emphasizing processes of collaboration and 'hybridization' between Greek newcomers and local indigenes, where the Greek-indigenous interaction shaped new, admixed cultures (Yntema, 2000; Malkin, 2002; van Dommelen, 2012). Although material culture is a valuable addition to ancient literary sources, it must be kept in mind that: 'pots are pots, not people'. The stylistic provenance of an object and the bio-geographic origin of its maker(s) may not be identical. Additionally, movements of object do not necessarily imply migration of people but can also result from trade or the diffusion and adoption of stylistic fashions.

Present-day DNA evidence

In recent years, geneticists have tried to tackle the question of the Greek colonization and its impact on southern Italy from a different angle. Using modern DNA data, several studies found that present-day southern Italians exhibit a genetic signature compatible with that found in modern Greeks (Di Gaetano et al., 2009; Sarno et al., 2014; Tofanelli et al., 2016; Sarno et al., 2017). Di Gaetano et al. (2009) estimated a Greek genetic contribution of ~ 37 % to the population of Sicily and attributed the influx to the Classical period (2380 years before present, with a 95 % confidence interval ranging between 6940 and 675 years ago). In another study, Tofanelli et al. (2016) estimated that the size of the original founding population was probably between 1,000 to 6,000 immigrants and attributed the influx to the Archaic period

(800 - 400 y BC). The latter study suggests fewer immigrants than the study of Scheidel (2003) who reckoned with 30.000 to 60.000 newcomers. Although genomic studies are highly interesting, the problem with present-day genetic distributions is that they may not accurately reflect the effects of past migration events due to movements of people in more recent times. Moreover, genomic estimates of the timing of past migration events generally have wide ranging confidence intervals spanning several centuries or even millennia. Studies using aDNA could help verifying these findings, but it remains to be tested if human skeletal remains from southern Italy provide ancient collagen of high enough quality to be DNA sequenced.

Osteological evidence

The most promising approach for studying the Greek genetic contribution to southern Italy is to directly analyze ancient biological data from archaeological human remains. As detailed above (Chapter 1.1.), biodistance analysis provides a powerful tool for assessing relationships among past human populations, when no aDNA is preserved. Surprisingly, only a few studies have utilized biodistance methods to address the impact of Greek colonization on southern Italy so far (Henneberg, 1998; Rubini et al., 1999). These studies found that the inhabitants of Greek colonies showed marked biological differences to indigenous Italian groups, possibly due to an influx of new genes. While this is an exciting finding, these studies were limited by several issues. First, they all employed few phenotypic variables, which generally reduces the accuracy of biological distance estimates (Sokal and Sneath, 1963). Second, all studies used either metric or nonmetric data; however, it has been shown that combining metric with nonmetric markers increases performance compared to using the features separately (Lease and Sciulli, 2005; Hefner et al., 2014). Third, none of the previous studies have used adequate comparative data from Greece which is essential to assess the degree of Greek genetic contribution to southern Italian populations.

In conclusion, a thoroughly performed biodistance analysis using a comprehensive osteological dataset based on samples from several sites − both colonial and indigenous − has great potential to refine our understanding of the Greek colonization of southern Italy.

2. Objectives, research questions and study design

The primary objectives of this dissertation were twofold: (1) to quantify the utility of dental phenotypes as a reliable proxy for neutral genomic data; and (2) to use dental biodistance analyses in an archaeological case study: the Greek colonization of southern Italy.

In order to perform this PhD research, I was awarded with a **3-year doctoral research scholarship from the Gerda Henkel Foundation**. Additional funding was provided by Prof. Dr. Richard Posamentir (Institute of Classical Archaeology, Tübingen University, Germany) and Prof. Dr. Katerina Harvati (Department of Paleoanthropology, Tübingen University, Germany).

To address the first objective, I **collaborated with an international team of experts** from the fields of Physical Anthropology and Population Genetics in order to compare existing large phenotypic and genomic datasets from modern worldwide human populations (see Figure 1 in Appendix A). The dental phenotypic data was provided by Prof. Dr. Tsunehiko Hanihara (Department of Anatomy, Kitasato University, Japan). Neutral genomic data was provided by Dr. Silvia Ghirotto (Department of Life Sciences and Biotechnologies, Ferrara University, Italy) and by Dr. Nicole Creanza (Department of Biological Sciences, Vanderbilt University, USA). All statistical analyses were performed by me at the Department of Paleoanthropology, Tübingen University, Germany. The work of this project is summarized in **Paper I**.

To address the second objective, I performed **fieldwork in southern Italy** to collect data on archaeological human remains. Between 2014 and 2018, I conducted six field campaigns, totaling approximately six months abroad. During that time, I collected a comprehensive dataset of 481 human skeletal remains with well-preserved dentitions from six archaeological sites from the coastal area of the Gulf of Taranto (for locations of the sites see Figure 1 in Appendix B; for site descriptions see Supplementary Information 1 in Appendix C). Data was collected at the storages of the National Archaeological Museums of Policoro, Metaponto and Taranto. Most of the time, I was accompanied by Dr. Giulia Saltini Semerari (Institute of Classical Archaeology, Tübingen University, Germany) who provided contact with the local museums and fundamentally helped to locate and assign the skeletal material of interest by checking excavation diaries and storage lists. Six undergraduate students from Tübingen and Leiden University completed fieldwork internships as part of this project and greatly assisted in sorting, washing, and labeling the skeletons prior to the osteological data collection. Post-processing and analysis of the gathered data was conducted at the Department of Paleoanthropology, Tübingen University, Germany.[3] The work of this project is summarized in **Paper II** (a preliminary investigation at the start of the project) and **Paper III** (the final investigation at the end of the project).

Paper I (Appendix A): Can dental phenotypic data be used to adequately reconstruct neutral genomic relationships among populations?

In the first study of this cumulative dissertation, I quantified the utility of dental phenotypes as a proxy for neutral genomic variation. To do so, I assessed the correlation of distance estimates between worldwide modern human populations derived independently from dental phenotypic features and neutral genetic markers. I employed two different dental phenotypic datasets for comparison: dental metrics and dental nonmetric traits. Both data types are commonly employed in biodistance studies but have been suggested being useful to different degrees (Coppa et al., 1998). Moreover, I employed two different neutral genomic datasets: Single Nucleotide Polymorphisms (SNPs) and Short Tandem Repeats (STRs). Both data types are used in contemporary genetic studies due to their highly polymorphic nature (Rubicz et al., 2007). It has been proposed that phenotypic variation should be compared to both neutral genomic data types (Reyes-Centeno et al., 2017) since the mutational rate of sequence change and the apportionment of modern human genetic variation is different in SNPs and STRs (Holsinger and Weir, 2009). For comparison I first matched genomic and dental phenotypic population samples from around the world using large existing databases. Matched SNP and dental phenotypic data were available for 19 populations and matched STR and dental phenotypic data were available for a subset of 13 populations (see Figure 1 in Appendix A). I then used the R-matrix method (Relethford et al., 1997) to calculate pairwise population kinship coefficients (r_{ij}) utilizing the genomic and dental phenotypic datasets independently. R-matrix analyses, founded on population and quantitative genetic theory, are most useful for comparing patterns of biological similarity from different types of data (Relethford, 2012). Additionally, these allowed me to correct for the confounding

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[3] The dataset is freely available at: [URL to GitHub will be added here before the publication of this thesis]

effects of genetic drift in different regions of the world by including estimates of effective population sizes (N_e) (Relethford, 1996; Reyes-Centeno et al., 2014). Finally, the associations between genomic and dental phenotypic kinship estimates were statistically assessed using Mantel correlation tests (Mantel, 1967). Dow-Cheverud tests (Dow and Cheverud, 1985) were used to determine whether dental metrics or dental non-metric traits are better suited to track neutral genomic relationships as calculated from SNP and STR data.

Paper II (Appendix B): What was the genetic impact of the Greek colonization on southern Italy? Evidence from population-level biodistance estimates based on dental nonmetric traits

In the second study, I applied a dental biodistance analysis to an archaeological case study: the Greek colonization of southern Italy. Specifically, this study investigates whether there are biological differences between the inhabitants of the Greek colony of Metaponto and indigenous Italic populations in the surrounding hinterland. To do so, I collected dental nonmetric trait data from archaeological human skeletons from three indigenous Italic sites in the vicinity of Metaponto: Incoronata, Santa Maria d'Anglona and Passo di Giacobbe (900-350 y BC). I then compared the dental nonmetric trait frequencies of the indigenous populations to published dental nonmetric trait frequencies of two populations from the Greek colony of Metaponto: the 'urban' necropolis of Crucinia and the 'rural' necropolis of Pantanello (700-250 y BC) (Henneberg, 1998). I used the model-free MMD approach (Harris and Sjøvold, 2004) to assess significant population relationships across the five sampled populations. Estimates were further visualized using a multidimensional scaling plot (Gower, 1966) to facilitate the interpretation of distance results. Finally, the model-bound R-matrix method was used (Relethford et al., 1997) to calculate pairwise population kinship coefficients (r_{ij}) and between-population variation (F_{ST}).

Paper III (Appendix C): What was the genetic impact of the Greek colonization on southern Italy? Evidence from individual-level biodistance estimates based on dental metric and nonmetric traits

The third study takes my previous biodistance study on the Greek colonization of southern Italy to the next level. First, I expanded the study region by collecting additional samples from the Greek colonies of Metaponto, Siris and Taranto (700-200 y BC). Second, I employed a larger battery of dental phenotypic variables and combined metric with nonmetric traits to increase biodistance accuracy. Third, I investigated the study region by chronological time period to trace bio-historical developments through time. Fourth, I performed individual-level biodistance analyses, which are better suited to capture the significant amount of human genetic variation within groups than population-level biodistance estimates. Fifth, I integrated comparative data from Greece in order to identify individual ancestries and to quantify the Greek contribution to southern Italy. This study can be considered as an update of my previous investigation (Paper II) and should provide a more accurate reconstruction of the population history of southern Italy during the Greek colonization.

Specifically, this study examines southern Italian population structure for two chronological time periods: precolonial (900-700 y BC) and postcolonial (700-200 y BC). For each time period, I inferred population structure using adonis, betadisper and isolation-by-distance models (Wright, 1943; Oksanen et al., 2016) based on inter-individual Gower distance coefficients (Gower, 1971) using a mixture of dental metric and nonmetric traits. For the postcolonial period, I furthermore determined individual ancestries using naïve Bayesian classification (Meyer et al., 2017) based on dental nonmetric traits.

3. Results and discussion

This section summarizes the key results from the three papers forming this cumulative dissertation and reviews the main contributions of this PhD project to (1) dental biodistance research; and (2) the study of Greek colonization of southern Italy.

Paper I (Appendix A): Can dental phenotypic data be used to adequately reconstruct neutral genomic relationships among populations?

The first study assessed the level of correlation between dental phenotypic (metrics and nonmetric traits) and neutral genomic (SNPs and STRs) distance estimates across a large set of worldwide modern human populations for which all four datasets were available. Mantel correlation tests showed that distance estimates between human populations based on dental phenotypes are significantly correlated with those based on neutral genetic data (on average: $r = 0.574$, $p < 0.001$) (see Table 1 and Figure 3 in Appendix A). This relatively strong correlation validates tooth form as a proxy for neutral genomic markers. Despite the differences in the mutational change and evolution of SNPs and STRs, both genomic datasets showed the same pattern and a similar degree of correlation with dental phenotypic variation. Although all correlations between dental phenotypes and neutral genetic markers were highly significant, their squared correlation coefficients (r^2) indicated that only part of the dental phenotypic variation can be explained in terms of neutral genetic differences. Dental metric variation explained approximately 31 % of the neutral genetic differences among populations as calculated from SNPs and STRs. Dental non-metric variation explained about 40 % and 30 % of the neutral genetic differences among populations as calculated from SNPs and STRs, respectively. Thus, other non-stochastic factors account for a large portion of the variation in dental morphology of modern humans, and we reason that a substantial portion of the variation can be explained by natural selection on dental morphology. This interpretation is consistent with previous inferences and direct genomic evidence linking non-neutral gene variants with specific tooth characteristics (Park et al., 2012; Mizoguchi, 2013; Hughes et al., 2016).

The results of the Dow-Cheverud test indicated that dental metrics and dental nonmetric traits are both comparably well-suited in tracking neutral genetic relationships as calculated from SNPs and STRs. This result supports the previous finding that different dental phenotypic data types give concordant but varying results, and supports the conclusion that reconstructions of population history are best served when both lines of evidence are investigated (Hemphill, 2016). Although not explicitly tested in this study, this finding further indicates that combining metric with nonmetric markers increases performance compared to using the features separately. This has been shown and suggested by several previous studies (Lease and Sciulli, 2005; Hefner et al., 2014) and should motivate future researchers to integrate multi-scale datasets.

The level of agreement between distance estimates based on dental nonmetric traits and STRs found in this study is comparable to that previously found in the only other study that has tested the association of dental morphology and neutral genomic variation (Hubbard et al., 2015). Furthermore, the quantified degree of correspondence between dental and neutral genetic variation found in this study is similar to that found for other skeletal cranial elements, including the basicranium or the temporal bone (Roseman, 2004; Harvati and Weaver, 2006; Smith et al., 2007; Cramon-Taubadel, 2009; Smith, 2009; Cramon-Taubadel, 2011; Smith et al., 2013; Reyes-Centeno et al., 2017). From this it can be concluded that dental and cranial phenotypes are equally well-suited for reconstructing genetic relationships among populations.

It is important to point out that this study is biased toward finding insignificant correlations between variation in neutral genetics and dental phenotypes. First, we compared matched but unpaired datasets, such that dental samples were from different individuals than those sampled for SNP and STR sequencing. Although it is a common and practical procedure to compare unpaired data at a global scale (Roseman, 2004; Harvati and Weaver, 2006; Smith et al., 2007; Cramon-Taubadel, 2009; Smith, 2009; Cramon-Taubadel, 2011; Smith et al., 2013; Reyes-Centeno et al., 2017), it is likely that such an approach results in sampling bias given that genetic variation between human populations is low compared to within-population variation (Witherspoon et al., 2007). Second, it is possible that the dental metric and non-metric datasets used for this study do not capture enough phenotypic variation. The metric dental dataset used in this study comprised 28 well-established crown width and length measurements, but could be complemented with more and alternative measures, such as diagonal crown measurements and cervical diameters at the cement-enamel junction (Hillson et al., 2005), or other measurements that derive from innovative and more robust 3D imaging techniques not requiring the use of hand-held calipers. Likewise, the dental nonmetric dataset was limited to 12 ASUDAS traits while more than 30 ASUDAS traits have been identified as useful in detecting population relationships (Turner et al., 1991). Furthermore, the study utilized binary nonmetric dental trait counts, although research has shown that dichotomization of ordinal-scaled data into simplified binary categories may skew biological distance results (Nikita, 2015; Edgar and Ousley, 2016). Given the limitations of the study, the levels of association between neutral genetic and dental phenotypic distance estimates must therefore be considered as minimum values and not as exact correlations. This is exciting because it indicates that the true level of congruence may be higher than the relatively strong level of congruence found in this study.

Future work seeking to quantify the exact correlation between dental phenotypic and neutral genomic variation should focus on (1) analyzing paired neutral genetic and dental phenotypic datasets from the same individuals, (2) using a large set of globally distributed population samples, (3) collecting both conventional and alternative dental metric and non-metric traits, and (4) comparing patterns of biological relationships from genetic and dental phenotypic data using similar distance measurement methods. By performing several comparisons using different combinations of dental metric and non-metric traits, future work could potentially identify dental data combinations that are most useful for tracking human population structure and history.

Paper II (Appendix B): What was the genetic impact of the Greek colonization on southern Italy? Evidence from population-level biodistance estimates based on dental nonmetric traits

The second study aimed at reconstructing genetic relationships between the inhabitants of the Greek colony of Metaponto and indigenous Italic populations in the surrounding hinterland by performing a population-level biodistance analysis utilizing dental nonmetric trait frequencies. MMD results showed that the frequency profiles of all populations under investigation significantly differed from each other (see Table 3 and Figure 2 in Appendix B). Moreover, MMD, r_{ij} and F_{ST} estimates revealed greater biological variation between the two geographically proximate Metaponto samples than between the geographically more distant indigenous groups (see Tables 4 and 5 in Appendix B). This indicates that the Greek colony of Metaponto comprised large numbers of people with diverse geographical origins, whereas the indigenous Italic communities of the surrounding hinterland were relatively homogenous. This study is the first to provide direct biological evidence of the arrival of newcomers into the region using a newly gathered set of samples from colonial and indigenous contexts. The results are in general concordance with written sources, archaeological data and present-day DNA evidence, indicating migration influx from Greece from the Archaic period onwards (Yntema, 2000; Tofanelli et al., 2016). Our study showed that dental phenotypic evidence can produce interesting insights into the Greek colonization and should be considered a key tool to be integrated with material culture and ancient written sources-based studies of complex past interactions.

Interestingly, our results disagree with a previous biodistance study performed by Henneberg (1998). She compared the 'urban' and 'rural' samples from Metaponto to two samples from central Italy near Rome dating to the 2nd century BC. She found that the rural people from Metaponto were more closely related to the indigenous Italic populations than to the urban people from Metaponto. Our study, in contrast, revealed similar degrees of differentiation between both Metapontian samples and indigenous populations. This discrepancy could have come about due to Henneberg's use of comparative samples from Rome, which might be problematic in terms of distance in both space (> 500 km) and time (ca. 700 years). We argue that our newly sampled data from indigenous sites in the direct vicinity of Metaponto (< 20 km) with a closer temporal proximity (ca. 350 years difference) are better suited for comparative purposes.

Nevertheless, it is important to point out that the results of this study could potentially be affected by several sources of bias. First, the study compared dental nonmetric trait frequency data collected by different researchers. The indigenous Italic samples were studied by me, while the Metaponto samples were studied by Henneberg (1998). Using data from different observers can introduce inter-observer error owing to slight discrepancies in the scoring technique of ASUDAS traits. Unfortunately, an inter-observer test could not be performed as the primary Metaponto dental data were not accessible at that time. Second, although a variety of dental metric and nonmetric traits were recorded for the indigenous Italic samples, only 12 nonmetric traits could be used for the biodistance analysis. These particular traits were the only ones in the Metaponto samples that were dichotomized using the established ASUDAS thresholds and, therefore, allowed for standardized comparability. Relying on few phenotypic variables, however, is generally problematic as it may reduce the accuracy of biodistance estimates (Sokal and Sneath, 1963). Third, the study compared samples from different time periods. While the samples from Incoronata and Santa Maria d'Anglona date to precolonial times (900-750 y BC), the samples from Passo di Giacobbe, Crucinia and Pantanello date to postcolonial times (700-250 y BC). Comparing samples from different periods may bias biodistance estimates due to diversity introduced by temporal separation. Investigating the study region by chronological time period would have been a neat solution but would have resulted in insufficient study units (i.e. populations) per time slide. Thus, the results of this study should be treated with caution and future research considering the mentioned pitfalls is necessary to generate a more reliable reconstruction of the population history of southern Italy during Greek colonization.

Paper III (Appendix C): What was the genetic impact of the Greek colonization on southern Italy? Evidence from individual-level biodistance estimates based on dental metric and nonmetric traits

The third study takes our previous biodistance study on the Greek colonization of southern Italy further by (1) including additional samples, (2) employing a larger battery of dental phenotypic variables, (3) combining metric with nonmetric traits, (4) investigating the study region by chronological phase, (5) performing individual-level biodistance methods, and (6) integrating comparative data from Greece in order to identify individual ancestries and to quantify the Greek contribution to southern Italy.

