A Biomolecular Approach to the Study of Social Organisation during the Late Copper Age of Southern Germany

Jörg Wicke[1,2*], François Bertemes[1], Keri Brown[2], Terry Brown[2], Richard Harrison[3], Volker Heyd[3] and Kate Robson-Brown[3]

[1] Martin-Luther-Universität
Halle-Wittenberg
Brandbergweg 23c
D-06120 Halle / Saale
Germany

[2] Faculty of Life Sciences
Jackson´s Mill
The University of Manchester
PO Box 88
Manchester M60 1QD
UK

[3] Department of Archaeology and Anthropology
University of Bristol
43 Woodland Road
Bristol BS8 1UU
UK

Outline

Mors est quies viatoris…

The Copper Age (c. 4500-2000 BC) is a critical transition stage in the development of social organisation in Europe. Immediately before the Copper Age, the late Neolithic population lived in largely agrarian communities whose burial practices suggest that their society had a less developed hierarchical structure. By the end of the Copper Age, these communities had adopted more complex social organisations, with greater stratification and individualisation, sufficient to drive the subsequent emergence in the Early Bronze Age of the sophisticated, warrior-led societies that eventually evolved into the civilisations of classical times. The social development of the Copper Age was accompanied by increases in population density resulting from the gradual intensification of agriculture and the discovery of metallurgy, but these technological advances, which are relatively amenable to study by science-based archaeology, tell us little about the social processes occurring at that time. These processes can only be understood by examining the societies themselves, in particular by describing the structures of individual, well-chosen communities.

Our project aims to gather knowledge about the mobility and biological relationships of individuals in the Late Copper Age of southern Germany, namely the eastern group of the Bell Beaker Culture.
We will use methods of molecular biology and analytical isotope chemistry on human remains, starting with two cemeteries of the Bell Beaker Culture. Alburg-Lerchenhaid (18 individuals) and Irlbach (21 individuals) are approximately 20km apart from each other situated in the vicinity of Straubing (Bavaria), immediately south of the Danube. Both were in use for 8-10 generations (ca. 2450-2250BC, HEYD 2000) and provided well preserved skeletons.

Fig. 1: The situation of Alburg and Irlbach. Map taken from Microsoft Encarta 2001

In the initial stage, our research is attempting to answer the following questions:

Was social status inheritable?
As far as we can infer from grave studies, status differences in the late Copper Age were not very high pronounced though clearly recognizable. For example, some wooden chamber graves had stone, copper and even amber and gold items whilst other, simply installed burials contained no grave goods at all. The social status´ scale of burials between those two extremes could partially be differenciated into strata, some objects for example the copper daggers and the armament of bowmen could be claimed as prestige goods and/or rank attributes. Apart from these objects, there seems to be a smooth transition between the "richer" and the "poorer" of the populations. Interestingly, some of the "richer" burials contained subadults (e.g. Landau-Südost, 5-7y, male with dagger and bowman armament, HUSTY 2004 or Irlbach Gr 11, juvenis, male with pottery, animal bones and slingshot stones). This suggests, that material and social status could be inherited, but this hypothesis can be verified only by using scientifical methods. The possibilities of a molecular kinship analysis could not only show us if there are parallels between biological relationship and social status, but in combination with chronological data how this status developed (increased/decreased/stagnated) over time. Thus, dynamics in material and social status within and between families would be observable.

In which dimensions is mobility of individuals traceable?
Perhaps the most famous example for a widely travelled prehistoric individual is the "Archer" of Amesbury, whose oxygen isotope ratio in tooth enamel suggests that he spent his childhood in continental Europe, probably about 1000km or more away from his burial place (WESSEX ARCHAEOLOGY 2005). Many more skeletons from this period exhibit unusual isotope ratios, especially Sr87/Sr86. Unusual, because the values of those individuals do not correlate with other burials on the same cemeteries and/or show high differences between tooth enamel and dentin/bone ratios respectively (GRUPE et al 1997, WINTERHOLLER 2004, PRICE et al 2004). Therefore the people did not spend their whole lifetime in the population they have been buried with.
We will try to research isotope ratios (Sr^{87}/Sr^{86} and O^{18}/O^{16}) in our samples, in order to identify the outliers. Luckily the geological circumstances of the region near Straubing present two very different substrates, involving different strontium isotope ratios in the ground, which will be reflected in the organisms subsisting on them: north of the Danube the underlying rock is magmatic/metamorphic, whilst south of the river *Löß* soils predominate. Due to the vicinity of the cemeteries to this geochemical border which is also a topographical barrier the probability of finding different isotope patterns in the samples is good.
In addition, by comparing tooth-enamel samples with dentin/bone-samples of each individual, we should be able to isolate an age range during which the individuals moved.

