DNA ANALYSIS OF BONES FROM GRAVE CIRCLE B AT MYCENAE: A FIRST REPORT[1]

INTRODUCTION

EVER since its excavation, scholars have discussed the relationships between the individuals buried in the three or four groups of graves in Circle B at Mycenae, and their links with the slightly later Grave Circle A.[2] In an article published in the centenary volume of *BSA* one of us (AJNWP) described how we had used the technique of facial reconstruction to pick out similarities (and differences) in the appearance of seven individuals from Grave Circle B in order to identify possible family relationships among them by the simple method of testing whether they looked alike. The basic technique of reconstructing a face on a skull is totally objective, and so this apparently crude approach could provide evidence for suggesting some familial connections—and thus perhaps dynastic links—between the groups of graves. Given that only seven skulls proved suitable for reconstruction, the results were very encouraging: two individuals from the late grave Γ, a man and a woman, shared the same heart-shaped face with wide-set cheekbones and eyes and delicate features; while a third person in this grave had a long face with high forehead, lantern jaw, and narrow features that were also found on the champion buried two or three generations earlier in grave Z. At least one other distinctive facial type could be identified, in grave B, while other connections could be argued with greater or lesser conviction.[3]

There is, however, a limit to what can be deduced from such an approach even within a small group like this: there comes a point where all faces begin to share features. Further, of the 35-odd individuals buried in Grave Circle B, only seven skulls were suitable for reconstruction: the rest were generally too fragmentary, or missing altogether. None of the skulls from Grave Circle A had survived intact enough to provide the basis for a reconstruction. Since 1985, when the project was first conceived in the wake of the Manchester team's successful reconstruction of the skull from Tomb II at Vergina and its concomitant identification as that of Philip II of Macedon, other biological and bioanthropological methods of determining population affinities and familial relationships have advanced greatly, and we thought that it would be interesting to carry out a methodological comparison with two of these, epigenetic variation and DNA profiling. The first of these forms part of a wider study of Aegean Bronze Age populations by one of us (LML) and will be reported on another occasion. The pilot project in DNA analysis of the Grave Circle B material has successfully been carried out, and the results are described here.

[1] We owe grateful thanks to several bodies and individuals for their help: notably the Institute for Aegean Prehistory for support in this further step on our journey to meet the families of the Grave Circles; the staff of the Ephorate of Antiquities in Nafplion for making the material available to us once again; and Dr Elizabeth French, Director of the British School at the time this project was conceived, for her encouragement.

The following special abbreviation is used in this article:

Mylonas, *Grave Circle B* = G. E. Mylonas, *Ὁ Ταφικός Κύκλος Β τῶν Μυκηνῶν* (Athens, 1973).

[2] e.g. J. L. Angel in Mylonas, *Grave Circle B*, 389–90; O. T. P. K. Dickinson, *The Origins of Mycenaean Civilisation* (SIMA 49, Göteborg, 1977), 40–49, 50–51; I. Kilian-Dirlmeier, 'Beobachtungen zu der Schachtgräbern von Mykenai und zu der Schmuckbeigaben mykenischer Männergräber', *Jahrbuch des römisch-germanischen Zentralmuseums Mainz*, 33 (1986), 156–98.

[3] J. H. Musgrave, R. A. H. Neave and A. J. N. W. Prag, 'Seven faces from Grave Circle B at Mycenae', *BSA* 90 (1995), 107–35, especially 125–31; also John Prag and Richard Neave, *Making Faces Using Forensic and Archaeological Evidence* (London, 1997, rpr. 1999), ch. 6, especially 139–45.

Analysis Procedures

As a matter of practical and procedural necessity the analysis fell into three stages, and coincidentally the bone samples were chosen and collected by LML on three occasions. In stage one five samples were taken in 1994 for an initial study by KAB, whose goal was the apparently simple but crucial one of verifying the presence of preserved ancient DNA in the remains of the Grave Circle B occupants, and of ensuring that this DNA had not been unwittingly contaminated by the hands of excavators or researchers.

Following the success of that study, samples from nearly all available specimens were collected in two batches, nine in 1996 and thirteen in 1997, in order first to identify the sex of the dead, and second to investigate the possibility of tracing kinship. Samples were normally taken from cortical bone taken from the long bones: all were selected from pre-existing bone fragments, so that no sawing, cutting, or breaking was required. Of the seven skulls which had undergone facial reconstruction, Σ131 was not sampled, to our great regret. The skull is in near-perfect condition (and it is the only intact skull which still retains its mandible), and at this experimental stage we could not justify even the small damage that sampling would cause, while the post-cranial bones have become commingled with those of three other individuals (Υ132, $\Lambda_2$133, and $\Lambda_2$134).