Our results showed that southern Italy was characterized by moderate levels of population stratification during precolonial times (900-700 y BC) but became a place of high mobility during postcolonial times (700-200 y BC) (see Figure 2, Tables 2 and 3 in Appendix C). This result is in concordance with the arrival of Greek settlers in the region as indicated by numerous ancient written sources, archaeological finds and present-day DNA evidence (Yntema, 2000; Tofanelli et al., 2016). Moreover, we estimated a Greek contribution of ~ 10 % to the population of southern Italy. Our estimated Greek contribution is concordant with the size spectrum proposed by historical demographers, who reckoned with ~ 10 % Greeks in pre-Roman southern

Italy (Beloch, 1886). Our results also broadly agree with preliminary strontium isotopic evidence for the study region which indicates that 5 % of the postcolonial individuals are of non-local origin.[4] Interestingly, our results disagree with genomic estimates of the ancient Greek contribution to southern Italy derived from present-day DNA profiles. Di Gaetano et al. (2009) used the haplogroup lineage E-V13 to estimate a Greek contribution of ~ 37 % to the population of Sicily and attributed the migration influx to 2380 years before present (CI: 6940 to 675 years ago). A potential explanation for the discrepancy between our estimate and their genomic reconstruction could be that proportions of specific genetic lineages surviving in present-days populations may not be good proxies for assessing the effects of past migration events (see Tofanelli et al., 2016). Moreover, their genomic estimate of the timing of the influx has a wide ranging confidence interval spanning several millennia. The estimate provided in this study is directly derived from archaeological human remains and can therefore be considered a more precise estimate.

We found that Greek immigrants and their descendants were equally distributed across sites and did not live in isolated colonial enclaves. This finding points towards a gradual colonization process with substantial involvement of local populations. This result supports current postcolonial theories about the Greek colonization and matches several archaeological discoveries indicating close interaction (Yntema, 2000; Burgers, 2004). At the colony of Metaponto, for example, an indigenous-style hut was found with mostly Greek-style pottery and a smaller percentage of indigenous pottery (Siena, 1986). Moreover, at the indigenous site of Passo di Giacobbe, several burials contained Greek-style pottery (Schojer, 2010). Thus, our finding fits well the archaeological evidence, suggesting coexistence between a small number of Greek newcomers and indigenous Italic populations.

Lastly, it has to be noted that our results deviate from the findings of our previous dental nonmetric distance study (Paper II), where we found a significant separation between the inhabitants of indigenous Italic sites and the colony of Metaponto which evidenced a higher amount of variability. This discrepancy can be explained as the results of three potential sources of bias in our previous study: (1) bias due to inter-observer error; (2) bias due to the use of a limited number of traits; and (3) distortion

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[4] Isotopic analyses investigating strontium signals of human skeletal remains (n = 20) from the sites of Metaponto and Siris were performed by Anouk Vos (Leiden University) as part of her MSc thesis in 2017.

of the ‘true’ variability of the groups by using group centroid estimates for comparative purposes. The present study overcomes these potential sources of bias. It further highlights the need for individual-level analyses which are better suited to capturing the true variabilities of groups, and cautions against group-level analyses based on simplified centroid estimates, especially in geographically fine-scaled contexts where genetic differences between individuals are subtle.

Our results might be affected by several possible sources of bias. First, our study is limited to archaeological sites from the Gulf of Taranto region. Expanding the study area to Calabria and Sicily and including additional indigenous Italic samples from the inland would help to generate a more comprehensive picture of the colonial history of whole *Magna Graecia*. This study provides a conceptual template for future research in this area and the provided raw data allow for repeatability. Second, there is a need to improve the reference samples used for the individual ancestry determinations. Specifically, this study used two reference samples as potential ancestry sources, ‘Italians’ and ‘Greeks’. However, the high degree of Mediterranean mobility involving a multiplicity of actors in addition to Greek seafarers (for example Phoenicians and Etruscans) leaves open the possibility that multiple agents might have been involved in the colonial process, not all of them necessarily coming from ancient Greece. Given our simplified two-sample study design, such individuals would be forced into one of the two ancestry reference samples, ‘Italian’ or ‘Greek’, regardless of their true ancestry. Using a large number of robust reference samples from all over the Mediterranean would allow us to overcome this problem and, furthermore, opens the possibility to explicitly test for the bio-geographical origin of the colonists. Third and last, it could be that dental phenotypic data may not capture adequate neutral genetic variation for geographically fine-scaled analyses. In fact, dental phenotypic data are considered to be most effective at higher geographic scales of study, particularly global and continental (Scott and Turner, 1997). However, in our study, we use a comprehensive set of mixed metric and nonmetric data with a trait battery outnumbering the variables employed in most dental biodistance studies so far. We therefore think our approach is adequate for detecting subtle genetic structures on a local scale. Nevertheless, future studies should systematically test the association of mixed metric and nonmetric dental datasets and neutral genomic variation on a local scale to identify dental data combinations that are most useful for reconstructing local population structure and history.

4. Concluding remarks and areas for future research

Using human dental remains to reconstruct the population structure and history of past civilizations is a challenging and exciting endeavor with many possible applications for future archaeological and anthropological research. It requires careful and comprehensive data collection and thoughtfully performed data analysis. Today, a wide range of conventional and innovative data collection techniques allow for precise metric and nonmetric data recording and numerous software packages enable researchers to perform highly sophisticated and complex biodistance analyses.

This dissertation demonstrates that even the simplest dental data types (i.e. measurements collected with a hand-hold caliper and nonmetric traits observed with the naked eye) can be used to reliably reconstruct genetic relationships among population. It further motivates researchers to combine metric and nonmetric datasets for biodistance analyses, as an integrative approach increases performance when compared to using the features separately. The author is confident that future work seeking to quantify the association between dental phenotypic and neutral genomic variation has great potential to identify dental data combinations that are most useful for tracking human population structure and history.

This dissertation furthermore applies dental biodistance analyses to an exciting archaeological case study: the Greek colonization of southern Italy. Results support a gradual colonization model with substantial involvement of local populations and contradict the hypothesis that Greek colonies were homogenous enclaves within conquered territories. The case study shows that dental phenotypic evidence can produce new and highly interesting insights into the Greek colonization and should be considered a key tool to be integrated with material culture-based studies of complex past interactions. Expanding the study area to Calabria and Sicily has great potential to generate a detailed picture of the colonial history of the whole *Magna Graecia.* Moreover, including reference samples from several areas in ancient Greece and elsewhere in the Mediterranean opens up the possibility to explicitly test for the bio-geographical origin of the colonists. The case study in this dissertation provides a conceptual template for future research on the Greek colonization of southern Italy and the provided raw data allow for repeatability.

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Appendix A

Reconstructing human population history from dental phenotypes

Hannes Rathmann, Hugo Reyes-Centeno, Silvia Ghirotto, Nicole Creanza,
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Published in Scientific Reports (2017) 7: 12495[1]
DOI: 10.1038/s41598-017-12621-y

SCIENTIFIC REPORTS

OPEN

Reconstructing human population history from dental phenotypes

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Received: 6 July 2017
Accepted: 7 September 2017

Dental phenotypic data are often used to reconstruct biological relatedness among past human groups. Teeth are an important data source because they are generally well preserved in the archaeological and fossil record, even when associated skeletal and DNA preservation is poor. Furthermore, tooth form is considered to be highly heritable and selectively neutral; thus, teeth are assumed to be an excellent proxy for neutral genetic data when none are available. However, to our knowledge, no study to date has systematically tested the assumption of genetic neutrality of dental morphological features on a global scale. Therefore, for the first time, this study quantifies the correlation of biological affinities between worldwide modern human populations, derived independently from dental phenotypes and neutral genetic markers. We show that population relationship measures based on dental morphology are significantly correlated with those based on neutral genetic data (on average $r = 0.574$, $p < 0.001$). This relatively strong correlation validates tooth form as a proxy for neutral genomic markers. Nonetheless, we suggest caution in reconstructions of population affinities based on dental data alone because only part of the dental morphological variation among populations can be explained in terms of neutral genetic differences.

In archaeological and paleontological studies, dental phenotypic data are often used to estimate biological relatedness among past human groups, in order to reconstruct migration events, population histories, or hominin phylogenies[1–11]. Dental morphology has become a favored dataset primarily because teeth are generally well preserved in the archaeological and fossil record, even when associated skeletal and DNA preservation is relatively poor. Their better state of preservation results in teeth being recovered in higher quantities and, therefore, allows studies to employ larger samples and more robust statistical analyses. Furthermore, tooth form has been proposed to be highly heritable, selectively neutral, and evolutionarily conservative, thus, providing an excellent proxy for neutral genetic data[12,13]. Tooth crowns develop relatively early in the life of an individual and their form is not altered after full formation, except by wear or pathology. Finally, dental phenotypic data can be sampled in a non-destructive, cost-efficient, and straightforward manner using crown width and length measurements (hereafter, dental metrics) or visual scoring of well-established crown and root shape variants (hereafter, dental non-metric traits).

Despite the popularity of population genetic studies utilizing dental phenotypes as proxies for genetic markers, less than a handful of studies have attempted to directly test the level of congruence between population distance measures based on these two data types[14–17]. Those previous investigations found contradicting results, with some of them reporting weak to strong correlations, whereas others found that dental and genetic distances produced fundamentally different patterns of group relationships. Thus, the utility of dental morphology as an efficient proxy for genetic data, formally tested in human population genetic analyses, is currently unresolved. It also has to be noted that those previous studies were limited by several factors. First, most used serological data as genetic markers; however, contemporary genetic studies commonly utilize either single nucleotide polymorphisms (SNPs) or short tandem repeats (STRs) due to their highly polymorphic nature[18]. In fact, it has been proposed that phenotypic variation should be compared to both neutral genomic data types[19–21] since the mutational rate of sequence change and the apportionment of modern human genetic variation is different in SNPs and

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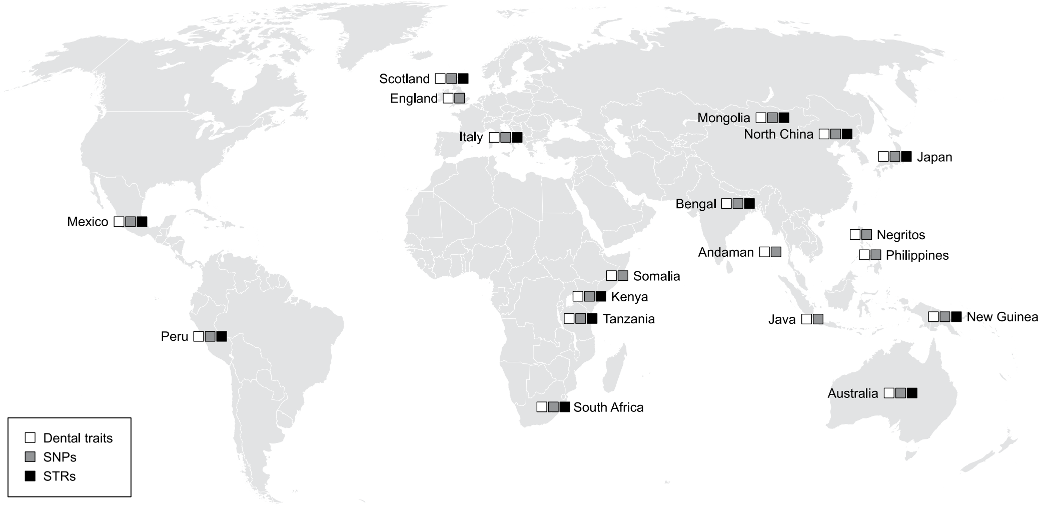

Figure 1. Location of the modern human population samples used in this study. White squares indicate that the population was sampled for dental metric and non-metric traits. Grey squares indicate that the population was sampled for single nucleotide polymorphisms (SNPs). Black squares indicate that the population was sampled for single tandem repeats (STRs). Word map modified from BlankMap-World6, available at https://commons.wikimedia.org/wiki/File:BlankMap-World6.svg (Public Domain).

STRs[22]. Second, most previous studies were limited to dental non-metric trait data; however, dental metrics are another important data source for biological distance studies and some researchers argue that crown measurements may be collected with lower observer error than dental non-metric traits[23]. Third, all previous studies were limited to regional scales, with some of them analyzing only a few population samples, which reduces the power of statistical correlation tests between dental and genetic distance estimates. A study seeking to investigate dental morphological and neutral genetic correspondence with a large set of globally distributed population samples is still pending.

Here, for the first time, we seek to test for correlations of biological affinities among globally distributed modern human populations, derived independently from diverse dental phenotypic markers (metrics and non-metric traits) and neutral genetic loci (SNPs and STRs). To do so, we first matched genomic and dental phenotypic population samples from around the world using existing databases. Matched SNP and dental phenotypic data were available for 19 populations and matched STR and dental phenotypic data were available for a subset of 13 populations (Fig. 1). We then used the R-matrix method[24] to calculate pairwise population kinship coefficients (r_{ij}) utilizing the genomic and dental phenotypic datasets independently. R-matrix analyses, founded on population and quantitative genetic theory, are most useful for comparing patterns of biological similarity from different types of data and, additionally, allowed us to correct for the confounding effects of genetic drift in different regions of the world by including estimates of effective population sizes (N_e)[19,24,25]. Finally, we statistically assessed the associations between genomic and dental phenotypic kinship estimates using Mantel correlation tests. Dow-Cheverud tests were then used to determine whether dental metrics or dental non-metric traits are better suited to track neutral genomic relationships as calculated from SNP and STR data.

Results

Figure 2 illustrates biological affinities among globally distributed modern human populations, derived independently from dental phenotypic markers and neutral genetic loci. While SNPs and STRs gave largely concordant results, dental metric and non-metric traits revealed a somewhat different pattern. Overall, dental phenotypes successfully classified populations in broader geographic and continental areas.

Our results show that kinship estimates between human populations based on dental phenotypes are significantly correlated with those based on neutral genetic data (Table 1, Fig. 3). The correlation values were relatively strong and similar for all four data type comparisons. Dental metric variation explained approximately 31% of the neutral genetic differences among populations as calculated from SNPs and STRs. Dental non-metric variation explained about 40% and 30% of the neutral genetic differences among populations as calculated from SNPs and STRs, respectively.

Table 1 also presents the results of the Dow-Cheverud test, which determined whether dental metrics or dental non-metric traits are significantly more strongly correlated with either SNP or STR markers. None of the comparisons were significant, indicating that dental metrics and dental non-metric trait data are both comparably well-suited in tracking neutral genetic relationships as calculated from SNPs and STRs.

Discussion

Our results validate the use of dental phenotypic data to infer neutral genetic relationships among human populations. This, at least to some extent, confirms the previous hypothesis[13] that the worldwide human dental variation was primarily generated by random processes of genetic drift. We also found that different dental phenotypic data types, i.e. metric and non-metric traits, are both well-suited in serving as proxies for neutral genetic markers. This

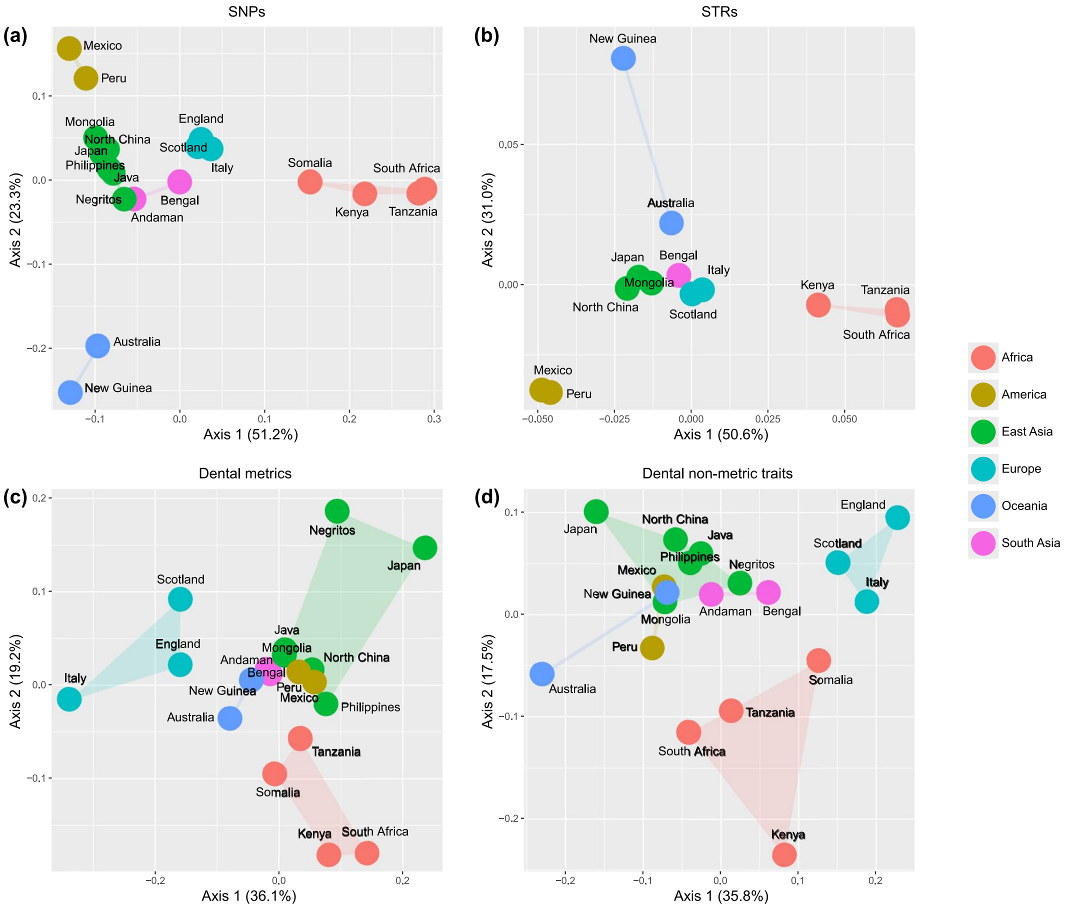

Figure 2. Biological distances among human populations (d_{ij}) generated from neutral genetic and dental phenotypic data. Figures show scatterplot of the first two principal coordinates of: (**a**) d_{ij} distances generated from SNPs; (**b**) d_{ij} distances generated from STRs; (**c**) d_{ij} distances generated from dental metrics; and (**d**) d_{ij} distances generated from dental non-metric traits.

	SNPs (19 populations)	**STRs (13 populations)**
Dental metrics	0.558 (<0.001)[1]	0.556 (<0.001)[1]
Dental non-metric traits	0.635 (<0.001)[1]	0.547 (<0.001)[1]
Dental metrics vs. Dental non-metric traits	0.074 (0.155)[2]	−0.008 (0.464)[2]

Table 1. Mantel and Dow-Cheverud tests. [1]Mantel test of dental phenotypic kinship coefficients (r_{ij}) against neutral genetic r_{ij}. Reported values are Pearson correlation coefficients (r) and two-tailed significance (p, in parentheses) after 10,000 permutations. All comparisons are statistically significant after Bonferroni correction for multiple testing at $\alpha = 0.025$. [2]Dow-Cheverud test of dental metric r_{ij} versus dental non-metric r_{ij}. Reported values are correlation coefficients ($p1Z$) and two-tailed significance (p, in parentheses) after 10,000 permutations. Positive correlation values indicate that dental metrics are more strongly correlated with neutral genetics. Negative correlation values indicate that dental non-metric traits are more strongly correlated with neutral genetics. None of the results are statistically significant at $\alpha = 0.05$.

result supports the previous finding that different dental phenotypic data types give concordant but varied results, and the conclusion that reconstructions of population history are best served when both lines of evidence are investigated[23]. Although all correlations between dental phenotypes and neutral genetic markers were highly significant, their correlation coefficients indicated that only part of the dental phenotypic variation can be explained in terms of neutral genetic differences. Other non-stochastic factors therefore account for a large portion of the variation in dental morphology of modern humans. Because we controlled for the effect that sexual dimorphism, pathology, and wear can have on teeth, we reason that a substantial portion of the variation can be explained by natural selection on dental morphology. This interpretation is consistent with previous inferences and direct genomic evidence linking non-neutral gene variants with specific tooth characteristics[26–28].