In all we hope to clarify at least some of the biological relationships between the two cemeteries Alburg-Lerchenhaid and Irlbach, in order to find out, whether a genetic exchange between those two populations existed. Closely related to this question is the following:

Can we detect a dimension of mobility in marriage patterns?
Recent studies in strontium isotope ratios found, that small possibly family groups travelled as some of the outliers are children. In addition, there might be more of migrating females, which suggests patrilocality (GRUPE et al 1997) - the brides where chosen from a different population and moved after marriage to the groom´s group. Although this assumption has been retracted (e.g. PRICE et al 2004), it still remains to be tested by molecular biology.
Strontium ratio analysis has two disadvantages in that it is limited by the ratio-homogeneity of large areas and also provides only indirect evidence of migration. Therefore, female exogamy is directly provable by the occurrence of different maternal lineages, particularly if new lineages appear during the use of the cemetery.

Finally, we have to keep in mind, that our interpretations about mobility inferred from strontium isotope ratios are only a secondary aspect, depending primarily on the diet. A preliminary study on 15 individuals from Irlbach revealed a slight but verifiable change in Sr^{87}/Sr^{86} ratios over time, which suggests rather a change in subsistence than a migrational event (WINTERHOLLER 2004). This hypothesis remains to be scrutinized with the aid of additional isotopic ratios, esp. oxygen.

And generally: ***Do the results gathered from molecular biology, biological anthropology and archaeology confirm each other?***
By using scientific methods of different disciplines on the same material we try not only to answer archaeological questions as those mentioned above but also we wish to compare the methods themselves. Are we able to find consistency in the results of research in isotope ratios, molecular biology and methods of biological anthropology? The conclusions enabled by those disciplines separately should be comparable or, in the case of missmatching, at least coherently explainable. After all we should be able to compare the scientific results with the archaeological hypotheses or paradigms, e.g. those about sex and gender in the late Copper Age: Men and women are buried in opposite positions, although some individuals have been anthropologically identified as being the "wrong" sex for their position. This identification is questionable (NORTHE et al 2002), however many archaeologists use this data still in social-theoretical discussions.

	mol. biology	Isotopes	biol. anthropology	archaeology
sex/gender	X		X	X
kinship	X		X	
migration	X	X	(X)*	X

Tab 1: The three scientific disciplines plus archaeology and their comparable results.
** - a special skull shape on some of the Bell Beaker skeletons has repeatedly accounted for ethnospecific and thus traceable evidence*

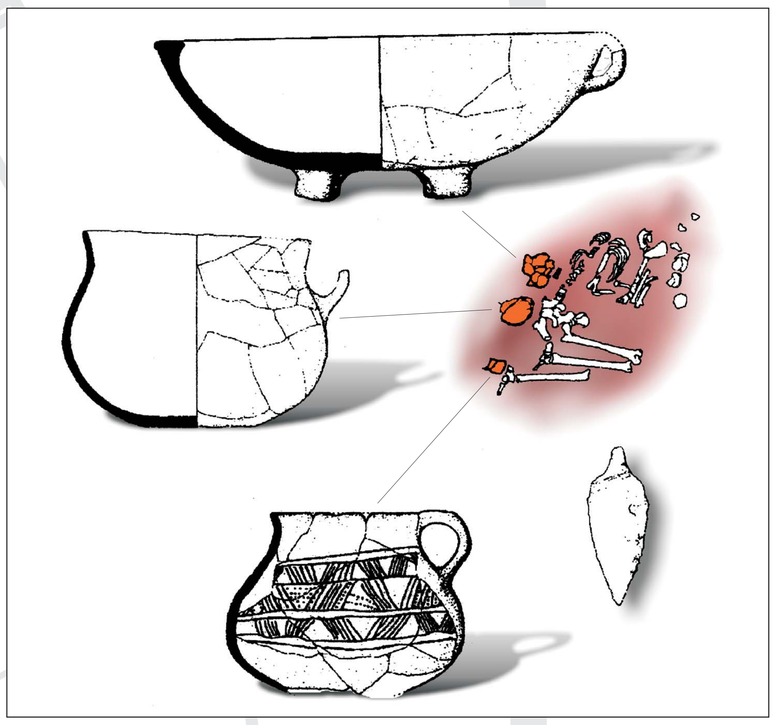

Fig 2.: Grave number 14 fom Irlbach. The burial of this adult male contained three ceramic vessels, a copper dagger and slingshot stones. After HEYD (2000).

Molecular Biology

The first phase of the project started in Jan 05 with aDNA-research at the University of Manchester, Faculty of Life Sciences. The first ojective is to detect and to sequence mitochondrial DNA from the hypervariable regions I (HVR I) and II (HVR II). HVR I is divided into 5 subsections (119-168 basepairs long), HVR II is divided into 2 subsections (117 and 137bp) each section is associated with a special pair of primers for single PCR.

