Research Title: Examining evidence for genetic continuity among Holocene Khoesan skeletons

Researcher: Prof RR Ackermann, University of Cape Town
Collaborators: Dr. Wendy Black, Iziko Museums of South Africa; Prof Eske Willerslev, Centre for Geogenetics, Copenhagen

Project Description and Motivation:

For well over a century, anthropological research has focussed on the question of genetic continuity/discontinuity in the South African Holocene. Early research into various subsistence and cultural changes in the archaeological record (e.g. (Goodwin and Van Riet Lowe 1929)), as well as studies of differences in cranial variation (Louw 1960; Meiring 1937), attributed differences seen among cultural assemblages and skeletal phenotypes to population migrations/replacements. Later work with fuller and better contextualised assemblages favoured population continuity, with shifts in the archaeological record attributed to internal developments (e.g. (Deacon 1984a; Deacon 1984b)), although some continued to argue that differences (the introduction of herding in particular) could be attributed to the influx of new people (Kinahan 1994; Smith 1983), and by extension genes. Recent research into the crania, teeth and postcrania of Holocene Khoesan skeletons from the Western Cape has supported archaeological evidence for continuity through time, with no strong evidence for the migration of new people into the region until fairly recently (e.g. (Black 2014; Sealy and Pfeiffer 2000; Stynder 2006)). Although size and trait frequency changes do occur during this time period, they are largely attributed to external influences, such as nutrition.

This conclusion of regional continuity has important implications for understanding the emergence of the Khoesan phenotype, and may provide some insight into the emergence of modern humans. Yet despite the importance of this research both regionally and globally, no genetic evidence has yet been applied to the task of understanding Holocene Khoesan continuity. This is important as genetic evidence is likely to be a more sensitive indicator of admixture than the phenotype, allowing us to definitively test the hypothesis of continuity and determine whether minor changes seen in the skeleton over time were indeed due to environmentally-influenced plasticity as opposed to gene exchange. Because modern Khoesan peoples are admixed to varying degrees, it remains imperative to examine ancient remains in order to address this question of admixture/continuity.

This application is for permission to sample and export Khoesan Holocene specimens for extraction and analysis of ancient DNA. Increasing sophistication of DNA extraction techniques means that only small samples are necessary for destructive analysis. Here, we request one or (when available) two teeth from each of the Khoesan specimens. Samples span the Holocene. Samples have been chosen based on preservation, as well as chronology. Tooth casts will be made before destructive sampling and returned to the museum. The tooth crown (i.e. the visible part of the tooth when *in situ*) of each tooth will also be returned to the museum, although the root will need to be dissolved and therefore permanently destroyed. For the three specimens in which AMS dates have not yet been obtained, we also request to sample a small piece of compact bone (2cm x 1cm) – rib or femur – for permanent destruction. Samples will be hand carried by airplane to Copenhagen by collaborator Dr. Wendy Black.

Extraction and analysis of ancient DNA will be done in the laboratory of Prof. Dir. Eske Willerslev, Center for GeoGenetics, University of Copenhagen, Denmark. AMS dating will be

done in the collaborative laboratory of Prof Thomas Strafford, University of Aarhus AMS 14C Dating Centre, Dept of Physics & Astronomy, Denmark. Prof Willerslev's lab has been at the cutting edge of ancient DNA extraction and analysis, and has outputted numerous ground-breaking discoveries in recent years. Among other things, his lab has extracted, sequenced and analysed the oldest genome from a prehistoric organism (Orlando et al. 2013). This 700,000 year old horse was preserved in the permafrost of Yukon, Canada; their work pushed back the date for recovery of DNA by an order of magnitude (previously 70K). They have also sequenced non-permafrost material, including the recently announced genome of the terminal Pleistocene (~12.5K) Clovis baby (Rasmussen et al. 2014). This work indicates a direct link between these remains and modern Native Americans, and importantly for the purpose of this research confirms the capabilities of the laboratory for doing full genome extraction/analysis on comparably aged and preserved skeletal material. Other work in their lab has focussed on the interaction between hunters and farmers in Europe (Skoglund et al. 2012). Additionally, they have played an instrumental role in understanding the migration of east Asians and Aboriginals (Raghavan et al. 2013; Rasmussen et al. 2011) and have explored the details of ancestral/descendent states for immune and pigmentation genes in a 7000-year-old European individual (Olalde et al. 2014). More information on this and other recent genomic research can be found on their laboratory website: http://geogenetics.ku.dk/. As this body of work indicates, this team is well-poised to successfully extract and analyse DNA for the purpose described here.

Silica based DNA extraction protocols will be used, followed by shotgun and genome capture, followed by Next Generation Sequencing. We aim for whole genome sequencing, in order to fully understand the relationships between these individuals and other sequenced material (including both ancient and modern peoples). Analyses will include the application of established methods such as treemix, d-statistics, f3-statistics, admixture and PCA plots; the lab will also develop new methods for understanding the demographic history of these particular ancient individuals using modern genome and SNP chip data as reference material.