While it has been shown or suggested that linguistic and skeletal phenotypic variation can correlate differentially with genomic variation based either on SNPs or STRs[19,29], our results do not suggest this to be the case

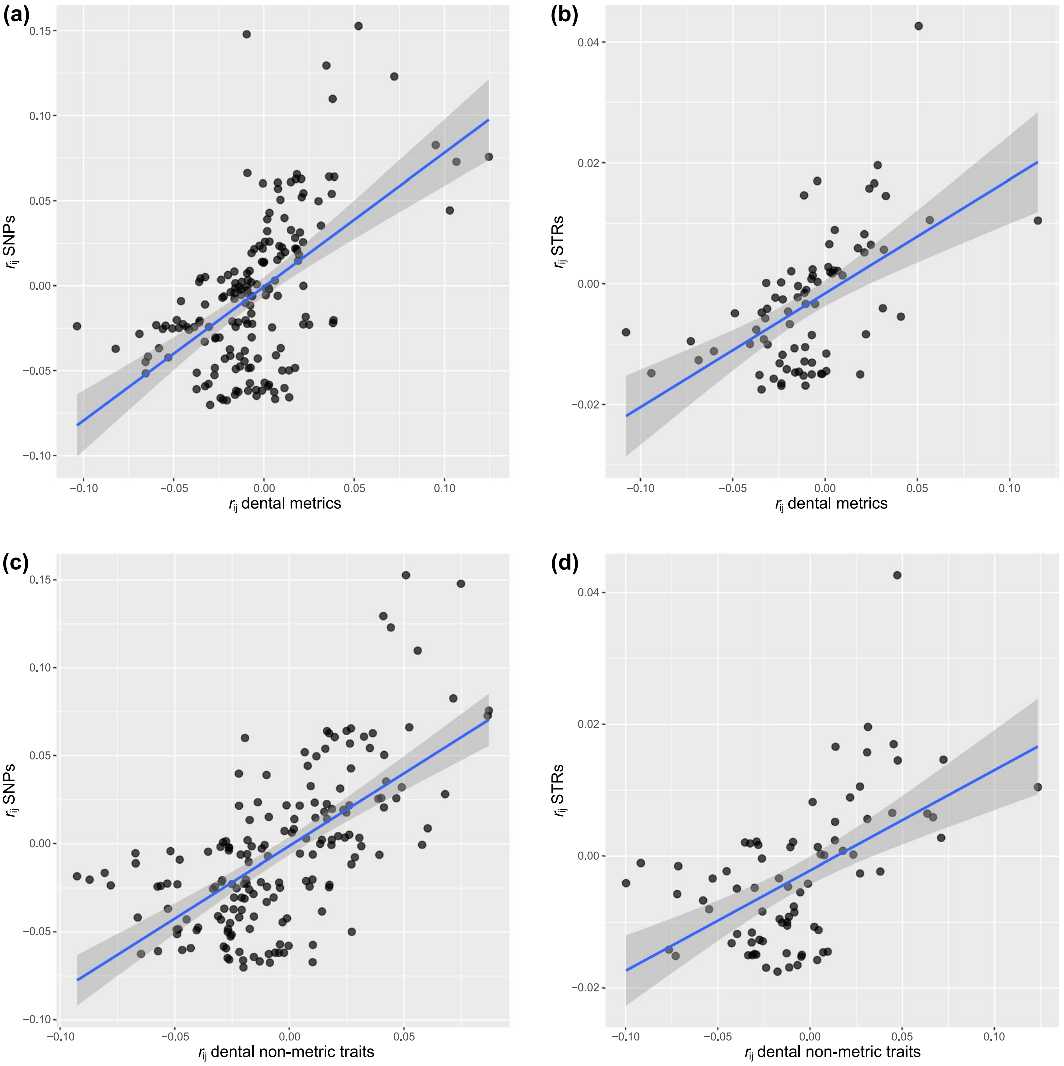

Figure 3. Regression of pairwise kinship coefficients among human populations (r_{ij}) generated from neutral genetic and dental phenotypic data. Figures show scatterplot, linear regression line, and 95% confidence interval of: (**a**) r_{ij} values generated from SNPs versus r_{ij} values generated from dental metrics; (**b**) r_{ij} values generated from STRs versus r_{ij} values generated from dental metrics; (**c**) r_{ij} values generated from SNPs versus r_{ij} values generated from dental non-metric traits; and (**d**) r_{ij} values generated from STRs versus r_{ij} values generated from dental non-metric traits.

for dental phenotypic variation. Despite the differences in the mutational change and evolution of SNPs and STRs[30,31], both genomic datasets showed the same pattern and a similar degree of correlation with dental phenotypic variation.

The level of agreement between kinship estimates based on dental non-metric traits and STRs found in this study is comparable to that previously found in the only other study that has tested the association of dental morphology and neutral genomic variation[17]. That study compared dental and genetic distances among four modern groups in Kenya using paired data from 295 individuals. They calculated dental distances using a Mahalanobis-type (D^2) distance for binary data[32] derived from nine non-metric crown traits. Genetic distances were estimated using a delta-mu squared (Ddm) distance[33] utilizing 42 STR loci. They compared both distance matrices with a Mantel test and found a moderate to strong positive correlation between the two distance types, although this result was not significant ($r = 0.500$, $p = 0.21$). The correlation coefficient reported here is slightly higher ($r = 0.547$, $p < 0.001$). This could be due to the larger battery of dental traits employed (12 traits vs. 9 traits), the higher geographic scale of analysis (global scale vs. regional scale), and/or the use of different biological relationship measures (R-matrix comparisons vs. D^2 against Ddm). Moreover, the correlation reported here is highly significant, whereas the correlation presented by ref.[17] was not, albeit that result was probably due to the Mantel test design based on only four populations.

More broadly, the quantified degree of correspondence between dental and neutral genetic variation reported here is similar to that found for other skeletal cranial elements[19,34–40]. Dental and cranial phenotypes are therefore equally well-suited for reconstructing genetic relationships among populations. However, we caution that previous studies on the association of cranial and genomic variation are not directly comparable to ours since different populations have been sampled and diverse methodological approaches have been employed.

It is important to point out that our study is biased toward not finding significant correlations between variation in neutral genetics and dental phenotypes. First, we compared matched but unpaired datasets, such that dental samples were from different individuals than those sampled for SNP and STR sequencing. Although it is a common and practical procedure to compare unpaired data at a global scale[19,34–40], it is likely that it results in sampling bias given that genetic variation between human populations is low compared to within-population variation[41]. Second, it is possible that the dental metric and non-metric datasets employed in this study do not capture adequate phenotypic variation. Our metric dental dataset comprises well-established crown width and length measurements, but could be complemented with alternative measures, such as diagonal crown measurements and cervical diameters at the cement-enamel junction[42], or other measurements that derive from innovative and more robust 3D imaging techniques not requiring the use of hand-held calipers. Likewise, our dental non-metric dataset was limited to 12 traits while more than 30 traits have been identified as useful in detecting population relationships[43]. Furthermore, we utilized binary non-metric dental trait counts, although recent research has shown that dichotomization of ordinal-scaled data into simplified binary categories may skew biological distance results[44,45]. Given the limitations of our study, the levels of association between neutral genetic and dental phenotypic kinship estimates reported here must therefore be considered as minimum values and not as exact correlations. Paired data from individuals sampled worldwide, as has been employed at a smaller scale[17], would provide a more accurate estimate of genetic and dental phenotype associations.

In conclusion, our results confirm that dental phenotypic data can be used as a proxy for neutral genomic data in studies of population relatedness, although we suggest caution and careful choice of dental features because only part of the dental variation among populations can be explained in terms of neutral genetic differences. Future work should focus on (1) analyzing paired neural genetic and dental phenotypic datasets from the same individuals, (2) using globally distributed population samples, (3) collecting both conventional and alternative dental metric and non-metric traits, and (4) comparing patterns of biological similarity from genetic and dental phenotypic data using the same quantitative genetic model. By performing several comparisons using different dental fields and different combinations of dental metric and non-metric traits, future work could potentially identify dental data combinations that are most useful for tracking human population history.

Materials and Methods

Matching population samples. Materials for this study comprise four different types of data: SNP allele frequencies, STR allele frequencies, dental metrics, and dental non-metric traits. All data were taken from existing databases. We matched datasets for several globally distributed modern human populations for which both genetic and phenotypic data were available (Fig. 1, Supplementary Table S1). Populations were chosen for inclusion in this study based on two criteria: first, availability of sufficient number of dental phenotypic specimens (i.e. both dental metrics and non-metric traits); and, second, availability of neutral genetic data (i.e. SNPs and/or STRs). In instances where exact population matches could not be achieved, a geographically similar population with ethno-linguistic affinities was selected. Matched SNP and dental phenotypic data were available for 19 populations; however, STR data were only available for a subset of 13 populations. We note that the matched populations are unpaired samples; that is, dental samples derive from different individuals than the genetic samples.

Neutral genetic data. SNP allele frequencies were collated from various datasets[46–55] for 19 populations comprising $n = 1652$ individuals sharing 1778 markers. The SNP data were merged using the plink 1.07 software[56] and polymorphisms possibly causing strand ambiguities (A/T or C/G) were removed. We then exploited the extent of linkage disequilibrium (LD) observed in each population to obtain an estimate of the effective population size (N_e) through time. Linkage disequilibrium levels have been estimated independently in each population using all SNP markers available for that population. We evaluated for each SNP the genetic map position, and for each pair of SNPs separated by less than 0.25 cM we quantified LD as the r^2_{LD}, calculated in plink 1.07. All observed r^2_{LD} values were then binned into one of 250 overlapping recombination distance classes, from 0.005 cM to 0.25 cM. Following refs[25,57], pairs of SNPs separated by less than 0.005 cM were not considered, and the adjusted r^2_{LD} values were corrected for sample size. We finally calculated the effective population size in each recombination distance class through the formula: $N_e = (1/4c)[1/r^2_{LD} - 2]$, which corresponds to the effective population size 1/2c generations ago, where c is the distance between loci, expressed in Morgans[58]. The long-term N_e for each population was then calculated as the harmonic mean of the values of N_e over all the recombination distance classes. The estimated N_e values for each population are reported in the Supplementary Table S1.

In addition to the SNP data, we analyzed a dataset of STR allele frequencies that combined data from several studies; the merging of data is described in ref.[59]. Specifically, we used their MS5255 dataset, which has genotype data from 645 loci for 265 worldwide populations. At each locus, allele sizes are recorded for each individual. Following refs[60,61], we tested for individual outliers by generating a matrix of individuals by alleles, performing a principal components analysis on this matrix, and defining an outlier as an individual with a score more than six standard deviations from the mean of any of the first four principal components. None of the individuals met these criteria, so all individuals were considered for further population-level analyses. We then restricted the dataset to $n = 265$ individuals in the 13 populations with both STR and dental data as described above.

Dental phenotypic data. The dental phenotypic data were collected by one of us (T.H.) and comprise dental metrics and dental non-metric traits from mostly the same individuals. Several samples are from collections of known age and sex. When demographic data were not available, age and sex were determined by T.H. using standard osteological methods[62]. When possible, approximately equal numbers of adult males and females were measured for both dental datasets for each population. However, we note that overall, the datasets are biased in representing more males. Detailed information on the composition of the morphological datasets, such as country of origin, ethnic affiliation, and cultural background is given elsewhere[63,64]. We excluded samples older than 2.000 years in order to avoid temporal bias.

The dental metric dataset consists of mesio-distal and bucco-lingual crown diameters of all teeth recorded for each individual (up to a total of 28 metric variables, excluding third molars). Only right teeth were measured, but when a right tooth was missing, damaged, or affected by wear or pathology, the corresponding left antimere was measured. All measurements were recorded according to the procedures of ref.[12] using a digital sliding caliper accurate to 0.01 mm. T.H. quantified his level of intra-observer error by separately re-measuring a Japanese sample; measuring error was found to be insignificant[63]. Because not every tooth could be observed for each individual due to poor preservation or pathology, the dataset comprises large amounts of missing values. The multivariate statistical methods performed here require complete datasets; however, removing individuals with missing values would eliminate the bulk of the sample. Thus, missing data were imputed following ref.[65] using the *k*-nearest neighbor (kNN) algorithm, conducted in the software R 3.3.1[66] using the VIM package[67]. The kNN algorithm searches the entire dataset for cases most similar to the one with missing data and generates a mean to replace the missing value(s). Prior to imputation, individuals with more than half of the measurements missing were removed from the analysis. In this way we ensured that less than 22% of the final dataset requires imputation (down from 56%). Raw measurements were then converted into shape variables by dividing each measurement by the geometric mean for all the measurements in each individual[68]. This standardization procedure removes gross size from the data in order to assess differences in the proportionate contribution of individual variables to overall tooth size. This procedure also has the advantage to adjust for size differences between individuals that may result from sexual dimorphism. A table listing the summary statistics of the dental metric dataset is provided in the Supplementary information (Table S2).

The dental non-metric trait dataset consists of observations for 15 morphological variables in the permanent dentition according to procedures detailed in ref.[64]. The 15 traits include characteristics attributed to the Asian[69], European[70], and sub-saharan African dental complex[71,72], as well as the key crown traits that distinguish continental Southeast Asians from island Southeast Asians[73]. Most (14 of 15) traits follow the widely used Arizona State University Dental Anthropology System (ASUDAS) described by ref.[43]. This system has as reference set of dental casts illustrating expression levels for various traits and specific instructions that ensure a standardized scoring procedure that minimizes observer error. Although observations were made on both antimeres, scoring followed the individual count method[74], where a trait was counted only once per dentition, regardless of whether or not the trait appeared bilaterally. In cases where a trait was expressed asymmetrically, we followed the standard ASUDAS protocol and scored the side with the highest expression level[4,5,75,76]. The dental observations were originally scored in a graded fashion and were subsequently dichotomized into simplified categories of presence or absence following the dichotomization thresholds detailed in ref.[64]. Thus, our final dataset consists of binary dental trait information (i.e. 0 = absent, 1 = present) for each individual. The multivariate statistical methods performed here can handle incomplete datasets; however, the amount of missing data should be reduced as much as possible in order to prevent non-positive-semidefinite dispersion matrices[44]. We therefore removed the most incomplete variables and individuals from the analysis in a systematical stepwise manner so that the final dataset consists of less than 40% missing data (down from 60%). Most dental traits listed in the ASUDAS have low or no sexual dimorphism[13], which allows for pooling of sexes[4,64,76]. A table listing the final dental non-metric dataset is given in the Supplementary information (Table S3).

Generating population affinity matrices. We independently estimated genetic and dental phenotypic affinities between the sampled populations using the R-matrix method. The R-matrix method was originally developed to work with allele frequency data[77] and was later modified for use with morphometrics[24] and non-metric traits[78]. These extensions make R-matrix analyses most useful for comparing patterns of biological relationships from different types of data[79]. The off-diagonal elements of an R-matrix quantify the biological relationship between population pairs with values ranging from +1 to −1. Those values are covariances about the regional centroid and are defined as average kinship coefficients (r_{ij}). Positive r_{ij} values indicate that two populations exhibit greater biological similarity than on average, and negative r_{ij} values denote that two populations are more distinct than on average. Moreover, the R-matrix can be scaled by weighing the samples by their population sizes in order to account for the confounding effects of genetic drift on small populations. Here, we included point values of effective population size (N_e) derived from levels of genetic linkage disequilibrium (values are reported in the Supplementary Table S1). The phenotypic R-matrices were calculated with a heritability estimate of $h^2 = 0.5$, reflecting the approximate average of various heritability estimates of dental anatomy based on twin and family studies[63,64].

Genetic R-matrices were generated from the allele frequency data using the RMAT 1.2 software, following the model described by ref.[77]. Genetic R-matrices were constructed for all 19 populations using the SNP data and for the subset of 13 populations using the STR data. Dental metric R-matrices were generated from the crown width and length measurements using the RMET 5.0 software, following the model described by ref.[24]. We constructed two dental metric R-matrices; one for the 19-population setup and a second for the 13-population subset. Dental non-metric R-matrices were generated from the discrete crown traits in R 3.3.1, following the methodology detailed in refs[78,80,81]. As with the dental metric dataset, we constructed two dental non-metric R-matrices; one for the 19-population dataset and a second for the 13-population subset. All estimated R-matrices are reported in the Supplementary information (Tables S4–S9).

Comparing population affinity matrices. To measure the degree of association between genetic and dental phenotypic population kinship coefficients, we followed the protocol set forth by refs[35,40] and compared the off-diagonal R-matrix values using Mantel tests. Mantel tests measure the congruence between two matrices against a null model and assess statistical significance via a permutation procedure[82]. Genetic R-matrices based on SNPs and STRs were compared independently against the phenotypic R-matrices based on dental metrics and dental non-metric traits. The Mantel tests were conducted in R 3.3.1 using the vegan package[83]. Correlation significance was determined after 10.000 random permutations and significance levels were set to $\alpha = 0.025$ to correct for multiple comparisons (Bonferroni correction: $\alpha = 0.05/2$). We interpreted correlation strength following the convention of ref.[84]. We furthermore visualized the association of R-matrix values in regression plots, generated in R 3.3.1 using the ggplot2 package[85]. In addition to the Mantel tests, we performed Dow-Cheverud tests[86] in order to determine whether dental metrics or dental non-metric traits could be considered significantly more strongly correlated with neural genetic variation as calculated from SNPs and STRs. Dow-Cheverud tests were conducted in R 3.3.1. Correlation significance was determined after 10.000 random permutations and significance levels were set to $\alpha = 0.05$. We furthermore visualized population affinities generated from neutral genetic and dental phenotypic data by deriving pairwise distances from the R-matrices[24] and plotting them using principal coordinates analysis in R 3.3.1 employing the vegan[83] and ggplot2[85] packages.

Data availability. The data that support the findings of this study are available from T.H., S.G., and N.C. but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of T.H., S.G., and N.C.

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Acknowledgements

This work was supported by the German Research Foundation (DFG FOR 2237: Project ‘Words, Bones, Genes, Tools: Tracking Linguistic, Cultural, and Biological Trajectories of the Human Past’) and by a Gerda Henkel Foundation doctoral research grant (awarded to H.R.). We thank Mark Grabowski, Lyle Konigsberg, John Relethford, and Charles Roseman for kindly making available software packages and computer programming code used in this study. We also thank Mei-Shin Wu for assistance in processing the genomic data.

Author Contributions

T.H. collected data; H.R., H.R.-C., S.G. and K.H. designed research; H.R., H.R.-C., S.G., and N.C. performed research and analysed data; H.R., H.R.-C., S.G., N.C., and K.H. wrote the paper.

Additional Information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-017-12621-y.

Competing Interests: The authors declare that they have no competing interests.

Appendix B

Evidence for migration influx into the ancient Greek colony of Metaponto: A population genetics approach using dental nonmetric traits

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Published in International Journal of Osteoarchaeology (2017) 27: 453-464[1]
DOI: 10.1002/oa.2569

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International Journal of Osteoarchaeology
Int. J. Osteoarchaeol. **27**: 453–464 (2017)
DOI: 10.1002/oa.2569

Evidence for Migration Influx into the Ancient Greek Colony of Metaponto: A Population Genetics Approach Using Dental Nonmetric Traits

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ABSTRACT Ancient Greek colonies were founded by Greek seafarers all along the Mediterranean coast as early as the eighth century BC. Despite extensive archaeological and historical research, the population structure of the inhabitants of Greek colonies and their relationship to indigenous populations are still debated. Here, we perform a biodistance analysis to reconstruct migration and gene flow between the ancient Greek colony of Metaponto (southern Italy) and indigenous groups in the surrounding hinterland (900–250 BC). We collected dental nonmetric trait data of 355 human skeletons from the indigenous Italic sites of Santa Maria d'Anglona, Incoronata and Passo di Giacobbe. This data set is compared with an urban and rural sample of the Greek colony of Metaponto comprising 351 individual dentitions. The R-matrix approach is used to estimate inter-population relationships and F_{ST}. The resulting kinship coefficients indicate that the three indigenous groups exhibit greater similarity to each other and possess lesser similarity to the two Metapontian samples. Interestingly, the two samples of Metaponto are least similar to each other, although they are geographically very close. The F_{ST} estimates confirm this pattern and reveal greater biological variation between the two nearby Metaponto samples ($F_{ST} = 0.0603$) than between the three geographically fairly distant indigenous groups ($F_{ST} = 0.0389$). We conclude that the Greek colony of Metaponto included large numbers of people with diverse geographical origins, whereas the indigenous Italic communities of the surrounding hinterland exhibited high levels of homogeneity and cohesion. Copyright © 2016 John Wiley & Sons, Ltd.