We should not only be able to identify the mitochondrial haplogroups, but also family inherent mutations in HVR I and II in order to find the matrinlinear kinship between the individuals within Alburg-Lerchenhaid and Irlbach. Samples were taken from dental pulps (COBB 2002) and compact pieces of longbones (extraction method after YANG et al. 1998).
Sex identification will be performed by amplification of the Amelogenin-locus on the X- and Y-chromosomes and if results are positive tested by sequencing of the products.

So far, tooth-samples from ten individuals from the cemetery of Irlbach have been tested for the loci in single PCRs. In addition bone samples from four of these individuals have been tested.
In the first test series, only the primer pair mtW with the shortest product yielded molecules of the expected length (117bp, in HVR II) in the samples of four individuals:

Grave number	Tooth sample	Bone sample
4	-	
5	-	
6	-	
7	-	
8	-	-
10	-	+
11	-	
14	+	
19	+	+
21	-	+

Tab 2: The results of mtDNA screening so far, "-" indicates negative results for the primer pair mtW, "+" positive results, blank space means, no samples provided/not tested yet.

On first glance, these results raise the question whether we have to pay the advantage of a lower contamination risk in tooth-samples with the disadvantage of a smaller chance to find any traces of aDNA in them. This might be due to the fact, that an average tooth sample consists of 0.005g, an average bone sample of 0.5g - due to the mere quantitiy of material the probability of recovering biomolecules should be higher in the latter. But the quality, i.e. whether those products represent ancient human DNA or not, remains to be proven by the following steps:

After a first PCR the products of the expected length are purified (QUIAGEN) and transferred into molecular vectors (Lambda DNA). These vectors are then inducted into cells which are spread on a culture medium for proliferation into colonies. Since each vector carries one molecule of the amplified product and each cell absorbs usually one vector and finally each colony has grown from one cell, this method provides the separation of different kinds of strands. Afterwards a minimum of five colonies per sample is transferred to another PCR to amplify the strands and to prepare the sequencing PCR, which is the final step leading to a readable printout of the sequences (sequencing at OCMS, MRC, Oxford).

Since the results so far seem to indicate the presence of DNA in the bones, we will try to increase both the number of positive tested individuals and the loci examined. The later will require the use of nested PCR´s.

Further Work

The second phase of the project will start in the third quarter of 2005 and will involve isotopic-analytical as well as biological-anthropological research. These parts will be carried out at the University of Bristol, Department of Archaeology and Anthropology and Department of Earth Sciences.

We will start with measuring Sr^{87}/Sr^{86} ratios on tooth enamel and bone (dentin respectively) samples of each of the individuals from Irlbach and Alburg Lerchenhaid; local reference values (animal bones) will be gained from publications.

Preliminary research by Bärbel WINTERHOLLER (2004) shows, that we can expect at least one outlier in Irlbach - a female adult buried in the last phase of the cemetery. Additionally she showed a development of strontium ratios during the use of the burial ground, which might be accounted as a change in subsistence rather than mobility. To test this hypothesis and her other results, we will measure O^{18}/O^{16} ratios as well as research the second cemetery from Alburg Lerchenhaid for both strontium and oxygen istope ratios. As this burial ground covers the same period of time as Irlbach and is situated in the same microregion, this second study will help us understand the pattern and the development of isotope ratios in a small area.

Furthermore we will examine the material with "conventional" methods of biological anthropology, starting with macroscopic morphognostic approaches. Main points will be the diagnosis of the age at death and sex identification. So called "epigenetic traits" (for example traits on teeth crowns), which are thought to be mostly genetic and developed without any environmental influences, shall be examined in order to compare those indicators of possible biological relationship between individuals with the results of our biomolecular study. The appearence of a distinctive trait, a short and steep headed skull, the so called "*planoccipitales Steilhaupt*" (GEHRHARDT 1976) is especially connected with the Bell Beaker "folk" and will thus be an object of our researches and discussions as well.

Finally we will try to collect more samples from surrounding burials of the Bell Beaker Culture as well as their predecessors, the Corded Ware Culture and their cultural genetic successors, the populations of the Early Bronze Age. Due to the fact that these cultural groups did not suddenly displace each other but overlap in time, the synchronous relations between those groups will be of interest for us, too. The supplementary samples will be added to the second phase of the project in order not only to look for differences/concordances in the distribution of their measurements compared to our two cemeteries but also to research whether the observed development is continuous or staggered.

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contact: joerg.wicke@praehist.uni-halle.de /|\ homepage: www.glockenbecher.de.vu