DNA analysis was carried out with 22 samples by CEF, who was unaware of the results of the conventional sex identifications. Approximately 500 mg of bone powder were subjected to an extraction procedure based on standard methods for purification of ancient DNA.[4] Extractions were prepared in a laboratory dedicated to this type of work, in three batches. Two or three blank extractions (carried out without any bone) were prepared in parallel with each batch to check for contamination of the working environment with modern DNA. All of these blank extractions gave negative results when tested by the polymerase chain reaction (PCR), confirming that our operating procedures were sound. As stage two of the study, sex identification was carried out by PCRs directed at the sex-dimorphic amelogenin gene, the preferred method for sex identification in ancient DNA studies and in forensic science.[5] All samples were subjected to at least one PCR but because of the limited amount of material available it was not possible to repeat these PCRs for all samples. The nature of the experiment is such that a male result is considered more definite than a female result because the latter can sometimes be confused with a false result arising from partial failure of the PCR.[6]

For the third and final stage PCRs were also directed at two microsatellite loci to assess the potential of DNA analysis in kinship studies with these bones.[7]

[4] R. Boom, C. J. A. Sol, M. M. M. Salimans, C. L. Jansen, P. M. E. Wertheim-van Dillen, and J. van der Noordaa, 'Rapid and simple method for purification of nucleic acids', *Journal of Clinical Microbiology*, 28 (1990), 495–503; M. Höss and S. Pääbo, 'DNA extraction from Pleistocene bones by a silica-based purification method', *Nucleic Acids Research*, 21 (1993), 3913–14. Full details of the DNA analysis methods, including the procedures used to avoid and monitor contamination with modern DNA, are available on request from TAB at the Department of Biomolecular Sciences, UMIST, Manchester M60 1QD, UK.

[5] K. A. Brown, 'Gender and sex: what can ancient DNA tell us?', *Ancient Biomolecules*, 2 (1998), 3–15; K. M. Sullivan, A. Mannucci, C. P. Kimpton, and P. Gill, 'A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin', *BioTechniques*, 15 (1993), 636–41.

[6] C. Lassen, S. Hummel, and B. Herrmann, 'PCR-based sex identification of ancient human bones by amplification of X- and Y-chromosomal sequences: a comparison', *Ancient Biomolecules*, 1 (1996), 25–33.

[7] The two loci are HUMTH01 and D5S818. M. Polymeropolous, H. Xiao, D. S. Rath, and C. R. Merril, 'Tetranucleotide repeat polymorphism at the human tyrosine hydroxylase gene', *Nucleic Acids Research*, 19 (1991), 3753; L. Jin, P. A. Underhill, M. R. Buoncristiani, and J. M. Robertson, 'Defining microsatellite alleles by genotyping global indigenous human populations and non-human primates', *Journal of Forensic Science*, 42 (1997), 496–9.

Results and Discussion

From the archaeologist's standpoint the immediate advantage of DNA sampling is that a far greater number of individuals can be studied than by facial reconstruction, simply because one is not restricted to individuals with reasonably intact skulls. Further, it should provide a means of sexing individuals where more traditional methods such as anatomical study of the bones or examination of associated grave goods have failed to give an answer. Of the 22 samples that we studied by DNA analysis, nine gave positive results for sex identification (TABLE 1), a level of success (41%) similar to that obtained in other projects using PCR with bones from Bronze Age Greece and elsewhere.[8] The nine positive results included one burial that had not previously been assigned a sex and eight that had been sexed by conventional methods. With six of these eight the DNA and conventional methods gave the same sex, but with two there was a disagreement. One of these, $A_1$69, J. L. Angel reported as a strongly-built woman of about 30 of good average stature (158.8 cm), whose skull was missing. He based his sex identification on the morphology of the *os pubis*, one of the more reliable indicators of sex, as well as the general size and robusticity of the post-cranial skeleton. No grave-goods were recovered from this grave, all presumably lost when grave A was cut through it.[9] The DNA analysis identified $A_1$69 as 'definite male', which on balance is perhaps not a controversial result. This is less easy to say in the case of Γ55, where both the finds (which included a number of weapons as well as the famous electrum mask) and anatomical study of remains entail a male burial but the DNA result is 'possible female'.[10] In view of the reduced certainty of female results, as alluded to above, and the fact that the only répeat of this PCR failed, we believe that the male assignment should stand. However, in a more general context it is worth reporting that in a study of larger group of British burials (currently being prepared for submission to *Antiquity*) one of us (CEF) has discovered an unexpectedly high frequency of incompatibility between the biological sex of a burial determined by PCR and the gender affiliation of its grave-goods, raising the possibility that archaeologists have placed too great a degree of trust in the latter, indirect method of sex identification.