Key words: biological distance; dental nonmetric traits; Greek colonisation; migration; southern Italy

Introduction

Starting from the eighth century BC, people coming from ancient Greece started to settle along the coasts of the Mediterranean. Greek colonies were founded at the shores of the Black Sea, Anatolia, southern Italy, Sicily, North Africa and along the coast of southern France and Spain. Archaeological evidence suggests that they initially settled in small groups, sometimes embedding themselves within the local indigenous populations. With time, however, Greek colonies became increasingly urbanised and culturally distinct settlements. The Greek colonisation has been called 'one of the most important cultural encounters in world history' (De Angelis, 2016: 101), and its consequences in Mediterranean history were profound and long-lasting. It contributed to the creation of a Mediterranean-wide network of exchanges (Malkin, 2005), to the development of urbanisation along its shores (Malkin, 1994) and to the spread of the alphabet (Boardman, 2014).

Decades of extensive archaeological and historical research have greatly enhanced our understanding of this process, yet no consensus has been reached by researchers regarding its interpretation. Especially contentious are questions concerning the geographical origin of the colonisers, and whether and to what extent indigenous populations actively participated in the colonial process (see, for example, contributions in

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Received 17 February 2016
Revised 9 July 2016
Accepted 29 September 2016

Donnellan *et al.*, 2016a, 2016b, with bibliography). Although focused on interaction, this debate has mostly been waged using ancient written sources, standard typological associations of artefacts, burial practices or architectural elements. Surprisingly, large-scale biological investigations, which could provide crucial information regarding migration and admixture, remain rare (with a few recent exceptions, e.g. McIlvaine *et al.*, 2014; Tofanelli *et al.*, 2015).

This paper evaluates the population structure of the colonised coastal area along the Gulf of Taranto in southern Italy (Figure 1). This region is home to three major Greek colonies that developed in the course of the late 8th to 6th centuries BC (Metaponto, Taranto and Siris). It also provides extensive archaeological evidence of indigenous sites and 'mixed' Greek-indigenous contexts dating to the earliest phases of colonisation (De Siena, 1986; Berlingò, 1993; Carter, 2006; Burgers & Crielaard, 2007; Denti, 2009; Bianco, 2012; Crielaard & Burgers, 2012). Here, we performed a biological distance analysis to reconstruct migration and gene flow between the ancient Greek colony of Metaponto and indigenous populations in the surrounding hinterland. According to written sources, the colony of Metaponto was founded in the course of the seventh century BC by Achaeans from the northern Peloponnese (Carter, 1990a, 1990b). Biological distances were generated on the basis of the R-matrix approach (Relethford & Blangero, 1990) using dental nonmetric trait data (Turner *et al.*, 1991). Our analysis addressed the following questions: (i) How are colonial and indigenous populations biologically related to each other? (ii) Is there evidence for Greek immigration into the colony of Metaponto? (iii) Did the inhabitants of the colony admix with the indigenous people of the surrounding hinterland?

The debate over Greek colonisation

Traditional approaches have focused on the relationship between colonies and their putative 'mother cities' (city from which the colonisers allegedly originated as indicated by ancient literary sources), searching for similarities in their cults, traditions and material culture (Boardman, 1964; Dunbabin, 1968; contributions in Pugliese Carratelli, 1985, 1996; Greco, 2002; Lombardo, 2009). Accounts of the early phases of colonisation were often directly derived from literary sources, as the archaeological evidence dating to that time was still scarce. Central to these studies was the idea that colonies were 'well-organised state enterprises' (Graham, 1981: 314), whose foundation had been directed by the mother city and whose population was substantially homogeneous. In this view, the role of local populations, if at all considered, was essentially passive. They were deprived of land, resources and (possibly) women to fulfil the colonies' needs while being the object of gradual acculturation (or 'hellenisation') (Burgers, 2004; Shepherd, 2005a). However, this perspective has changed considerably in the last two decades owing to an increase in archaeological

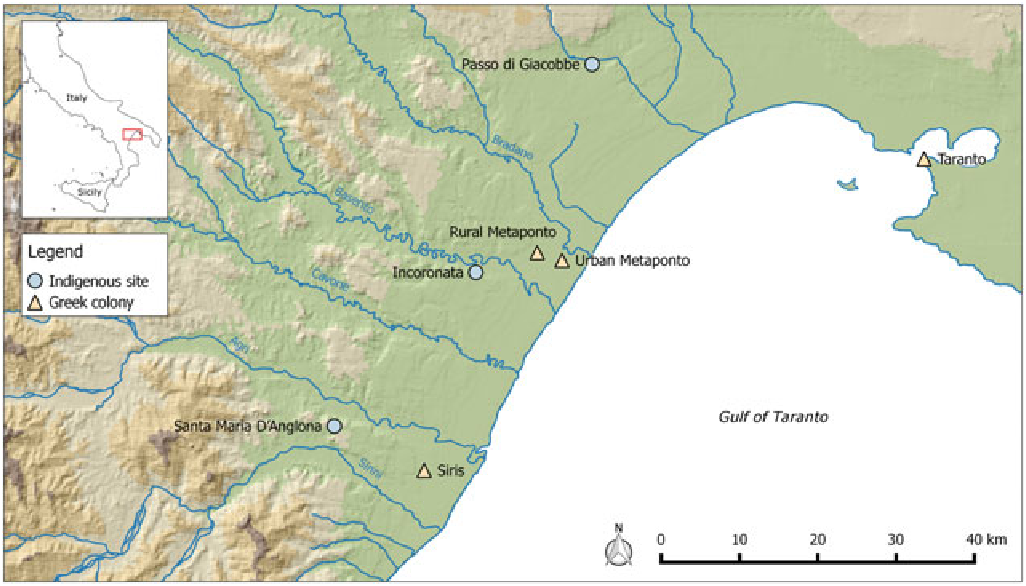

Figure 1. Map of the coastal area along the Gulf of Taranto showing archaeological sites mentioned in the text. [Colour figure can be viewed at wileyonlinelibrary.com]

data and to the reconsideration of traditional narratives through the lens of new theories.

Archaeological discoveries in almost every region where Greek colonisation occurred show a complex picture that resists simple associations between assemblages and specific mother cities (Shepherd, 2005b; Yntema, 2011). For example, painted pottery typical of different areas of Greece, such as Corinth, Athens, Euboea and Laconia, were often both imported and locally produced within one colony (Osborne, 1998; Yntema, 2000). Moreover, elements from local 'indigenous' material cultures and customs have also been found in the early phases of most colonies (Buchner, 1975; Berlingò, 1993; Bianco, 2012, to name just a few). In Metaponto, for example, an indigenous-style hut was found with mostly Greek-style pottery and a smaller percentage of indigenous productions (De Siena, 1986). Conversely, some non-colonial sites show evidence of 'mixed' assemblages, possibly pointing to coexistence between Greeks and indigenes (Burgers & Crielaard, 2007; Crielaard & Burgers, 2012; Isler, 2012). One of those sites, which produced some of the most compelling evidence of coexistence, is Incoronata. Structures in both Greek and indigenous building traditions were found (Carter, 2006), as well as a large pottery production site that included locally produced Greek and indigenous wares, alongside with some interesting 'hybrid' examples (Denti, 2009). Thus, the archaeological evidence associated with the early colonisation points to more complex realities that contrast with the proposed homogeneity of traditional colonial narratives.

The direct involvement of local populations in the process of colonisation has also come into sharper focus with the adoption of postcolonial theories in archaeological discourse (Van Dommelen, 1997, 2012; Burgers, 2004; Malkin, 2004). Guided in part by these theoretical insights, some archaeologists have shown that local populations underwent independent patterns of socioeconomic development and political strife (Peroni, 1994; Vanzetti, 2002; Burgers, 2004). They also highlight the existence of patterns of close interaction and 'mixing', whether interpreted within the theoretical framework of frontiers (Lombardo, 1999; Spatafora & Vassallo, 2002; Greco, 2011; De Angelis, 2016), middle ground (Malkin, 2002) or hybridisation (Van Dommelen, 2005). More recently, the idea that such interactions may have impacted concepts of identity beyond a binary coloniser–colonised system has come to the fore and led to new conceptualisations of contacts as fluid and characterised by a multiplicity of agents (Hodos, 2009; Giangiulio, 2010; Kistler, 2012). In sum, views of the Greek colonisation have gradually shifted from a centrally organised event involving a uniform group of individuals to a less homogeneous, long-term process strongly shaped by interactions. However, these arguments have not gained universal traction and are still hotly contested, especially when it comes to the relation between Greeks and indigenous populations (see, for example, the most recent contributions in Greco, 2011; Nijboer, 2011; Yntema, 2011; De Angelis, 2016; Donnellan *et al*., 2016a, 2016b).

Materials and methods

To evaluate admixture between Greek colonists and indigenous Italic populations, we examined dental nonmetric traits. Dental nonmetric traits are particularly useful in detecting relationships between past human groups, because twin and family studies have shown that many of them are under strong genetic control (Alt, 1997; Scott & Turner, 1997). In a recent study, Hubbard *et al*. (2015) showed that relationship patterns among groups based on dental morphology are correlated with those obtained by nuclear DNA. Thus, dental morphology can be used as a proxy for genomic data when none are available. Furthermore, teeth are generally well preserved in archaeological contexts, even where overall skeletal preservation is relatively poor.

The dental samples

This study uses two sources of data: (i) data gathered by the first author and (ii) summary data from the literature. We analysed 355 skeletons with well-preserved dentitions from three indigenous sites in the surrounding hinterland of the Greek colony of Metaponto: Incoronata, Santa Maria d'Anglona and Passo di Giacobbe. This data set was compared with samples from the urban and rural areas of the Greek colony of Metaponto published in Henneberg (1998) comprising 351 individual dentitions. The locations of the archaeological sites from where the skeletal samples were drawn are shown in Figure 1. Summary information about the samples, including cultural affiliation, dating, number of examined individuals and the researcher who collected the data, are listed in Table 1. All skeletal samples were dated based on pottery and grave good assemblages.

The urban Metaponto skeletal sample (URME) was obtained from the Crucinia burial ground, an urban cemetery just outside the city walls, and comprises 175 individuals. The rural Metaponto skeletal sample (RUME) comes from the surrounding area in close

Table 1. Composition of the samples

Site	Abbreviation	Cultural affiliation	Dating	Sample size	Observer
Incoronata	INCO	Indigenous Italic	900–750 BC	141	Rathmann (present study)
Santa Maria d'Anglona	SMDA	Indigenous Italic	850–750 BC	104	Rathmann (present study)
Passo di Giacobbe	PADG	Indigenous Italic	625–350 BC	110	Rathmann (present study)
Rural Metaponto	RUME	Greek colonial	600–250 BC	176	Henneberg (1998)
Urban Metaponto	URME	Greek colonial	700–300 BC	175	Henneberg (1998)

proximity to the city of Metaponto. It is composed of four cemeteries: most skeletons come from Pantanello (*n* = 158), fewer from Saldone (*n* = 11), Sant'Angelo (*n* = 6), and Celeste (*n* = 1) (Carter, 1990a, 1990b, 1998; Henneberg, 1998). The skeletal sample of Incoronata (INCO) comes from two cemeteries situated along the associated indigenous settlement and comprises 141 individuals (Chiartano 1983, 1994a, 1994b). The skeletal sample of Santa Maria d'Anglona (SMDA) was obtained from three cemeteries in the vicinity of the corresponding indigenous settlement: Conca d'Oro (*n* = 84), Cocuzzolo Sorigliano (*n* = 16) and Valle Sorigliano (*n* = 3) (Frey, 1991). The skeletal sample of Passo di Giacobbe (PADG) comes from two burial grounds situated along the indigenous settlement and consists of 110 individuals (Schojer, 2010). It should be noted that combining different burial grounds into a single bioarchaeological sample may introduce error in the representativeness of skeletal samples and can lead to increased intra-group biological heterogeneity. On the other hand, this procedure increases sample sizes, and large samples are necessary to detect phenotypic differences between populations on a regional scale. We believe that pooling geographically close cemeteries into a single sample is reasonable in our study, because archaeological findings suggest that they belong to the same settlement (see aforementioned references).

Dental trait recording

We scored dental nonmetric traits in the permanent dentition of each individual. All traits were recorded according to the reference standards of the Arizona State University Dental Anthropology System (ASUDAS) described by Turner *et al.* (1991). This system comprises a set of dental casts illustrating expression levels for various traits and specific instructions to ensure a standardised scoring procedure. Scoring followed the 'individual count method', where a trait was counted only once per dentition, regardless of whether or not the trait appeared bilaterally. In cases where a trait was expressed asymmetrically, the side with the highest expression level was scored (Turner & Scott, 1977; Turner, 1985; Irish & Guatelli-Steinberg, 2003; Irish, 2005; Edgar, 2007; Irish & Konigsberg, 2007). Although this method has been claimed to underestimate trait frequencies in poorly preserved samples (Korey, 1980), it remains the standard protocol in ASUDAS studies. To ensure accuracy, any observation that was potentially affected by dental wear, caries or calculus was treated as missing data. The ordinal-graded trait scores were dichotomised into binary categories of 'present' or 'absent' in order to calculate trait frequencies. The applied dichotomisation thresholds are in line with established breakpoints (Turner, 1987; Scott & Turner, 1997; Irish & Guatelli-Steinberg, 2003).

Although a variety of traits were recorded for the indigenous Italic samples, only 13 could be used for the comparison with the Metaponto samples (Table 2). These particular traits are the only ones in the Metaponto samples that were dichotomised using the established ASUDAS thresholds.

To assess sexual dimorphism of the dental traits, we performed Fischer's exact tests. However, the test could only be performed for 7 out of 13 traits (54%) owing to the large number of individuals of unknown sex in our sample. Any sex differences in trait expressions were found to be statistically non-significant and thus negligible (results not presented here). This finding is concordant with the statement that most of the dental traits listed in the ASUDAS have low or now sexual dimorphism (Scott & Turner, 1997). Thus, we adopted the standard procedure and pooled the sexes (e.g. Irish, 2005; Irish & Konigsberg, 2007).

Quantitative analysis

The dental nonmetric data set was analysed within a framework of population genetic models utilising the R-matrix approach. The R-matrix model was originally developed to work with allele frequency data by Harpending and colleagues (Harpending & Jenkins, 1973; Harpending & Ward, 1982) and was later modified for use with morphometric data (Relethford & Blangero, 1990; Relethford *et al.*, 1997). The

Table 2. Dental nonmetric traits used in the analysis, with their dichotomisation breakpoints (absent/present), frequencies of positive observations (%) and number of individuals scored (n) for the five population samples

			INCO	SMDA	PADG	RUME	URME
Dental traits	Absent[a]	Present[a]	% (*n*)	% (*n*)	% (*n*)	% (*n*)	% (*n*)
UC Tuberculum dentale	0	1–6	26.82 (41)	22.85 (35)	7.50 (40)	56.52 (69)	13.41 (82)
UM2 Hypocone	2–5	0–1	23.07 (52)	15.78 (38)	22.85 (35)	17.34 (98)	35.63 (87)
UM1 Cusp 5	0	1–5	13.15 (38)	26.41 (53)	4.76 (21)	34.48 (29)	0.00 (30)
UM1 Carabelli's trait	0–4	5–7	28.88 (45)	20.33 (59)	23.33 (30)	25.39 (63)	29.62 (54)
UI2 Peg-shaped	0	1–2	0.00 (40)	2.43 (41)	2.08 (48)	3.33 (90)	3.17 (63)
UM3 Congenital absence	0	1	4.76 (42)	34.61 (26)	20.00 (40)	0.00 (74)	2.06 (97)
LP2 Lingual cusp variation	0-1	2–9	56.41 (39)	45.83 (24)	45.45 (22)	30.95 (84)	48.38 (62)
LM2 Groove pattern	+,x	y	3.92 (51)	15.15 (33)	13.88 (36)	17.47 (103)	0.00 (94)
LM1 Protostylid	0	1–7	36.84 (57)	39.68 (63)	19.14 (47)	58.44 (77)	53.84 (65)
LM1 Hypoconulid	1–5	0	7.79 (77)	9.21 (76)	8.62 (58)	13.79 (87)	28.08 (89)
LM2 Hypoconulid	1–5	0	86.2 (29)	77.41 (31)	96.77 (31)	96.42 (112)	97.95 (98)
LM1 Cusp 6	0	1–5	0.00 (34)	5.66 (53)	11.53 (26)	0.00 (87)	1.12 (89)
LM3 Congenital absence	0	1	3.92 (51)	29.62 (27)	17.3 (52)	2.50 (80)	2.32 (129)

INCO, Incoronata; SMDA, Santa Maria d'Anglona; PADG, Passo di Giacobbe; RUME, Rural Metaponto; URME, Urban Metaponto.
[a]Columns show the ordinal graded dental nonmetric trait scores according to Turner *et al.* (1991) collapsed into binary categories of 'absent' or 'present'.

application of the R-matrix to discrete nonmetric data was discussed by Konigsberg (2006) and Irish (2010). The present study follows the latter approach. Unless otherwise noted, all calculations were performed in R, version 3.2.0.

The first step in order to generate an R-matrix from nonmetric data is to calculate a conventional model-free distance matrix. There are two distance statistics available that are commonly used by physical anthropologists working with nonmetric traits: the mean measure of divergence (MMD) (Sjøvold, 1977) and the pseudo-Mahalanobis D^2 (Konigsberg, 1990). Previous work has shown that both distance statistics yield comparable results (Irish, 2010; Nikita, 2015). The choice of which statistic to use depends on the nature of the data set, that is, sample sizes, number of traits and amount of missing data. In the present study, the MMD was used because it is an unbiased estimator of population divergence and is run with summary count data, allowing us to easily integrate the published grouped data of both Metaponto samples (Henneberg, 1998).

Prior to applying the MMD statistic, we removed highly intercorrelated traits to avoid over-representing variation from features that co-occur (Sjøvold, 1977). Inter-trait correlations were determined by submitting the ASUDAS rank-scale data to Kendall's tau-b correlation coefficient. Of strongly correlated traits ($\tau_b \geq 0.5$), the one with the fewest observations overall was removed from the analysis. UM3 congenital absence and LM3 congenital absence were found to be highly correlated ($\tau_b = 0.929$). Consequently, UM3 congenital absence was dropped from the analysis.

The MMD statistic converts a battery of trait frequencies into a nonlinear distance value between group pairs; low values indicate similarity and high values imply dissimilarity (Harris & Sjøvold, 2004). The MMD was calculated according to the formula in Sjøvold (1977):

$$MMD = \frac{1}{r}\sum_{i=1}^{r}\left\{(\theta_{1i}-\theta_{2i})^2 - \frac{1}{n_{1i}+0.5} - \frac{1}{n_{2i}+0.5}\right\}$$

where r is the number of traits used, n_{1i} and n_{2i} are the numbers of individuals examined for the ith trait in the two groups being compared and Θ_{1i} and Θ_{2i} are the transformed frequencies of the ith trait in the two groups. The transformed frequencies were calculated using the Freeman & Tukey (1950) angular transformation to correct for small sample sizes and low (≤ 0.05) or high (≥ 0.95) trait frequencies:

$$\theta = \frac{1}{2}\sin^{-1}\left(1-2\frac{k}{n+1}\right)+\frac{1}{2}\sin^{-1}\left(1-2\frac{k+1}{n+1}\right)$$

where k is the count of positive observations for a trait in a sample of size n. MMD distances can be considered significant if they are more than twice their standard deviation (see Harris & Sjøvold, 2004; formula 12).

The nonlinear distance matrix produced by the MMD was then submitted to multidimensional scaling (MDS) to calculate an optimally scaled distance matrix (Irish, 2010). Calculations were performed in SPSS, version 22.0. (IBM Corp., Armonk, N.Y., USA), running an interval-level Alscal procedure. This procedure scales the nonlinear MMD distances to the same scale as squared Euclidean distances. This step is crucial, because only squared Euclidean distances exhibit a direct relationship to average kinship and can be used to calculate the R-matrix (Konigsberg, 2006).