Specimens approved by HWC for sampling:

Acc. number	**Date**	**Lab no.**	**Locality**
SAM-AP 6332	980 ± 50	Pta-8767	Melkbosstrand
SAM-AP 3053	1990 ± 50	Pta-4411	Strand, Somerset West
SAM-AP 1142	2090 ± 27	OxA-V-2056-32	Strand, Somerset West
SAM-AP 4813	2140 ± 45	Pta-4202	Bokbaai, Darling
SAM-AP 34	2310 ± 25	Pta-6599	Knysna Cave
SAM-AP 1145	3120 ± 70	Pta-2284	Robberg
SAM-AP 3026	3980 ± 60	Pta-7925	Robberg
SAM-AP 4793	4110 ± 60	Pta-4694	Noordbaai, Saldanha
SAM-AP 6272	5830 ± 80	Pta 9082	Darling
SAM-AP 4182	6811 ± 36	OxA-V-2056-26	Coldstream Cave (Drury's Cave)
SAM-AP 4728a	7210 ± 30	Pta-6627	Coldstream Cave (Drury's Cave)
SAM-AP 4828	9830 ± 90	Pta-6605	Tucker's Cave
SAM-AP 4933			Stompneusbaai
SAM-AP 6082			Oakhurst Rock Shelter
SAM-AP 6365			Duynefontein

References:

Black W. 2014. Dental morphology and variation across Holocene Khoesan people of southern Africa: University of Cape Town.

Deacon J. 1984a. The Later Stone Age of southernmost Africa. Oxford: British Archaeological Reports.

Deacon J. 1984b. Later Stone Age people and their descendents in southern Africa. In: Klein R, editor. Southern African prehistory and palaeoenvironments. Rotterdam: Balkema. p 221-328.

Goodwin A, and Van Riet Lowe C. 1929. The Stone Age cultures of South Africa. Ann S Afr Mus 27:1-289.

Kinahan J. 1994. A new archaeological perspective on nomadic pastoralist expansion in south-western Africa. Azania 29/30:211-226.

Louw J. 1960. Prehistory of the Matjes River Rock Shelter. Res Nat Mus Bloem Memoir No. 1.

Meiring A. 1937. The "Wilton" skulls of the Matjies River Shelter. Soo Nav Nas Mus Bloem No. 1.

Olalde I, Allentoft ME, Sanchez-Quinto F, Santpere G, Chiang CWK, DeGiorgio M, Prado-Martinez J, Rodriguez JA, Rasmussen S, Quilez J et al. . 2014. Derived immune and ancestral pigmentation alleles in a 7,000-year-old Mesolithic European. Nature advance online publication.

Orlando L, Ginolhac A, Zhang G, Froese D, Albrechtsen A, Stiller M, Schubert M, Cappellini E, Petersen B, Moltke I et al. . 2013. Recalibrating Equus evolution using the genome sequence of an early Middle Pleistocene horse. Nature 499(7456):74-78.

Raghavan M, Skoglund P, Graf KE, Metspalu M, Albrechtsen A, Moltke I, Rasmussen S, Stafford Jr TW, Orlando L, Metspalu E et al. . 2013. Upper Palaeolithic Siberian genome reveals dual ancestry of Native Americans. Nature advance online publication.

Rasmussen M, Anzick SL, Waters MR, Skoglund P, DeGiorgio M, Stafford Jr TW, Rasmussen S, Moltke I, Albrechtsen A, Doyle SM et al. . 2014. The genome of a Late Pleistocene human from a Clovis burial site in western Montana. Nature 506(7487):225-229.

Rasmussen M, Guo X, Wang Y, Lohmueller KE, Rasmussen S, Albrechtsen A, Skotte L, Lindgreen S, Metspalu M, Jombart T et al. . 2011. An Aboriginal Australian Genome Reveals Separate Human Dispersals into Asia. Science 334(6052):94-98.

Sealy J, and Pfeiffer S. 2000. Diet, body size and landscape use among Holocene people in the southern Cape, South Africa. Curr Anthropol 41:642-655.

Skoglund P, Malmström H, Raghavan M, Storå J, Hall P, Willerslev E, Gilbert MTP, Götherström A, and Jakobsson M. 2012. Origins and Genetic Legacy of Neolithic Farmers and Hunter-Gatherers in Europe. Science 336(6080):466-469.

Smith A. 1983. Prehistoric pastoralism in the southwestern Cape, South Africa. World Archaeol 15:79-89.

Stynder D. 2006. A quantitative assessment of variation in Holocene crania from South Africa's western, south-western, southern, and south-eastern coasts and coastal forelands: University of Cape Town.