The one previously unassigned burial is that of the child aged two or three years in grave $Ξ_1$ (Angel's no. 57).[11] Using traditional osteological methods the sex of a child cannot be determined with any degree of confidence, and in this particular case the nature of the associated grave goods—four small vases—further limited speculation. For a burial such as this DNA analysis is the only method of discovering the sex of the dead person, and $Ξ_1$57 was identified by PCR as a 'definite male'.

As is evident in TABLE 1, there is some disagreement between the estimated number of individuals buried in and recovered from grave Λ. When LML carried out a detailed study of the crania in 1997 it became apparent that the box containing the skeletal remains from the grave included three individuals, rather than the single adult male, Λ70a Myc, identified by Angel as coming from this context. To complicate matters further, Mylonas in his original report and other scholars in subsequent studies identified only two individuals buried in the grave.[12] At present it is not clear to us

[8] K. A. Brown, 'Ancient DNA applications in human osteoarchaeology: achievements, problems and potential', in M. Cox and S. Mays (eds), *New Perspectives on Human Osteoarchaeology* (Greenwich Medical Media, London, in press).

[9] Mylonas, *Grave Circle B*, 34–5, 400 (Mylonas' internal cross-reference is wrong), pl. 23 α; Angel's report is on p. 379, with Table 1.

[10] ibid., 46–7, 379–80; Dickinson (n. 2), 45–6; *BSA* 90 (1995), 119–21.

[11] Mylonas, *Grave Circle B*, 185–6, 402, pl. 161, with Angel's report on p. 383.

[12] ibid., 129–31, 401, with Angel's report on p. 382; Dickinson (n. 2), 43; W. Cavanagh and C. Mee, *A Private Place: Death in Prehistoric Greece* (SIMA 125; Jonsered, 1998), 28; S. Dietz, *The Argolid at the Transition to the Mycenaean Age* (Copenhagen, 1991), 106–32. In 1997 the box of remains from tomb Λ contained (1) a small bag of bone fragments, including a male os pubis, on the outside of which was the crossed-out place-name 'Berbati'; and (2) the commingled cranial and post-cranial remains of three individuals, two adult males and one adult female.

TABLE 1. Grave Circle B, Mycenae, Human Skeletal Collection: Comparison of Sex and Age-at-Death Estimations

Inventory Number	Grave	DNA Analysis Sex	L. M. Little - Dissertation Sex	Age‡	J. L. Angel* Sex	Age
MYC 51	Γ	Definite male	Male	Young Adult	Male	28
MYC 52	B	Definite male	Male	Young Adult	Male	30
MYC 53	Π	–	Male	Middle Adult	Male	33
MYC 54	H	–	Male	Young Adult	Male	28
MYC 55	Γ	Possible female	Male	Young Adult	Male	33
MYC 56	Λ1	–	Male	Young Adult	Male	25
MYC 57	Ξ1	Definite male		Subadult	–	2
MYC 58	Γ	Possible female	Female	Middle Adult	Female	36
MYC 59	Z	–	Male	Old Adult	Male	49+
MYC 60	Δ	–	Male	Middle Adult	Female ?	40
MYC 61	Δ	–	Male	Young Adult	Male	33
MYC 62	A	–	Male ?	Young Adult	Male	23
MYC 63	Θ	Probable female	No Cranium	–	Female	35
MYC 66	N	Probable male	Male	Middle Adult	Male	45
MYC 66a	N	–	Male	Young Adult	Male	28
MYC 68	I	–	Male	Middle Adult	Male	42
MYC 69	A1	Definite male	No Cranium	–	Female	30
MYC 70	K	–	Male ?	Middle Adult	Male	45
MYC 70a-1	Λ?	–	Male ?	Young Adult	Male	38
MYC 70a-2	Λ?	–	Female	Middle Adult	–	–
MYC 70a-3	Λ?	Definite male	Male	Middle Adult	–	–
MYC 131	Σ	Not tested	Male	Old Adult	Male	55
MYC 132	Y	Not tested	Female	Middle Adult	Female	37
MYC 133	Λ2	Not tested	Male ?	Middle Adult	Male	37
MYC 134	Λ2	Not tested	–	Subadult	Male	5

‡ Age categories used in Little's analysis are those recommended and defined in J. E. Buikstra and D. H. Ubelaker, *Standards for Data Collection from Human Skeletal Remains* (Arkansas Archaeological Survey Research Series No. 44; Fayetteville, Arkansas, 1994), 36: Young Adult = 20–34 years; Middle Adult = 35–49 years; Old Adult = 50+ years.