Next, a codivergence matrix was derived from the optimally scaled distance matrix, according to the formula presented in Konigsberg (2006):

$$C = -0.5(I - 1w')D^2(I - 1w')'$$

where I is an identity matrix with the dimensions $g \times g$ (g is the number of groups), 1 is a $g \times 1$ column vector of 1s, D^2 is the optimally scaled distance matrix and w is a $g \times 1$ column vector of relative population sizes. Weighting with relative population sizes is recommended to correct for genetic drift on small populations. We assumed that the effective population size of the Greek colony of Metaponto was at least three times the size of the surrounding indigenous Italic villages, as indicated by settlement survey data and architectural structure counts (Carter, 1990b; De Angelis, 2003). The relative weights of the populations (w) are therefore $3/9 = 0.333$ for both Metaponto samples and $1/9 = 0.111$ for the three indigenous samples. *Minimum* F_{ST} was calculated from the codivergence matrix as follows (Konigsberg, 2006):

$$\min F_{ST} = \frac{w' diag(C)}{2t + w' diag(C)}$$

where diag(C) is the diagonal of the codivergence matrix converted into a column vector and t is the number of traits. F_{ST} is a summary measure of genetic differentiation between populations, taking into account the total among-sample variation. *Minimum* F_{ST} is the most conservative way of estimating F_{ST}, because complete heritability of the used traits is assumed. The *estimated* F_{ST} was calculated according to the formula in Relethford (1994):

$$F_{ST} = \frac{\min F_{ST}}{\min F_{ST} + h^2(1 - \min F_{ST})}$$

where h^2 is a heritability estimate for dental nonmetric traits. For the present study, a heritability value of $h^2 = 0.5$ was used, which is the average of various heritability estimates of dental traits based on twin and family studies (Hanihara, 2010).

The R-matrix was then computed as follows (Konigsberg, 2006):

$$R = \frac{C(1 - F_{ST})}{2t}$$

The R-matrix diagonal elements (r_{ii}) are distances from population i to the regional centroid and indicate the degree of population divergence from the pooled genetic average. Populations near the centroid exhibit greater internal variation, and those farther away have less variation as a result of genetic drift and lower migration rates (Konigsberg, 2006). The off-diagonal elements (r_{ij}) are covariances about the regional mean and are defined as average kinship coefficients between population i and j. Positive values indicate greater genetic similarity and negative values lesser similarity than average (Relethford & Harpending, 1994).

Results

The resulting MMD distance matrix is provided in Table 3. All populations differ significantly at $p < 0.025$. The indigenous Italic samples show relatively small MMD distances to one another (between 0.045 and 0.058), whereas the Greek colonial samples of Metaponto are much more divergent (0.231). Figure 2 presents the biological distance results obtained by the MMD analysis implemented in an MDS plot, where Kruskal's stress value is 0.021 and $r^2 = 0.995$.

Table 4 provides the R-matrix from the MDS optimally scaled MMD distances. The diagonal values (r_{ii}) indicate that PADG and SMDA are farthest away from the genetic centroid, INCO is closest and RUME and URME possess intermediate values. The off-diagonal kinship coefficients (r_{ij}) indicate that the indigenous Italic populations exhibit greater genetic similarity to each other and possess lesser genetic similarity to the populations of the Greek colony of Metaponto. The

Table 3. Mean measure of divergence distance matrix between indigenous Italic and Metapontian populations based on 12 dental nonmetric traits[a]

	INCO	SMDA	PADG	RUME	URME
INCO	0				
SMDA	0.0453	0			
PADG	0.0588	0.0464	0		
RUME	0.0903	0.1254	0.2289	0	
URME	0.0777	0.2291	0.1256	0.2313	0

INCO, Incoronata; SMDA, Santa Maria d'Anglona; PADG, Passo di Giacobbe; RUME, Rural Metaponto; URME, Urban Metaponto.

[a] All distances are statistically significant at $p < 0.025$.

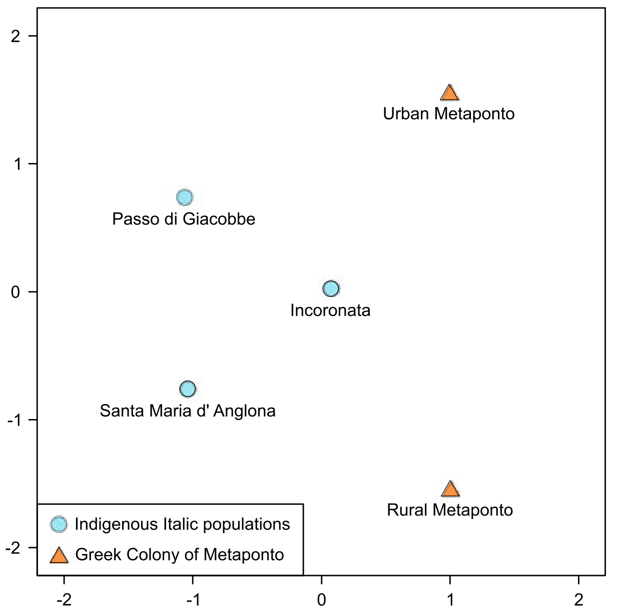

Figure 2. Two-dimensional MDS plot of 12-trait mean measure of divergence dental distances between Metapontian and indigenous Italic populations. [Colour figure can be viewed at wileyonlinelibrary.com]

two populations of Metaponto show the least genetic similarity to each other.

Table 5 lists the F_{ST} measures to evaluate genetic differentiation between populations. When all five sites are considered as a single sample, an F_{ST} value of 0.0706 is obtained. The value considerably decreases when the three indigenous Italic sites are considered alone ($F_{ST} = 0.0389$). When the two Greek colonial sites are considered alone, the value is more than one and a half times higher ($F_{ST} = 0.0603$). This indicates that there is greater biological variation between the sites of the Greek colony of Metaponto than between the indigenous Italic sites in the hinterland.

Table 4. R-matrix from MDS optimally scaled mean measure of divergence distances between indigenous Italic and Metapontian populations estimated using an overall heritability of $h^2 = 0.5$ for 12 dental nonmetric traits

	INCO	SMDA	PADG	RUME	URME
INCO	0.0229				
SMDA	0.0060	0.0439			
PADG	0.0039	0.0166	0.0445		
RUME	−0.0064	−0.0020	−0.0196	0.0346	
URME	−0.0045	−0.0202	−0.0020	−0.0252	0.0341

INCO, Incoronata; SMDA, Santa Maria d'Anglona; PADG, Passo di Giacobbe; RUME, Rural Metaponto; URME, Urban Metaponto.

Table 5. Estimated F_{ST} values for indigenous Italic and Metapontian populations

Grouping	Number of sites	F_{ST}
Total area	5	0.0706
Indigenous Italic	3	0.0389
Greek colonial	2	0.0603

Discussion

Our study has produced three important results: (i) the gene pool of the Greek colony of Metaponto differed from those of indigenous populations in the vicinities; (ii) there was significant biological diversity between the inhabitants of the rural and urban areas of Metaponto; and (iii) the indigenous populations were relatively homogeneous.

We are aware that our results could potentially be affected by inter-observer error owing to discrepancies in the scoring technique of the two different researchers. An inter-observer test could not be performed as the primary Metaponto dental data were not accessible. However, each observer followed the same definitions to score the dental traits, and both observers are experienced in collecting data of this nature. Nichol & Turner (1986) reported that most dental traits can be observed with adequate inter-observer reliability. In their study, misclassification of ranked traits by more than one grade was low (6–10%) for between-observer comparisons. Additionally, the performed trait dichotomisation reduces intra-observer error by collapsing the ordinal graded trait scores into simplified categories of 'present' or 'absent' in such a way that slight scoring discrepancies are eliminated. Thus, we consider that inter-observer error, although potentially present, cannot solely account for our results.

The biological distinction between colonial and indigenous populations (Table 4), and substantial diversity between the samples of Metaponto despite their close proximity to each other (Figure 1), suggests gene flow from external sources into the colony. Two plausible scenarios might account for the diversity in Metaponto: first, a high degree of variability was already present in the 'founding' population, and second, population influx throughout the settlement's duration could have brought increasing diversity to the group of original settlers. These scenarios are not mutually exclusive.

The first scenario is that the high degree of diversity can be attributed to an already variable 'founding' population, originating from different regions of Greece. This scenario would support the hypothesis that the Greek colonisation, especially in its initial phase, was largely the product of a non-centrally organised, relatively chaotic process that developed from a period of intense mobility across the Mediterranean (the end of the Early Iron Age). This hypothesis was first outlined by Osborne (1998), who suggested that the early colonisation was the

> product of a world in which many were constantly moving across the seas, [...] and where individuals and small groups out of their own gain from time to time came to believe that more or less permanent settlement on foreign shores was both in their immediate best interest and was sustainable (Osborne, 1998: 268; but see Moggi, 2008; Greco, 2011).

The second scenario is that population influx throughout the colony's duration brought increasing biological variability to the original settler group. Given the urban development and important economic role of Metaponto, the fact that it would have attracted people from different regions seems reasonable. This would not necessarily imply direct participation in the political life of the colony, as for example, women, slaves or foreigners would have been excluded to varying degrees. Ultimately, this result lends greater weight to the idea, put forward by Ridgway (2006, 2007) and Shepherd (2005b), that the cohesion of colonies as communities was based on the construction of a common *colonial* identity (as expressed through material culture) that went beyond strict ethnic boundaries and biological realities (see also Lombardo, 2009).

Future work investigating the Metaponto dental remains by chronological phase has the potential to shed light on whether biological diversity in the colony originated in the earliest phase of its settlement, in the course of its history or both.

It is not possible at present to determine whether indigenous people were also part of the colonial population at Metaponto. A high degree of extra-local gene flow into the colony would cause the colonial gene pool to remain distinct from those of indigenous populations even in the case of admixture. Thus, extra-local gene flow into Metaponto must have been higher than gene flow from indigenous populations. This leaves the question of Greek-indigenous admixture still open. Interestingly, a modern DNA study revealed that there is indeed a Greek genetic signature in southern Italian present-day populations that can, by modelling the typical mutation rate over the centuries, be attributed to a migration influx somewhere between the eighth and fifth century BC (Tofanelli *et al.*, 2015). We hope that future comparative work on bioarchaeological data from the supposed mother cities in Greece will bring us closer to understand the timing and degree of Greek-indigenous admixture at Metaponto (for example, McIlvaine *et al.*, 2014).

Interestingly, the indigenous populations were relatively homogeneous ($F_{ST} = 0.039$) in comparison with the colonial populations of Metaponto ($F_{ST} = 0.071$). This is especially striking when the fairly distant geographical locations of the indigenous sites are taken into account (Figure 1). This result suggests that a long-term history of interconnectedness existed between indigenous communities, involving not only economic and political relations but also mobility and intermarriage. This implies that the sociopolitical landscape of indigenous communities was more complex and dynamic than usually assumed. It also serves to direct future research towards considering the development of indigenous sites in relation to each other as well as to the Greek colonies. The existence of such well-connected networks between indigenous communities certainly affected the way Greek-indigenous dynamics played out. It would, for example, have affected the capacity of indigenous populations to generate an organised response to the arrival of the first settlers, their openness to interaction and the degree to which contact would have been destabilising.

Conclusion

This study is the first to apply a biological distance analysis utilising the R-matrix method to the study of ancient Greek colonisation. By comparing samples from colonial and indigenous contexts in southern Italy, we could show that the gene pool of the Greek colony of Metaponto differed from those of indigenous populations in the surrounding hinterland. The distinction was likely the result of extra-local gene flow into Metaponto originating from (i) the diverse origin of the earliest colonial settlers and/or (ii) the continued influx of new migrants into the colony throughout its history. This suggests that Greek colonies were dynamic places able to generate social cohesion based on colonial identity rather than strictly geographical origin. This situation stands in contrast to the closer than expected biological affinity between indigenous populations that were geographically relatively distant. Such homogeneity needs to be further investigated and included in our reconstructions of colonial dynamics. Although the scope of our study is limited, our results show that bioarchaeological evidence can produce important new insights into Greek colonisation and should be considered a key tool to be integrated with material culture-based studies of complex past interactions.

Acknowledgements

The following individuals and institutions graciously provided access to the human skeletal collections in their care: Dr De Siena, Dr Cinquantaquattro and Dr Bianco (Soprintendenza di Basilicata), and Dr La Rocca, Dr Schojer and Dr Masiello (Soprintendenza di Apulia). We also thank the staff of the national archaeological museums in Policoro and Metaponto, as well as the staff of the Magazzini S. Antonio in Taranto for their assistance. We thank Professor Posamentir (University of Tübingen) and Professor Burgers (University of Amsterdam) for their support and advice. Hannes Rathmann and Giulia Saltini Semerari are supported by research grants from the Gerda Henkel Foundation. Data collection was funded by the State Ministry for Science, Research and Arts of Baden-Württemberg, Germany, (Project "Interdisciplinary Analysis of Culture Contacts in Ancient Peripheries") granted to Richard Posamentir. Katerina Harvati's research is funded by the German Research Foundation (DFG FOR 2237: Project "Words, Bones, Genes, Tools: Tracking Linguistic, Cultural and Biological Trajectories of the Human Past"). Finally, we thank our anonymous reviewers for providing helpful suggestions to improve this article.

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Appendix C

Population history of southern Italy during Greek colonization inferred from ancient skeletons

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Manuscript close to submission[1]

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[1] The final submitted manuscript may deviate slightly from the version in this thesis

Title:

Population history of southern Italy during Greek colonization inferred from skeletal remains

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ABSTRACT

Objectives: Around the 8th century BC, Greek seafarers founded several colonies along the coastal area of southern Italy. While the impact of the Greek colonization is clearly visible in the cultural heritage of the region, researchers have argued for decades about their genetic contribution. Especially contested is the numerical size of the migration from Greece and the degree of admixture between Greek colonists and indigenous populations.

Materials and Methods: To address this debate, we collected osteological data from 481 human skeletons from six archaeological sites along the Gulf of Taranto, dating to precolonial (900-700 y BC) and postcolonial periods (700-200 y BC). For both time periods, we inferred population structure using adonis, betadisper and IBD models based on inter-individual Gower distance coefficients based on 42 dental metric and nonmetric traits. For the postcolonial period, we furthermore determined individual ancestries using naïve Bayesian classification based on 28 dental nonmetric traits.

Results: Precolonial southern Italy was characterized by moderate levels of population stratification. During postcolonial times, the area became a place of high mobility, hosting ~ 10 % Greek immigrants and their descendants. Greek newcomers were equally distributed across Greek colonies and indigenous Italic settlements.

Discussion: Our study provides unique insights into the Greek colonization of southern Italy by using biological data from archaeological human remains. Our results support a gradual colonization model with substantial involvement of local populations and contradict the theory that Greek colonies were homogenous enclaves within conquered territories.

KEYWORDS

Greek colonization
Southern Italy
Biodistance
Dental metrics
Dental nonmetric traits

INTRODUCTION

During the eighth century BC, people coming from ancient Greece started to settle along the coasts of the Mediterranean. They founded more than 500 Greek colonies at the shores of the Black Sea, Anatolia, southern Italy, Sicily, North Africa and along the coast of southern France and Spain (Hansen and Nielsen, 2004). Archaeological evidence suggests that they initially settled in small groups, sometimes embedding themselves within the local indigenous populations. Over time, Greek colonies became increasingly urbanized and culturally distinct settlements. The Greek colonization has been called "*one of the most important cultural encounters in world history*" (Angelis, 2016: 101), and its consequences in Mediterranean history were profound and long-lasting. It contributed to the creation of a Mediterranean-wide network of exchanges (Malkin, 2005), to the development of urbanization along its shores (Malkin, 1994), to the spread of the alphabet (Boardman, 2014), and to the diffusion of Greek artistic and architectural traditions (Greco, 1992). Among the regions settled by the Greeks, southern Italy was one of the most densely populated areas and eventually became known as *Megálē Hellás* or *Magna Graecia* (Greater Greece).

Centuries of extensive research have greatly enhanced our understanding of the Greek colonization of southern Italy. Nevertheless, the nature of early settlements, the scale of demographic impact and the Greek genetic legacy are still largely debated. Historians, archaeologists, demographers, and geneticists have proposed different degrees of Greek genetic contribution, with scenarios ranging from a colonization process driven by a few Greek colonists living in biologically isolated enclaves within the conquered territories, to scenarios with substantial migration from Greece to Italy and intensive interaction between newcomers and locals (e.g. Pugliese Carratelli, 1996; Osborne, 1998; Yntema, 2000; Greco, 2002; Scheidel, 2003; Donnellan et al., 2016b, 2016a; Tofanelli et al., 2016).

Early interpretations on the causes and modes of the Greek colonization were primarily based on survived texts from ancient Greek writers (Beloch, 1886; Pais, 1894). Drawing from these historical sources, it was thought that the colonies (or *apoikiai*) were centrally organized expeditions sent out by a 'mother city' under the auspices of the Delphian oracle. They were led by an official founder (the *oikistes*), who chose the location and proceeded to divide the land in regular allotments distributed to the colonists. The *oikistes* was also responsible for establishing local cults (largely derived from the mother cities) and public spaces. In this view, the role of local populations, if at all considered, was essentially passive. They were deprived of land, resources and women to fulfill the colonies' needs while being the object of gradual acculturation (or 'hellenisation') (Burgers, 2004; Shepherd, 2005). Central to those early interpretations was the assumption that Greek colonies formed culturally and biologically homogenous enclaves within the conquered foreign territories. Within this framework, archaeological evidence (being very scarce at these times) was only considered when it supported the written evidence. Wherever the two research sources did not correspond, excuses were found to discard the former (Yntema, 2000). Philological approaches are, however, somewhat problematic, since most ancient written sources postdate the Greek colonization by several centuries and portray it solely from a Greek perspective.

Over the course of time, increasing archaeological evidence from early colonial contexts (7th century BC) pointed to a more complex picture that contradicted the proposed settlement homogeneity of the traditional colonial narratives. Specifically, local indigenous material culture was found in the early phases of many Greek colonies and, conversely, some non-colonial sites showed evidence of mixed Greek-indigenous assemblages (Siena, 1986; Berlingò, 1993; Carter, 2006; Crielaard and Burgers, 2012; Denti, 2018). Based on those findings, the colonization process was considered an interplay of actions of both colonizers and colonized, and a more balanced investigation of both Greek and indigenous roles in the colonization process was advocated. These developments led to an interpretation of Greek colonization emphasizing processes of collaboration and 'hybridization' between Greek newcomers and local indigenes, where the Greek-indigenous interaction shaped new, admixed cultures (Yntema, 2000; Malkin, 2002; van Dommelen, 2012). Although material culture is a valuable addition to ancient written sources, it must be kept in mind that: ‘pots are pots, not people’. The stylistic provenance of an object and the bio-geographic origin of its maker(s) may not be identical. Additionally, movements of objects do not necessarily imply migration of people but can also result from trade or the diffusion and adoption of stylistic fashions.

Another interesting line of evidence was provided by scholars making use of population demographic modeling to calculate the numerical size of the migration from Greece that resulted in the establishment of early colonies in southern Italy. For example, Scheidel (2003) used census counts of Greeks living in southern Italy during Classical times (ca. 400 y BC) and by modeling population growth rates over the centuries, he estimated that the original founding population during Archaic times (7th century BC) was likely around 30,000 to 60,000 immigrants. Admittedly, the order of magnitude of such estimates must be taken with caution as they are based on approximated census counts primarily derived from historical enumerations of Greek military forces documented in ancient texts (Beloch, 1886). Although these numbers need not be wide of the mark, they certainly cannot be treated as the truth.

In recent years, geneticists have tried to tackle the question of the Greek colonization and its impact on southern Italy from a different angle. Using modern DNA data, several studies found that present-day southern Italians exhibit a genetic signature compatible with that found in modern Greeks (Di Gaetano et al., 2009; Sarno et al., 2014; Tofanelli et al., 2016; Sarno et al., 2017). Di Gaetano et al. (2009) estimated a Greek genetic contribution of ~ 37 % to the populations of Sicily and attributed the influx to the Classical period (2380 years before present, with a 95 % confidence interval ranging between 6940 and 675 years ago). In another study, Tofanelli et al. (2016) estimated that the size of the original founding population was probably between 1,000 to 6,000 immigrants and attributed the influx to the Archaic period (800 - 400 y BC). Although these studies are highly interesting, the problem with present-day DNA profiles is that they may not accurately reflect the effects of past migration events due to movements of people in more recent times. Moreover, genomic estimates of the timing of past migration events generally have wide ranging confidence intervals spanning several centuries or even millennia. Studies using ancient DNA (aDNA) could help verifying these findings, but it remains to be tested if human skeletal remains from southern Italy provide ancient collagen of high enough quality to be DNA sequenced.

The most promising approach for studying the Greek genetic contribution to southern Italy is to directly analyze ancient biological data from archaeological human remains. Biodistance analysis (hereafter, biodistance) based on phenotypic features of the cranium or dentition provides a powerful tool for assessing relationships among past human populations, when no aDNA data is available. Surprisingly, only a few studies have utilized biodistance methods to addressed the impact of the Greek colonization on southern Italy (Henneberg, 1998; Rubini et al., 1999; Rathmann et al., 2017b). These investigations found that inhabitants of Greek colonies showed marked biological differences to indigenous Italian groups, possibly due to an influx of new genes. While this is an exciting finding, these studies were limited by several issues. First, they all employed few phenotypic variables, which generally reduces the accuracy of biological distance estimates (Sokal and Sneath, 1963). Second, all studies used either metric or nonmetric data; however, it has been shown that combining metric with nonmetric markers increases performance compared to using the features separately (Lease and Sciulli, 2005; Hefner et al., 2014). Third, all studies performed group-level biodistance analyses; however, individual-level biodistance methods are better suited for capturing the significant amount of human genetic variation within groups (Stojanowski and Schillaci, 2006). Fourth, none of the previous studies used adequate comparative data from Greece which is essential to quantify the degree of Greek genetic contribution to southern Italy.

Here, we are taking previous biodistance studies on the Greek colonization of southern Italy to the next level by (1) analyzing a new dataset from several sites along the coastal area of the Gulf of Taranto region, (2) employing a large battery of phenotypic variables, (3) combining metric with nonmetric traits, (4) performing individual-level biodistance analyses, and (5) integrating comparative data from Greece in order to identify individual ancestries and to quantify the Greek contribution to southern Italy. Specifically, this paper examines changes in southern Italian population structure from the precolonial period (900-700 y BC) to the postcolonial period (700-200 y BC). Population structure for both time periods is inferred using adonis, betadisper and IBD models based on inter-individual Gower distance coefficients based on a mixture of dental metric and nonmetric traits. For the postcolonial period, we furthermore determine individual ancestries using naïve Bayesian classification based on dental nonmetric traits.

This research has broad implications for understanding the Greek colonization of *Magna Graecia*. Our results contribute to the long-lasting debate about the Greek genetic signal found in southern Italy during postcolonial times and the degree of admixture between colonists and colonized.

MATERIALS AND METHODS

Skeletal samples

We collected osteological data from 481 human skeletons with well-preserved dentitions from six archaeological sites from the coastal area of the Gulf of Taranto, southern Italy, dating from pre- to postcolonial periods (900-200 y BC) (Figure 1). The dataset was collected by a team from the University of Tübingen between the years

2014 and 2018 at the storages of the National Archaeological Museums of Policoro, Metaponto and Taranto. Several of the skeletal remains were hitherto inaccessible and the majority has not been published before.

The study region is of special importance to scholars concerned with the Greek colonization of *Magna Graecia*, because it is home to several major Greek colonies and features a wealth of well-studied indigenous sites (Siena and Tagliente, 1986; Greco, 1999; Yntema, 2000; Carter, 2006; Bianco, 2012). Our dataset comprises skeletons from three indigenous Italic settlements: Incoronata, Santa Maria d'Anglona and Passo di Giacobbe. These sites share a distinct indigenous cultural package, defined by specific grave good assemblages, flexed burial positions, and gender-specific burial orientations. Moreover, our dataset includes skeletons from three Greek colonies: Metapontion, Siris and Taras. According to ancient written sources, the colonies were founded in the course of the late 8th to 6th centuries BC by Greeks settlers from Achaia, Ionia and Laconia (Strabo: 6.1.14, 6.1.15, 6.3.2). The early colonies gradually grew into full-fledged urban centers, amassed a great amount of wealth owing to fertile farmland, and erected large temples and monumental public buildings.

Table 1 gives summary information about the skeletal samples under study, including cultural affiliation, dating, total number of examined skeletons, as well as as subsamples used for different statistical analyses (see method section below). More detailed information and references about the individual archaeological sites are provided in the Supplements (Supplementary Information 1). All skeletons were dated based on grave good assemblages, including pottery and metal finds.

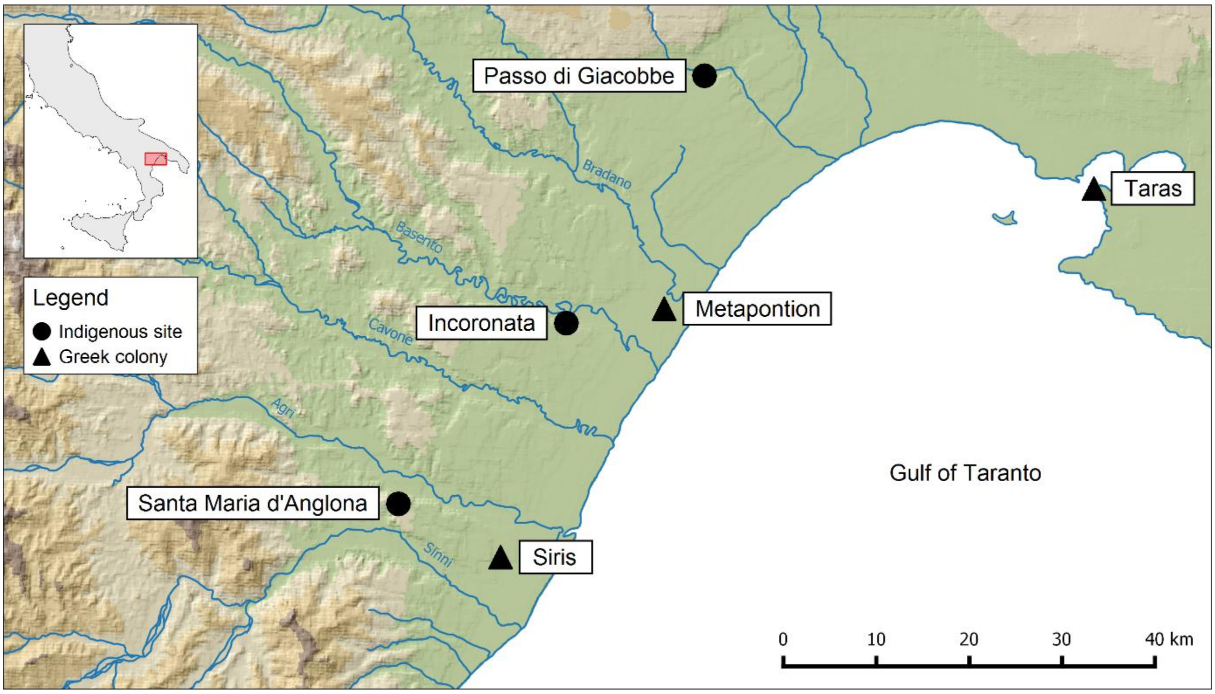

Figure 1. Map of the Gulf of Taranto region showing the geographic locations of the archaeological sites analyzed this study.

Table 1. Archaeological sites and sample sizes used in this study.

Site	Cultural affiliation	Time period	Dating	Number of skeletons[a]	Analytic subset 1[b]	Analytic subset 2[c]
Incoronata	Indigenous settlement	Precolonial	900-750 y BC	139	21	-
Santa Maria d'Anglona	Indigenous settlement	Precolonial	850-700 y BC	102	23	-
Passo di Giacobbe	Indigenous settlement	Postcolonial	625-300 y BC	105	20	14
Metapontion	Greek colony	Postcolonial	600-200 y BC	109	21	19
Siris	Greek colony	Postcolonial	700-500 y BC	19	8	7
Taras	Greek colony	Postcolonial	700-500 y BC	7	1	1

[a] Total number of skeletons for which at least one dental metric or nonmetric trait was recorded

[b] Subset of skeletons with well-preserved dentition used for the adonis, betadisper and Mantel test analyses

[c] Subset of skeletons with well-preserved dentition used for the naïve Bayesian ancestry determination

Data recording

Sex and age-at-death estimates are fundamental to any osteological analysis. Sex estimation was based on pelvic and cranial morphology using standard osteological techniques (Buikstra and Ubelaker, 1994). Age-at-death estimates were based on dental development, fusion of epi- and apophyses, cranial and palatal suture closure, and morphological changes of the pubic symphyseal and auricular surfaces (Buikstra and Ubelaker, 1994).

The biodistance analysis is based on dental morphology for a number of practical reasons: First, skeletal preservation of many southern Italian samples is poor, while teeth are generally well-preserved. Therefore, teeth were recovered from the sites in higher quantities than any other skeletal region, allowing us to employ large enough samples and robust statistical analyses. Second, tooth crowns develop relatively early in the life of an individual and their form remains unchanged once fully developed, except by wear or pathology. Third, tooth form has been proposed to be highly heritable and selectively neutral, thus providing an excellent proxy for neutral genetic data (Hillson, 1996; Scott and Turner, 1997). In fact, several recent studies have demonstrated the utility of dental phenotypic data in reconstructing genetic relatedness among populations on different geographic scales (Hubbard et al., 2015; Rathmann et al., 2017a) and even between individuals within a population (Ricaut et al., 2010; Paul and Stojanowski, 2015; Stojanowski and Hubbard, 2017). Finally, dental phenotypic data can be sampled in a non-destructive, cost-efficient and straightforward manner using crown width and length measurements (dental metrics) and visual scoring of crown and root shape variants (dental nonmetric traits).

The dental metric dataset consists of 32 mesiodistal and buccolingual crown and cervical diameters of the permanent teeth recorded for each individual. Only polar teeth were recorded (UI1, UC, UP3, UM1, LI2, LC, LP3, LM1) in order to reduce genetic covariation between traits, and to minimize potential effects of fluctuating asymmetry and ontogenetic plasticity on adult tooth size (Stojanowski, 2003). Only left teeth were measured, but when a left tooth was missing, damaged, or affected by wear or pathology, the corresponding right antimere was measured. All measurements were recorded according to the procedures detailed in Hillson et al. (2005) using a Mitutoyo pointed blade digital sliding caliper accurate to 0.01 mm.

The dental nonmetric trait dataset consists of observations for 34 morphological variables in the permanent dentition of each individual. All traits were recorded according to the reference standards of the Arizona State University Dental Anthropology System (ASUDAS) described in Turner et al. (1991). This system comprises a set of dental casts illustrating expression levels for various traits and specific instructions to ensure a standardized scoring procedure that minimizes observer error. Only traits on key teeth were recorded (Scott et al., 2016). Scoring followed the individual count method, where a trait was counted only once per dentition, regardless of whether or not the trait appeared bilaterally. In cases where a trait was expressed asymmetrically, the side with the highest expression level was scored (Turner and Scott GR., 1977; Turner, 1985; Edgar, 2007; Irish and Konigsberg, 2007). To ensure accuracy, any observation that was potentially affected by dental wear, caries or calculus was treated as missing data. We followed the standard procedure and dichotomized the ordinal-scaled dental trait scores into binary categories of absence (i.e. 0) or presence (i.e. 1) in order to reduce observer error and simplify data analysis. The applied dichotomization thresholds follow established breakpoints (Turner, 1987; Irish, 2016). For traits whose dichotomization breakpoints are not specified in the latter reference studies, we adopted the breakpoints used by anthropologists working in the Mediterranean (Parras, 2004; McIlvaine et al., 2014).

All osteological data were collected by the lead author (H.R.). The dataset is freely available on GitHub (link here). The data sheet provides individual-level information about sex, age-at-death, dental metrics, dental nonmetric traits scores, and dichotomized dental nonmetric traits.

Data preprocessing

A number of data preprocessing steps were used to ensure that patterns of dental phenotypic variation most closely approximate underlying genotypic variation. First, H.R. quantified his level of intra-observer error by re-measuring a subsample of individuals from Santa Maria D'Anglona (n = 30) in two sessions separated by an interval of one week. Dental measurements from the two sessions were compared using intra-class correlation coefficients using the *ICC* function from the psych package (Revelle, 2017) in R (R Core Team, 2016). The resulting coefficients ranged from 0.929 to 0.998 with a significance of $P < 0.05$ for all comparisons (Supplementary Information 2, Table S1). Dichotomized dental nonmetric traits from the two sessions were compared using Cohen's Kappa tests using the *cohen.kappa* function from the psych package in R. The resulting coefficients ranged from 0.621 to 1.000 with a significance of $P < 0.05$ for all comparisons (Supplementary Information 2, Table S2). All comparisons indicated that intra-observer error was negligible.

Second, sexual dimorphism on dental characters was analyzed using t-tests for metric variables and Fisher's Exact-tests for dichotomized variables performed in R. For comparison, we only included individuals with secure sex determinations ($n = 61$). We found that 53 % of the metric variables (15 of 28) showed significant differences between sexes with $P < 0.05$ (Supplementary Information 2, Table S3). For the dichotomized variables we found that 4 % (1 of 25) exhibited a significant difference between the sexes with $P < 0.05$ (Supplementary Information 2, Table S4). Hence, further analyses have to correct for sexual dimorphism on metric features, while the effect of sexual dimorphism on nonmetric features can be neglected as has been proposed by previous ASUDAS studies (Scott and Turner, 1997; Irish, 2016).

Third, inter-trait correlations between dental metric and nonmetric traits were analyzed using the *mixed.cor* function from the psych package in R. The *mixed.cor* function computes a heterogeneous correlation matrix consisting of Pearson correlations for metric variables, tetrachoric correlations for dichotomized variables, and biserial correlations for mixed variables. Correlations were generated using all observations with valid data for a pair of variables. The correlation matrix was then visualized using the *corrplot* function from the corrplot package in R (Wei and Simko, 2017) (Supplementary Information 2, Figure S1). Metric variables were highly correlated with each other, with 71 % of all 496 pairwise comparisons exceeding $r > 0.5$. Dichotomized variables were largely independent from each other, with only 7 % of all 465 pairwise comparisons exhibiting correlations of $r > 0.5$ or $r < -0.5$. Correlations between dichotomized and metric variables were found to be low as well, with only 5 % of all 942 pairwise comparisons exhibiting correlations of $r > 0.5$ or $r < -0.5$. In conclusion, we found a high amount of integration among dental metric descriptors, but general independence among dichotomized traits as well as among dichotomized and metric variables. Hence, further analyses have to correct for correlation among dental metric variables.

Analyzing population structure for pre-and postcolonial southern Italy

First, we computed biodistances among individuals using Gower distance coefficients following the protocol by Paul et al. (2013). Gower distance coefficients have been extensively used in anthropological kinship analyses (Ricaut et al., 2010; Stojanowski and Hubbard, 2017) because they can incorporate multiple variable scales (metric and nonmetric traits) and allow for missing data. Nevertheless, the amount of missing data should be reduced as much as possible in order to prevent that two individuals cannot be compared to each other because they have no traits in common. Because not every tooth could be observed in each individual due to poor preservation or pathology, our dataset comprises large amounts of missing values. We therefore removed the most incomplete variables and individuals from analysis in a systematical stepwise manner to ensure that no more than 33.3 % of variables were missing for any individual included in the final analysis. We removed all mesiodistal crown diameters and all diameters of the LI2 from the analysis because these variables contained too many missing values to be included. Furthermore, we removed dental nonmetric variables that were monomorphic and too data sparse. We also dropped duplicate dental nonmetric traits recorded on two key teeth, by removing the trait exhibiting fewer observations than the key tooth counterpart. This reduction procedure left us with a dataset of 94 individuals and 42 variables (21 metric and 21 nonmetric traits), comprising less than 15 % missing values overall. Missing metric data were imputed following Kenyhercz and Passalacqua (2016) using the *k*-nearest neighbor (kNN) algorithm using the *knn* function from the VIM package in R (Kowarik and Templ, 2016). The kNN algorithm searches the entire dataset for cases most similar to the one with missing data and generates a mean to replace the missing value(s). Raw measurements were then converted into shape variables by dividing each measurement by the geometric mean for all the measurements in each individual (Jungers et al., 1995). This standardization procedure removes gross size from the data in order to assess differences in the proportionate contribution of individual variables to overall tooth size. Furthermore, it has the advantage to adjust for size differences between individuals that may result from sexual dimorphism. Because the Gower distance analysis requires trait independence, we transformed the highly correlated 21 dental metric variables into seven uncorrelated factor scores with Eigenvalues greater 1 by performing principal component (PCs) analysis using the *principal* function from the psych package in R. Finally, we generated a matrix summarizing pairwise Gower distances values among individuals using the *daisy* function from the cluster package in R (Maechler et al., 2017).

After that, we assigned individuals to either the precolonial period (900-700 y BC) or the postcolonial period (700-200 y BC) based on the mean estimate of the burial's dating range. This procedure resulted in two partitioned matrices, summarizing inter-individual Gower distance values for precolonial individuals (n = 44) and for postcolonial individuals (n = 50).

For each time period, we tested whether populations were structured by archaeological site. To check for significant differences in individual compositions among sites, we used the *adonis* function from the vegan package in R (Oksanen et al., 2016). *Adonis* can identify significant relationships resulting from compositional differences in either location (mean) or dispersion (variance) across sites. We therefore used the *betadisper* function from the vegan package in R to check for homogeneity of dispersions among sites to confirm that significant predictors in our *adonis* analysis reflect differences in location rather than dispersion. Relationships among individual compositions across sites were visualized using principal coordinates analysis (PCoA). Dispersions among sites were visualized using boxplots. All graphics were created in R using the above mentioned functions and the ggplot2 package (Wickham, 2009).

For each time period, we further quantified whether population structure conforms to an isolation-by-distance model (IBD) (Wright, 1943). The model states that, if mobility was low, closely related individuals are expected to be buried closer together, whereas if mobility was high, closely related individuals are expected to be buried widely dispersed. To investigate this, we used Mantel tests to correlate inter-individual biodistances (B) and inter-burial spatial distances (G). We further used partial Mantel tests to correlate B and G, while controlling for variation in individual burial datings (T). Mantel tests were performed using the *mantel* and *mantel.partial* functions from the vegan package in R. Computationally, B is the matrix summarizing the pairwise Gower biodistances among individuals; G is a matrix of straight-line geographic distances among each individual burial, estimated with the *distm* function from the geosphere package in R (Hijmans, 2017); and T is a matrix of Euclidean distances between the mean ages of the date ranges of each individual burial.

Analyzing individual ancestries in postcolonial southern Italy

We determined the ancestry of southern Italian individuals postdating colonization (700-200 y BC) based on dichotomized ASUDAS traits using naïve Bayesian classification. Naïve Bayes is a simple yet powerful classification technique commonly used in biodistance research (Edgar, 2005; Hefner and Ousley, 2014; Herrmann et al., 2016; Scott et al., 2018), assigning individuals to pre-defined groups based on posterior probabilities. Naïve Bayes is well-suited for analyzing dental nonmetric trait datasets because, first, the algorithm can handle missing data and, second, it performs best with independent predictors, which generally holds true when using ASUDAS traits (Supplementary Figure S1). We trained our naïve Bayesian model with two ancestry reference groups, 'Italian' and 'Greek'. The Italian reference sample consists of pooled data from two precolonial sites in southern Italy (Incoronata and Santa Maria d'Anglona; 900-700 y BC). The Greek reference sample consists of pooled data from four sites from the Peloponnesus and Euboea (Asine, Lerna, Corinth and Karystos; 2000-200 y BC). The data from Asine and Lerna were obtained from the literature (Parras, 2004). The data from Corinth and Karystos were gathered by one of us (B.K.M). We only compiled ASUDAS data for our Greek reference sample which were gathered from key teeth (Scott et al., 2016) and which were dichotomized using the criteria detailed above. We chose to combine all four Greek samples into one large reference sample due to two reasons: (1) to ensure robust trait frequencies based on as many observations as possible, and (2) to allow for the largest possible trait battery, because some samples were scored for different dental trait setups. This procedure allowed us to employ a comprehensive battery of 28 ASUDAS traits for comparative purposes.