* In Mylonas, *Grave Circle B*, 379-97.

Note on DNA results:
The DNA analysis detects nucleotide sequences on the X and Y chromosomes. A male result is more definite than a female result because a 'false-female' identification will arise from a male sample if, by chance, DNA from the Y chromosome is not detected. Therefore female identifications are described as 'possible' if based on a single successful PCR experiment and 'probable' if based on two or more replicate PCRs. A 'definite male' is a sample for which all successful PCRs gave a male result. The 'probable male' (MYC 66) gave a male result in one PCR experiment, a female result in a second PCR, and no result in a third PCR. The HUMTH01 microsatellite was successfully amplified from MYC 51, MYC 57 and an unidentified member of the MYC 70a group, and the D5S818 microsatellite was amplified from MYC 57.

how these three skeletal specimens relate to the one or two discussed by previous scholars, or whether it is possible that the third was unidentified at the time of excavation or is a misplaced specimen from another grave within the circle or from another context altogether. Λ70a was sampled twice for our project, first in 1996, before it was clear that the storage box held three specimens rather than one and when the sample was naturally not identified with any particular individual; and again in 1997, when three separate and carefully identified samples were taken. Positive results for DNA sex identification were achieved for only one of these three and are consistent with the results of the morphological analysis. Λ70a–3 is a 'definite male'. By one of those inevitable ironies, only the sample taken in 1996 yielded positive results in the kinship portion of this study.

The microsatellite PCRs were less successful than the sex identifications, only three burials giving positive results (Γ_{51}, $\Xi_{1}57$, and 'Λ70a'). This is too small a sample to make any

inferences about kinship, since these are dependent on PCR results for two or more different microsatellites for each of the burials being investigated. The results are nevertheless encouraging because they show that at least a few of burials contain DNA that is sufficiently well preserved to yield microsatellite data. Although a substantial amount of effort might be required to obtain enough microsatellite results to investigate kinship between any pair of burials, our preliminary data suggest that this will be a fruitful avenue for further research. Although three successful microsatellite PCRs out of 22 samples studied might not appear to represent great success, we are encouraged by these results because in the work described we concentrated our attention on the sex identifications and did not attempt to determine the optimal experimental conditions for PCR of the microsatellite loci. We therefore anticipate a higher success rate in our future work, which will be directed specifically at these loci. The limited results described here are exciting because although successful microsatellite PCRs have been reported with historical material—for example the Romanov burials—there has only been one previous, unconfirmed, report with archaeological remains.[13]

SUMMARY

Bearing in mind the limited scope of our pilot project, we believe that our results provide grounds for considerable optimism for the future contribution which DNA analysis can make to our understanding of Bronze Age Mycenae. The continuing advances in molecular biology are stimulating the development of new and better techniques that we anticipate will enable smaller amounts of more degraded DNA to be studied in the future, extending the range of DNA analysis to burials that failed to yield results in the project reported here. We offer the tantalizing possibility that the language of the genes, coupled with the vast body of archaeological evidence, may help us decipher the biological relationships between the people buried in Grave Circle B. While such analysis will not on its own tell us what these people thought themselves to be—their ethnicity—it is tempting to think that in DNA may lie a clue to help unravel the legends of the migrations, the wars, and the *nostoi* that form such an important part of the story of the Argolid.[14]

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[13] The Romanovs: P. Gill, P. L. Ivanov, C. Kimpton, R. Piercy, N. Benson, G. Tully, I. Evett, E. Hagelberg and K. Sullivan, 'Identification of the remains of the Romanov family by DNA analysis', *Nature Genetics*, 6 (1994) 130–5. Microsateliite PCRs from archaeological remains: K. Kurosaki, T. Matsushita, and S. Ueda, 'Individual DNA identifications from ancient human remains', *American Journal of Human Genetics*, 53 (1993), 638–43.

[14] On the ethnicity of the Argolid, e.g. Jonathan M. Hall, *Ethnic Identity in Greek Antiquity* (Cambridge, 1997), ch. 4; more generally, Siân Jones, *The Archaeology of Ethnicity: Constructing Identities in the Past and Present* (London and New York, 1997).