Calculations were performed using the *naiveBayes* function from the e1071 package in R (Meyer et al., 2017). The estimated prior probabilities of the model were calculated as 0.536 for Italians and 0.464 for Greeks. Conditional probabilities for the analysis are presented in the Supplements (Supplementary Information 2, Table S5). Validation of the model was performed with a subset of training individuals from Italy (n = 30) and Greece (n = 5) for which sufficient individual-level data was available, i.e. no more than 33.3 % missing data. Italians and Greeks were correctly classified in 94 % of cases (Supplementary Information 2, Table S6). We then applied this trained naïve Bayes classification model to our test dataset consisting of 41 individuals from postcolonial southern Italy with no more than 33.3 % missing data.

Each individual was assigned to an ancestral group, either Italian or Greek, based on the estimated maximum class probability.

RESULTS

Precolonial period (900-700 y BC)

Our adonis analysis revealed a significant difference among the inhabitants of the two precolonial sites under investigation; Santa Maria d'Anglona and Incoronata (Figure 2a, Table 2). Our betadisper analysis indicated that this difference was due to differential within-site variability and estimated the average dispersion for Santa Maria d'Anglona as 0.211, whereas the average dispersion for Incoronata was estimated as 0.167. Thus, inhabitants of Santa Maria d'Anglona were about one and a half times more variable as those living in Incoronata. Mantel tests revealed low, though significant population stratification according to a geographical IBD model (Table 3). Results were still significant when we took temporal variation due to differential burial datings into account. Thus, precolonial southern Italy was characterized by a moderate pattern of population stratification.

Postcolonial period (700-200 y BC)

Our adonis analysis revealed that there was no significant patterning across the inhabitants of the three postcolonial sites under investigation; Passo di Giacobbe, Metapontion and Siris (Figure 2b, Table 2). The site of Taras was removed from analysis because it comprised only a single individual. Our betadisper analysis indicated equal levels of within-site variability. Mantel tests likewise did not reveal any significant population stratification according to a geographical IBD model (Table 3). Results were still not significant when we controlled for temporal variation due to differential burial dates. Thus, postcolonial southern Italy was characterized by a lack of population stratification, indicating high levels of mobility.

Although we inferred an increase in mobility in comparison to the precolonial period, a cross-period adonis and betadisper analysis revealed that the general biological composition and variability of the area did not change significantly through time (Table 2).

Our naïve Bayesian ancestry classifications revealed a presence of ~ 10 % Greeks within the area (4 of 41) (Supplementary Information 2, Table S7). Greek individuals were randomly scattered across the study region without any clear patterning being evident: ~ 21 % in Passo di Giacobbe (3 of 14), ~ 5 % in Metapontion (1 of 19), 0 % in Siris (0 of 7), and 0 % in Taras (0 of 1). Thus, Greek newcomers and their descendants were equally distributed across colonies and indigenous settlements.

Table 2. ANOVA tables for adonis and betadiper models testing differences in location (mean) and dispersion (variance) of inter-individual Gower distances across sites and time periods. Shown are factor and residual degrees of freedom (Df), sum of squares (SS), mean sum of squares (MSS), *F*-statistic values (F), proportion of explained variance (R^2) and probability values (p).

	Adonis						Betadisper			
Test	Df	SS	MSS	F	R^2	p	SS	MSS	F	p
Differences across sites in the precolonial period	1	0,134	0,134	3,273	0,072	**0,001**	0,021	0,021	8,319	**0,006**
Residual	42	1,714	0,041		0,928		0,107	0,003		
Differences across sites in the postcolonial period[a]	2	0,076	0,038	0,926	0,039	0,536	0,002	0,001	0,372	0,691
Residual	46	1,892	0,041		0,961		0,144	0,003		
Differences across pre- and postcolonial periods	1	0,067	0,067	1,610	0,017	0,135	0,000	0,000	0,162	0,688
Residual	92	3,854	0,042		0,983		0,255	0,003		

[a] To assess differences across sites during the postcolonial time period, we removed the single individual from Taranto because to produce results adonis and betadisper require that groups consist of at least two entities.

Bold values are statistical significant at the 0.05 level

Table 3. Mantel tests of isolation-by-distance (IBD) for pre- and postcolonial time periods. Simple Mantel tests correlating inter-individual biological distances (B) against geographical distances (G). Partial Mantel tests correlating B and G, while controlling for the effect of temporal distances (T). Shown are Person correlation coefficients (r) and probability values (p).

Time Period	IBD	r	p
Precolonial	B ~ G	0,081	**0,002**
	B ~ G, T	0,071	**0,012**
Postcolonial	B ~ G	0,001	0,487
	B ~ G, T	0,017	0,394

Bold values are statistical significant at the 0.05 level

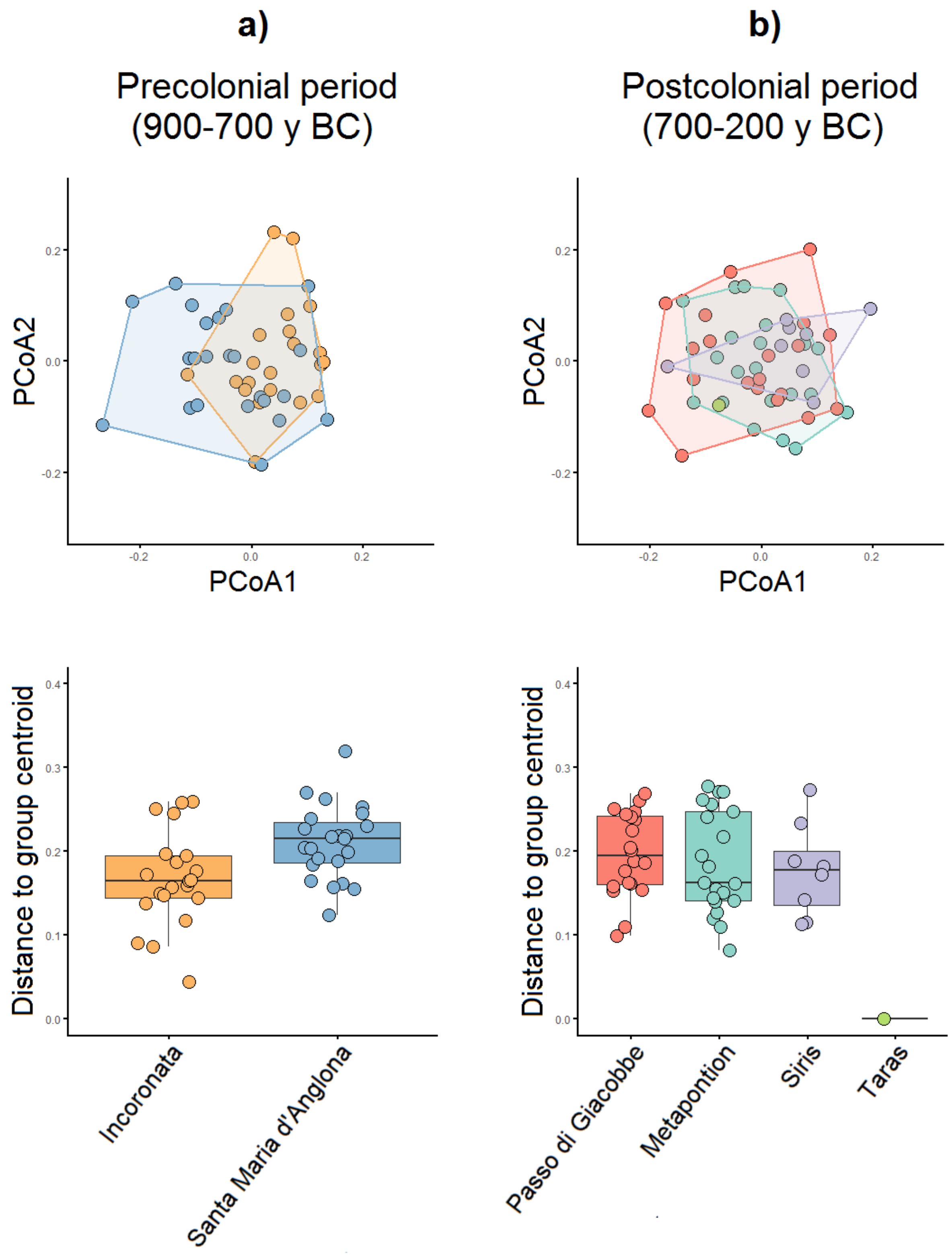

Figure 2. Population structure of southern Italy during a) precolonial and b) postcolonial times. Figures show PCoA plots of inter-individual Gower distances (top) and boxplots represent distances to group centroids (bottom). Color-coding indicates archaeological sites.

DISCUSSION

In this study we used heritable dental phenotypic markers from archaeological human remains as a proxy for aDNA to assess the population history of southern Italy during Greek colonization. Our study has produced three important results: (1) precolonial southern Italy was characterized by moderate levels of population stratification; (2) during postcolonial times, southern Italy became a place of high mobility, hosting ~ 10 % Greek immigrants and their descendants; and (3) Greek newcomers were equally distributed across Greek colonies and indigenous Italic settlements.

During precolonial times (900-700 y BC) southern Italy was characterized by a moderate pattern of population stratification. Although the inhabitants of the two sites under investigation, Santa Maria d'Anglona and Incoronata, shared a very similar genetic makeup, we uncovered a significant isolation-by-distance structure pointing to some levels of differentiation between the two spatially separated sites (ca. 20 km plus two river valleys). Moreover, Santa Maria d'Anglona showed significantly higher internal variability, indicative of more diverse inhabitants from different places as compared to Incoronata. This result indicates greater regional importance of Santa Maria d'Anglona. Interestingly, this interpretation matches the greater wealth of burial goods found in this place and is in line with ancient written sources reporting that the settlement (also called *Pandosia*) was the seat of a king (or *basileus*) (Greco, 1992: 34–40). The lower internal variability found in Incoronata matches the higher degree of equality in grave goods found in this place and supports the theory of a relatively homogenous population inhabiting the site (Siena, 1990).

During postcolonial times (700-200 y BC) mobility of the inhabitants of southern Italy increases. This result is in concordance with the arrival of Greek settlers in this region as indicated by numerous written sources and archaeological evidence (Yntema, 2000). We estimate a Greek contribution of ~ 10 % to the population of southern Italy. Our estimated Greek contribution agrees with the size spectrum proposed by historical demographers, who reckoned with ~ 10 % Greeks in pre-Roman southern Italy (Beloch, 1886). Our results also broadly agree with preliminary strontium isotopic evidence, indicating that 5 % (n=20) of the postcolonial individuals in the study region are of non-local origin (Vos et al. forthcoming). Our results disagree with genomic estimates of the ancient Greek contribution to southern Italy derived from present-day DNA profiles. Di Gaetano et al. (2009) used the haplogroup lineage E-

V13 to estimate a Greek contribution of ~ 37 % to the population of Sicily and attributed the migration influx to 2380 years before present (CI: 6940 to 675 years ago). A potential explanation for the discrepancy between our estimate and their genomic reconstruction could be that proportions of specific genetic lineages surviving in present-days populations may not be good proxies for assessing the effects of past migration events (see Tofanelli et al., 2016). Moreover, their genomic estimate of the timing of the influx has a wide-ranging confidence interval spanning several millennia. The estimate provided in this study is directly derived from archaeological human remains and can therefore be considered more precise.

We found that Greek immigrants and their descendants were equally distributed across sites and did not live in isolated colonial enclaves. In fact, only ~ 4 % of the inhabitants of Metapontion, Siris and Taras were of Greek origin and ~ 96 % were locals. This finding points towards a gradual colonization process with substantial involvement of local populations. This result supports current postcolonial theories and models about the Greek colonization and matches several archaeological discoveries indicating close interaction (Yntema, 2000; Burgers, 2004). At the colony of Metapontion, for example, an indigenous-style hut was found associated with mostly Greek-style pottery and a smaller percentage of indigenous pots (Siena, 1986). Moreover, at the indigenous site of Passo di Giacobbe, several burials contained Greek-style pottery (Schojer, 2010). Thus, our finding fits well the archaeological evidence, suggesting coexistence between a small number of Greek newcomers and indigenous Italic populations.

Despite the finding of ~ 10 % Greek contribution to southern Italy, it does not seem that the presence of newcomers and their descendants significantly impacted and reshaped the south Italian gene pool. Instead, the general biological composition and variability of the area remains relatively constant across pre-and postcolonial times. This could either be the result of the relatively low number of Greek newcomers or shared ancestry of Greeks and Italians making it difficult to observe significant biological differences.

Lastly, it has to be noted that our results deviate from the findings of a previous dental nonmetric distance study from our working group (Rathmann et al., 2017b), where we found a significant separation between the inhabitants of indigenous Italic sites and the colony of Metapontion which evidenced a higher amount of variability. This discrepancy can be explained as the results of three potential sources of bias in our previous study: (1) bias due to inter-observer error; (2) bias due to the use of a limited number of traits; and (3) distortion of the 'true' variability of the groups by using group centroid estimates for comparative purposes. The present study overcomes these potential sources of bias. It further highlights the need for individual-level analyses which are better suited to capturing the true variabilities of groups, and cautions against group-level analyses based on simplified centroid estimates, especially in geographically fine-scaled contexts where genetic differences between individuals are subtle.

Limitations to the study and areas for future research

Our results might be affected by two possible sources of error: (1) sampling bias; and (2) methodological issues related to biological distance analyses based on dental phenotypes. We will discuss each aspect below and highlight productive areas for future research.

First and foremost, our study is limited to archaeological sites from the Gulf of Taranto region. Expanding the study area to Calabria and Sicily and including additional indigenous Italic samples from the inland would help to generate a more comprehensive picture of the colonial history of whole *Magna Graecia*. This study provides a conceptual template for future research in this area and the provided raw data and R scripts in the supplements allow for repeatability.

Second, we believe that there is a need to improve the reference samples used for the naïve Bayesian ancestry determinations. In our study, we used two large reference samples as potential ancestry sources; 'Italians' and 'Greeks'. However, the high degree of Mediterranean mobility involving a multiplicity of actors in addition to Greek seafarers (for example Phoenicians and Etruscans) leaves open the possibility that multiple agents might have been involved in the colonial process, not all of them necessarily coming from ancient Greece. Given our simplified two-sample study design, such individuals would be forced to be classified as either 'Italian' or 'Greek', regardless of their true ancestry. Maybe this was the reason why validation of the model revealed a misclassification of Italians and Greeks 6 % of the time? Using a large number of robust reference samples from all over the Mediterranean would allow us to overcome this issue and, furthermore, opens the possibility to explicitly test for the bio-geographical origin of the colonists.

Finally, it could be that dental phenotypic data may not capture adequate neutral genetic variation for geographically fine-scaled analyses. In fact, dental phenotypic data are considered to be most effective at higher geographic scales of study, particularly global and continental (Scott and Turner, 1997). Recent research has confirmed this and identified congruence in dental phenotypic and neutral genetic datasets from globally distributed populations, with correlations as high as r = 0.635 (Rathmann et al., 2017a). Slightly lower correlations have been found for regionally distributed populations, with an agreement of r = 0.500 (Hubbard et al., 2015). At the within-population level, mixed results have been found, ranging from moderate to strong concordance (Paul and Stojanowski, 2015; Stojanowski and Hubbard, 2017). However, it has to be noted that all previous studies used either metric or nonmetric data, though it has been shown that combining metric with nonmetric markers increases performance compared to using the features separately (Lease and Sciulli, 2005; Hefner et al., 2014). In our study, we use a comprehensive set of mixed metric and nonmetric data with a trait battery outnumbering the variables used in all above mentioned previous studies. We therefore think our approach is adequate for detecting subtle genetic structures on a local scale. Nevertheless, future studies should systematically test the association of large mixed metric and nonmetric dental datasets and neutral genomic variation on a local scale to identify dental data combinations that are most useful for reconstructing local population structure and history.

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Supplementary Information 1

Sample description

Incoronata: Incoronata is an indigenous site covering a broad terrace between the Basento and Cavone rivers and is located roughly 8 km from the coast. The site consists of a large settlement distributed across the terrace and flanked by two extensive necropoleis. Archaeological evidence indicates that the settlement originated in the 9th century BC and came to an end at the end of the 7th century BC. Individuals were buried in a typical indigenous manner: bodies were buried in flexed positions, with gender-specific burial orientations. Males, as identified by their grave goods, faced right, while females faced left. The necropoleis have been largely published by Chiartano (1983, 1994b, 1994a). Further details about the settlement and the necropoleis can be found in Carter (1998), Orlandini (1999) and Denti (2018). We collected osteological data from 139 skeletons coming from the necropoleis of Incoronata indigena (n = 99) (GPS: 40.372589, 16.730201) and San Teodoro (n = 40) (GPS: 40.360514, 16.739261) dating from the 9th to the mid-8th century BC. The data were collected at the National Archaeological Museum of Metaponto. Dating of individual burials was based on grave good assemblages reported in unpublished museum inventory lists and on grave goods reported in Chiartano (1983, 1994b, 1994a).

Santa Maria d' Anglona: Santa Maria d' Anglona is an indigenous site situated on a hill between the Agri and Sinni rivers, about 14 km from the coast. The site consists of a settlement and two large necropoleis in the close vicinity of the hill. Archaeological evidence indicates that the settlement originated in the 9th century BC and came to an end in the 7th century BC. The material culture and flexed burial practice of the necropoleis follow indigenous traditions and are compatible with those found in Incoronata. Part of the necropoleis of Santa Maria d' Anglona have been published by Frey (1991). We collected osteological data from 102 individuals obtained from the Conca d' Oro necropolis (n = 85) (GPS: 40.243458, 16.551738) and the Valle Sorigliano necropolis (n = 17) (GPS: 40.236805, 16.560341) dating from the mid-9th to the final 8th century BC. The data were collected at the National Archaeological Museum of Policoro. Dating of individual burials was based on burial assemblages reported in Frey (1991) and based on information provided by Dr. Salvatore Bianco (personal communication).

Passo di Giacobbe: Passo di Giacobbe is an indigenous site located on a small hill between the Gravina di Laterza and Brandano rivers, roughly 12 km from the coast. The site consists of a small settlement and a large necropolis encircling the eastern and western slopes of the hill. The site was occupied over a long period from the end of the 7th to the final 4rd century BC. The material culture and flexed burial rites of the necropolis follow indigenous traditions, however, several colonial pottery finds point to close contacts to the two nearby Greek colonies of Metapontion and Taras. Summary information about the site is reported in Schojer (1990, 1991, 1992, 1994, 2010). We collected osteological data from 105 skeletons coming from the necropolis at the slope of the settlement plateau (GPS: 40.555282, 16.859746). The data were collected at the National Archaeological Museum of Taranto. Dating of individual burials was based on the date ranges reported in Schojer (1990, 1991, 1992, 1994, 2010) and based on information provided by Dr. Teresa Schojer (personal communication).

Metapontion: Metapontion is a Greek colony at the shores of the Taranto Gulf located between the Bradano and Basento river mouths. The colony is directly situated in the flat coast area bordering the sea, with a belt of necropoleis surrounding the city core. According to ancient written sources, the colony was founded at the end of the 7th century BC by Greeks from Achaia sent for by the Achaian colony of Sybaris in order to block the expansion of the Laconian colony of Taranto in the north (Strabo: 6.1.15). Additional information about the ancient written sources concerning Metapontion can be found in Yntema (2000). Archaeological evidence suggests that the area where Metapontion came into being was uninhabited before. Early Metapontion was probably a dispersed settlement with mixed Greek and indigenous material culture. The early village grew gradually, amassed a great amount of wealth owing to fertile farmland, and quickly developed a Greek urban center (*polis*) with several temples and a large *Ekklesiasterion*. The Metapontion area has been extensively surveyed and several detailed publications about the colonial urban center and the hinterland (*chora*) are available (Carter, 1998; Siena, 2001; Carter, 2006; Carter and Prieto, 2011). We collected osteological data from 109 individuals compiled from three necropoleis surrounding the colony of Metapontion: the Crucinia necropolis (n = 94) (GPS: 40.392593, 16.809456), the Pantanello necropolis (n = 12) (GPS: 40.391642, 16.777538), and the Torre di Mare necropolis (n = 3) (GPS: 40.370341, 16.813161). All data were collected at the National Archaeological Museum of Metaponto. Dating of individual burials from Crucinia was based on information provided by Prof. Dr. Angelo Bottini (personal communication). Individuals from Pantanello were dated based on the date ranges reported in Carter (1998). Dating of individual burials from Torre di Mare was based on unpublished museum inventory lists and information displayed in the museum exhibition.

Siris: Siris is a Greek colony on the coast of the Taranto Gulf located between the Agri and Sinni river mouths. The site consists of a settlement located on an elongated ridge and several burial grounds along the sides. According to ancient written sources, the colony was founded in the mid-7th century BC by Greeks from Ionia that left their home after the Lydian conquest (Strabo: 6.1.14). Additional information about the ancient written sources concerning Siris is summarized in Yntema (2000). Archaeological evidence suggests that the area where the town of Siris arose was not inhabited before. Early Siris was an open, dispersed settlement with mixed Greek and indigenous material culture. The settlement developed gradually into a *polis*, including fortifications and one or two temples. In the final 6th century BC, the city was destroyed. Summary information about the settlement and necropoleis is provided in Berlingò (1993) and Osanna (2012). We collected osteological data from 19 skeletons compiled from two burial grounds on both sides of the ridge: the Madonelle burial ground (n = 18) (GPS: 40.221702, 16.659968) and the Zona Castello burial ground (n = 1) (GPS: 40.216654, 16.680005) dating from the early 7th century to the final 6th century BC. The data were collected at the National Archaeological Museums of Policoro and Metaponto. Dating of individual burials was based on grave good assemblages reported in unpublished excavation reports and museum inventory lists and based on information provided by Dr. Antonio De Siena (personal communication).

Taras: Taras is a Greek colony at the shores of the Taranto Gulf located at a narrow entrance to a small lagoon-like bay providing a sheltered harbor. Unfortunately, most settlement traces and necropoleis from Taras were covered and probably destroyed by the intense occupation of the present-day city of Taranto. Thus, archaeological knowledge about the site is sparse. According to ancient written sources, the colony was founded in the late 8th centuries BC by Greeks from Laconia (Strabo: 6.3.2). Additional information about the ancient written sources concerning Taras are given in Yntema (2000). Archaeological evidence from Taras indicate that the site was continuously inhabited from the Bronze Age onwards. Early Taras was a settlement characterized by indigenous material culture and few Greek imports. By the late 6th century BC, the site developed into a *polis*. Summary information about the archaeological evidence is provided in Yntema (2000). The skeletal sample of Taras is composed of 7 individuals widely distributed across the ancient city core (GPS: 40.471209, 17.247914). The data were collected at the National Archaeological Museum of Taranto. Dating of individual skeletons was based on information provided by Dr. Antonietta Dell'Aglio (personal communication).

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Supplementary Information 2

Table S1. Intra-observer error test for dental measurements using intra-class correlation coefficients (ICC).

Dental measurement	n	ICC	sig
UI1-BL-CROWN	21	0.997	0.000
UI1-MD-CROWN	11	0.985	0.000
UI1-BL-CERVIX	23	0.992	0.000
UI1-MD-CERVIX	23	0.984	0.000
UC-BL-CROWN	29	0.998	0.000
UC-MD-CROWN	20	0.994	0.000
UC-BL-CERVIX	30	0.996	0.000
UC-MD-CERVIX	30	0.987	0.000
UP1-BL-CROWN	28	0.996	0.000
UP1-MD-CROWN	12	0.987	0.000
UP1-BL-CERVIX	27	0.993	0.000
UP1-MD-CERVIX	27	0.939	0.000
UM1-BL-CROWN	28	0.992	0.000
UM1-MD-CROWN	15	0.987	0.000
UM1-BL-CERVIX	30	0.991	0.000
UM1-MD-CERVIX	30	0.993	0.000
LI2-BL-CROWN	10	0.968	0.000
LI2-MD-CROWN	8	0.929	0.002
LI2-BL-CERVIX	10	0.978	0.000
LI2-MD-CERVIX	10	0.965	0.000
LC-BL-CROWN	27	0.990	0.000
LC-MD-CROWN	17	0.994	0.000
LC-BL-CERVIX	25	0.995	0.000
LC-MD-CERVIX	25	0.986	0.000
LP1-BL-CROWN	28	0.991	0.000
LP1-MD-CROWN	12	0.995	0.000
LP1-BL-CERVIX	27	0.991	0.000
LP1-MD-CERVIX	27	0.983	0.000
LM1-BL-CROWN	28	0.992	0.000
LM1-MD-CROWN	12	0.995	0.000
LM1-BL-CERVIX	30	0.992	0.000
LM1-MD-CERVIX	30	0.988	0.000

Table S2. Intra-observer error test for dental nonmetric traits using Cohen's Kappa tests.

Dental nonmetric trait	n	Kappa	sig
Shoveling (UI1)	21	0.786	0.000
Double Shoveling (UI1) [1]	22		
Interruption Groove (UI2)	18	1.000	0.000
Tuberculum Dentale (UI1)	21	1.000	0.000
Tuberculum Dentale (UC)	22	1.000	0.000
Mesial Ridge (UC) [1]	17		
Distal Accessory Ridge (UC)	8	1.000	0.005
Distal Accessory Ridge (LC)	7	1.000	0.008
Mesial and Distal Accessory Cusps (UP1)	17	0.850	0.000
Odontome (P1-P2)	9	1.000	0.003
Metacone (UM2) [1]	26		
Hypocone (UM2)	24	0.917	0.000
Cusp 5 (UM1)	16	1.000	0.000
Carabelli (UM1)	18	1.000	0.000
Parastyle (UM1-UM3)	27	1.000	0.000
Enamel Extension (UM1)	26	1.000	0.000
Enamel Extension (LM1)	27	0.926	0.000
Root Number (UP1)	22	1.000	0.000
Root Number (UM2)	24	1.000	0.000
Peg-shaped (UI2) [1]	21		
Peg-reduced-missing (UM3)	22	1.000	0.000
Lingual Cusp Variation (LP2)	18	1.000	0.000
Anterior Fovea (LM1)	10	1.000	0.002
Deflekting Wrinkle (LM1) [1]	6		
Groove Pattern (LM2)	23	1.000	0.000
Protostylid (LM1)	26	0.843	0.000
Hypoconulid (LM1)	24	1.000	0.000
Hypoconulid (LM2)	21	1.000	0.000
Cusp 6 (LM1)	11	0.621	0.026
Cusp 7 (LM1) [1]	21		
Root Number (LC)	24	1.000	0.000
Tome's Root (LP1)	23	0.881	0.000
Root Number (LM1) [1]	30		
Root Number (LM2)	24	1.000	0.000

[1] Cohen's Kappa could not be calculated because the trait was monomorphic in both scoring sessions (either always '0' or always '1').

Table S3. T-test for sexual dimorphism on dental measurements.

Dental measurement	n male	n female	t	df	sig
UI1-BL-CROWN	13	17	-0.553	28	0.585
UI1-MD-CROWN	2	5	-0.376	5	0.722
UI1-BL-CERVIX	15	18	-0.581	31	0.565
UI1-MD-CERVIX	15	18	-1.184	31	0.245
UC-BL-CROWN	19	23	-3.858	40	0.000
UC-MD-CROWN	4	10	-0.731	12	0.479
UC-BL-CERVIX	20	21	-5.516	39	0.000
UC-MD-CERVIX	19	21	-5.238	38	0.000
UP1-BL-CROWN	19	25	-1.638	42	0.109
UP1-MD-CROWN	3	6	-0.324	7	0.755
UP1-BL-CERVIX	21	23	-2.329	42	0.025
UP1-MD-CERVIX	21	23	-3.396	42	0.002
UM1-BL-CROWN	13	20	-3.424	31	0.002
UM1-MD-CROWN	2	4	-2.751	4	0.051
UM1-BL-CERVIX	19	24	-2.713	41	0.010
UM1-MD-CERVIX	19	24	-4.702	41	0.000
LI2-BL-CROWN [1]	0	2			
LI2-MD-CROWN [1]	0	2			
LI2-BL-CERVIX [1]	0	2			
LI2-MD-CERVIX [1]	0	2			
LC-BL-CROWN	21	23	-5.185	42	0.000
LC-MD-CROWN	2	9	-0.632	9	0.543
LC-BL-CERVIX	21	2	-6.003	42	0.000
LC-MD-CERVIX	21	23	-5.845	42	0.000
LP1-BL-CROWN	23	23	-1.565	39	0.126
LP1-MD-CROWN	9	18	1.307	11	0.218
LP1-BL-CERVIX	23	4	-3.080	43	0.004
LP1-MD-CERVIX	23	22	-3.775	43	0.000
LM1-BL-CROWN	19	22	-1.689	30	0.102
LM1-MD-CROWN	5	13	-1.708	5	0.148
LM1-BL-CERVIX	22	2	-3.147	36	0.003
LM1-MD-CERVIX	22	16	-2.936	36	0.006

[1] T-test could not be calculated because trait was present in only one sex

Table S4. Fisher's Exact test for sexual dimorphism on dental nonmetric traits.

Dental nonmetric trait	n male	n female	Fischer's Exact Test
Shoveling (UI1)	10	11	0.635
Double Shoveling (UI1) [1]	12	19	
Interruption Groove (UI2)	15	18	0.034
Tuberculum Dentale (UI1)	12	15	0.569
Tuberculum Dentale (UC)	13	17	0.070
Mesial Ridge (UC) [1]	8	10	
Distal Accessory Ridge (UC) [1]	1	3	
Distal Accessory Ridge (LC) [2]	0	4	
Mesial and Distal Accessory Cusps (UP1)	9	10	0.582
Odontome (P1-P2) [1]	4	7	
Metacone (UM2)	21	27	0.186
Hypocone (UM2)	16	24	0.740
Cusp 5 (UM1)	4	8	1.000
Carabelli (UM1)	11	8	0.074
Parastyle (UM1-UM3)	15	25	0.375
Enamel Extension (UM1)	18	23	0.679
Enamel Extension (LM1)	17	19	0.342
Root Number (UP1)	21	20	0.215
Root Number (UM2)	19	24	0.351
Peg-shaped (UI2)	19	21	0.475
Peg-reduced-missing (UM3)	26	27	0.250
Lingual Cusp Variation (LP2)	10	14	0.214
Anterior Fovea (LM1) [2]	0	3	
Deflekting Wrinkle (LM1) [2]	0	3	
Groove Pattern (LM2)	14	17	0.304
Protostylid (LM1)	12	19	1.000
Hypoconulid (LM1)	12	17	0.414
Hypoconulid (LM2)	6	12	0.515
Cusp 6 (LM1) [1]	3	4	
Cusp 7 (LM1)	5	15	1.000
Root Number (LC)	23	20	0.465
Tome's Root (LP1)	18	18	0.289
Root Number (LM1) [1]	22	24	
Root Number (LM2)	15	18	1.000

[1] Fisher's Exact Test could not be calculated because trait was monomorphic

[2] Fisher's Exact Test could not be calculated because trait was present in only one sex

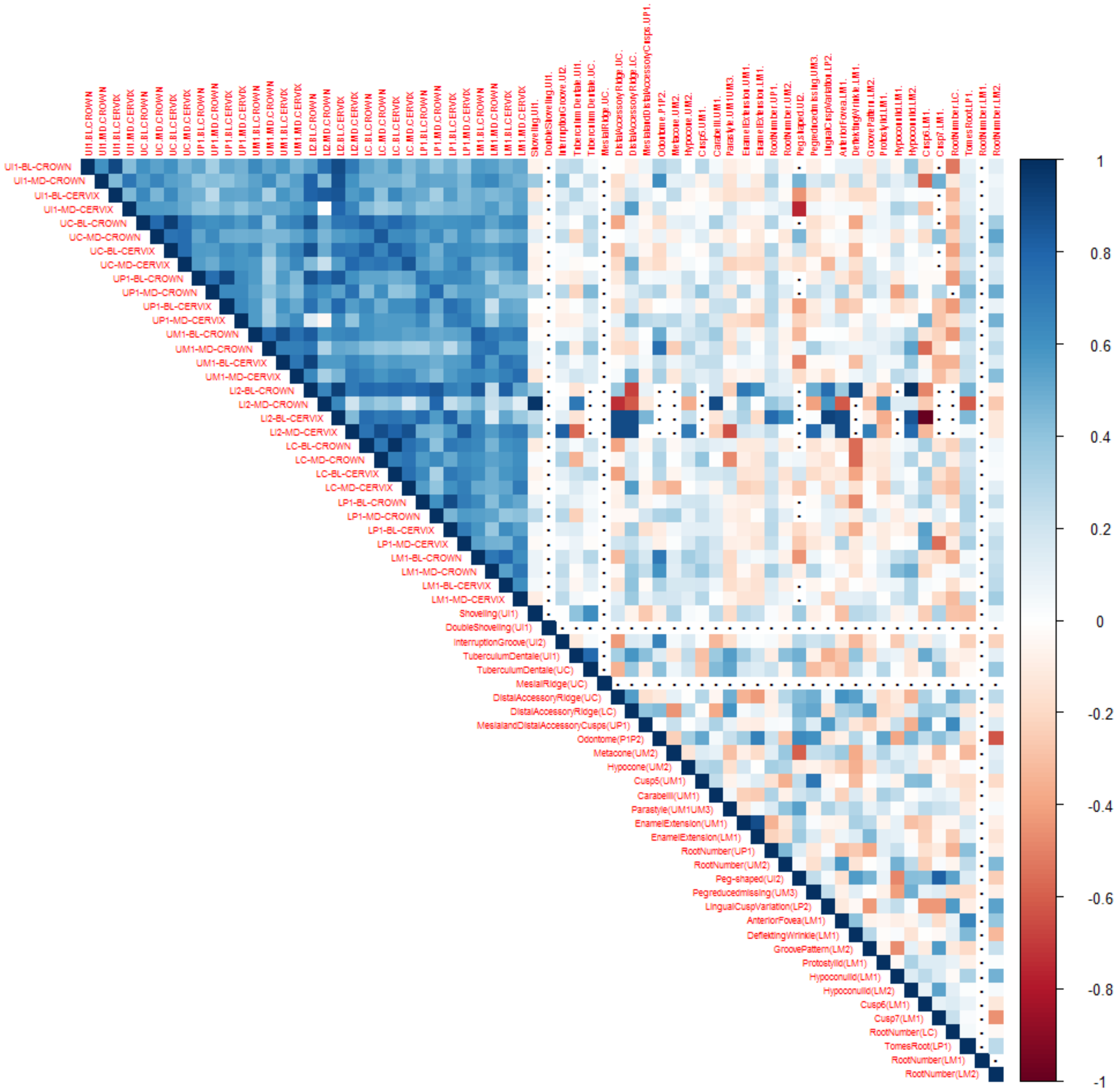

Figure S1. Correlations among dental metric and nonmetric traits. Dots indicate that pairwise correlation between binary traits could not be calculated because there was no variation.

Table S5. Conditional probabilities for naïve Bayesian classification

Shoveling_UI1		
	0	1
Greek	0,63	0,37
Italian	0,70	0,30
Interruption_Groove_UI2		
	0	1
Greek	0,74	0,26
Italian	0,74	0,26
Tuberculum_Dentale_UC		
	0,00	1,00
Greek	0,68	0,32
Italian	0,77	0,23
Mesial_Ridge_UC		
	0	1
Greek	0,95	0,05
Italian	1,00	0,00
Distal_Accessory_Ridge_UC		
	0	1
Greek	0,95	0,05
Italian	0,83	0,17
Mesial_and_Distal_Accessory_Cusps_UP1		
	0	1
Greek	0,96	0,04
Italian	0,83	0,17
Odontome_P1,P2		
	0	1
Greek	1,00	0,00
Italian	0,96	0,04
Metacone_UM2		
	0	1
Greek	0,01	0,99
Italian	0,03	0,97
Hypocone_UM2		
	0	1
Greek	0,14	0,86
Italian	0,40	0,60
Cusp_5_UM1		
	0	1
Greek	0,99	0,01
Italian	0,89	0,11
Carabelli_UM1		
	0	1

Greek	0,69	0,31
Italian	0,56	0,44
Parastyle_UM1,UM3		
	0	1
Greek	1,00	0,00
Italian	0,99	0,01
Enamel_Extension_UM1		
	0	1
Greek	0,59	0,41
Italian	0,81	0,19
Root_Number_UP1		
	0	1
Greek	0,85	0,15
Italian	0,44	0,56
Root_Number_UM2		
	0	1
Greek	0,02	0,98
Italian	0,32	0,68
Peg,shaped_UI2		
	0	1
Greek	0,98	0,02
Italian	0,99	0,01
Peg,reduced,missing_UM3		
	0	1
Greek	0,55	0,45
Italian	0,85	0,15
Lingual_Cusp_Variation_LP2		
	0	1
Greek	0,53	0,47
Italian	0,47	0,53
Anterior_Fovea_LM1		
	0	1
Greek	0,33	0,67
Italian	0,65	0,35
Deflekting_Wrinkle_LM1		
	0	1
Greek	0,90	0,10
Italian	0,86	0,14
Groove_Pattern_LM2		
	0	1
Greek	0,95	0,05
Italian	0,92	0,08
Protostylid_LM1		

	0	1
Greek	0,58	0,42
Italian	0,61	0,39
Hypoconulid_LM1		
	0	1
Greek	0,28	0,72
Italian	0,09	0,91
Hypoconulid_LM2		
	0	1
Greek	0,95	0,05
Italian	0,85	0,15
Cusp_6_LM1		
	0	1
Greek	1,00	0,00
Italian	0,99	0,01
Cusp_7_LM1		
	0	1
Greek	0,96	0,04
Italian	0,96	0,04
Root_Number_LC		
	0	1
Greek	0,91	0,09
Italian	0,93	0,07
Tomes_Root_LP1		
	0	1
Greek	0,92	0,08
Italian	0,90	0,10

Table S6. Confusion matrix from naïve Bayesian classification

		True		Total
		Greek	Italian	
Predicted	Greek	5	2	7
	Italian	0	28	28
Total		5	30	35

Table S7. Prediction results for 41 well-preserved individuals post-dating colonization

Burial_ID	prob,Greek	prob,Italian	pred,class
CRUC_140	0,0080	0,9920	Italian
CRUC_309	0,1078	0,8922	Italian
CRUC_319	0,0271	0,9729	Italian
CRUC_389	0,5426	0,4574	Greek
CRUC_397	0,0426	0,9574	Italian
CRUC_474_C	0,0023	0,9977	Italian
CRUC_541	0,0899	0,9101	Italian
CRUC_577	0,1697	0,8303	Italian
CRUC_580	0,2020	0,7980	Italian
CRUC_622	0,0037	0,9963	Italian
CRUC_82	0,0706	0,9294	Italian
PANT_130	0,1167	0,8833	Italian
PANT_277-4	0,0123	0,9877	Italian
PANT_292	0,0000	1,0000	Italian
PANT_320	0,0250	0,9750	Italian
PANT_356	0,0475	0,9525	Italian
TORR_76	0,0182	0,9818	Italian
TORR_89_1	0,0726	0,9274	Italian
TORR_91	0,0007	0,9993	Italian
PADG_?_Box_6	0,0003	0,9997	Italian
PADG_137	0,2640	0,7360	Italian
PADG_142	0,7762	0,2238	Greek
PADG_149_1	0,0094	0,9906	Italian
PADG_149_2	0,1850	0,8150	Italian
PADG_156	0,8723	0,1277	Greek
PADG_242	0,6612	0,3388	Greek
PADG_272	0,0601	0,9399	Italian
PADG_279	0,0570	0,9430	Italian
PADG_290	0,0233	0,9767	Italian
PADG_295	0,0873	0,9127	Italian
PADG_319	0,1559	0,8441	Italian
PADG_67	0,1947	0,8053	Italian
PADG_71	0,0001	0,9999	Italian
SIRI_1	0,0132	0,9868	Italian
SIRI_1083	0,0079	0,9921	Italian
SIRI_1222	0,1363	0,8637	Italian
SIRI_260	0,0140	0,9860	Italian
SIRI_813	0,0069	0,9931	Italian
SIRI_904	0,3671	0,6329	Italian
SIRI_948	0,2040	0,7960	Italian
TARA_115	0,0145	0,9855	Italian