

DNA8

The 8th International Conference
on Ancient DNA
and Associated Biomolecules



Uniwersytet Medyczny
Medical University of Łódź, Łódź, Poland
<http://www.umed.lodz.pl/>



Uniwersytet Łódzki
University of Łódź, Łódź, Poland

October 16 – 19, 2006

[Program and Abstracts](#)

Edited by Henryk W. Witas

Contents

1.	DNA8 Contact Information	3
2.	DNA8 Conference Venue	3
3.	Conference Office	3
4.	Conference Committee	4
5.	Other Important Information	4
6.	Program in short	6
7.	Detailed Program	7
8.	Public Day Presentations	11
9.	Oral and Poster Presentations	18
10.	Index of Authors	48
11.	Delegates	51

DNA8 Contact Information

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DNA8 Conference Venue

Public Day

Mowszowicz Lecture Theatre, Faculty of Biology and Environmental Sciences, Banacha 1/3 (no. 2.1 on the map) in the University of Łódź campus - you will reach it in 2 minutes walking to the left from your hotel.

Conference events and accommodation

The University of Łódź Training and Conference Centre
(no. 17 on the map)
Polish name: Centrum Szkoleniowo-Konferencyjne UŁ
ul. Kopcińskiego Street 16/18
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Conference Office

Registration Office and all necessary information you will find at the Desk in the Training and Conference Centre, University of Łódź

Conference Committee

Henryk W. Witas, Prof.	Head, Dept. of Molecular Biology Chair of Oncology Medical University of Łódź hww@poczta.onet.pl
Elżbieta Żądzińska, Prof.	Head, Chair of Anthropology University of Łódź elzbietz@biol.uni.lodz.pl
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Alicja Wajss-Rolczak, M.Sc.	Head, Training and Conference Centre University of Lodz

Other Important Information

Meals

Breakfasts, lunches, dinners (except Gala Dinner and Grill Party) will be served downstairs at the hotel restaurant - University of Łódź Training and Conference Centre. (Breakfast between 7:00 and 10:00,

Paper sessions

All speakers are pleased to give a copy of their Power Point presentation (preferably on PenDrive) at the front desk of Training and Conference Centre on Tuesday October 17, 2006 after Opening Addresses.

Poster session

Authors of posters are pleased to fix it during the lunch brake on Tuesday October 17, 2006.

Group photograph – will be taken during tea time on Wednesday October 18, 2006 in front of the Training and Conference Centre of the University of Łódź.

All information concerning the Conference Venue you can find on the web site: www.csk.uni.lodz.pl

Short Program

Sunday 15 October

13:00 – Arrivals and registration
Training and Conference Centre, University of Łódź (TCC)

Monday 16 October

8:00 – 9:00 Breakfast , TCC
9:30 – 15:30 Public Day
“**Evolution, Neandertals, Diseases and aDNA**”
Mowszowicz Lecture Theatre, Faculty of Biology and
Environmental Sciences, Banacha Street 1/3, block B
15:30 – 16:30 Dinner
17:00 – Free time (e.g. walk along the Piotrkowska Street)

Tuesday 17 October

9:00 – 9:30 Official Opening Addresses, TCCentre
9:30 – 10:45 Session 1: Population Genetics
10:45 – 11:15 Refreshments
11:15 – 12:30 Session 2: Population Genetics
12:30 – 13:30 Lunch
13:30 – 14:45 Session 3: Preservation of DNA
14:45 – 15:15 Refreshments
15:15 – 16:15 Applied Biosystems
18:00 – 21:00 Gala Dinner (Bidermann Palace)

Wednesday 18 October

9:20 – 9:30 Announcements
9:30 – 10:45 Session 4: Diseases I
10:45 – 11:15 Refreshments
11:15 – 12:30 Session 5: Diseases I
12:30 – 13:30 Lunch
13:30 – 14:45 Authenticity and Contamination – Panel Discussion
14:45 / 16:00 – 18:00 Business Meeting / Tour around Łódź
18:00 – 21:00 Grill Party

Thursday 19 October

9:20 – 9:30 Announcements
9:30 – 10:45 Session 6: Applications
10:45 – 11:15 Refreshments
11:15 – 12:30 Session 7: Diseases II
12:30 – 13:30 Lunch and Farewell

Detailed Program

Sunday 15 October

13:00 - Arrivals and registration
Training and Conference Centre, University of Łódź
(TCC)

Monday 16 October

“Evolution, Neandertals, Diseases and aDNA” - Public Day

Mowszowicz Lecture Theatre, Faculty of Biology and Environmental Sciences

9:30 – 10:00 Opening Addresses

Prof. Henryk W. Witas,
Dept. of Molecular Biology, Chair of Oncology Medical University of
Łódź

Prof. Mark Spigelman
Centre for Infectious Diseases and International Health, Department of
Infection, Windeyer Institute of Medical Sciences, UCL, London UK.
Department of Parasitology Kuvim Centre for the Study of Infectious
and Tropical Diseases Hebrew University - Hadassah Medical School
Jerusalem

“Evolution, Neanderthals, Diseases and aDNA”

10:00 – 11:00 **Milford H. WOLPOFF**
The Paleontology of Human Evolution -
How can DNA Analysis Help?

11:00 – 12:00 **Igor V. OVCHINNIKOV**
150 years of the discovery: from the odd bones
to the Neanderthal genome project

12:00 – 12:45 **Mark SPIGELMAN**
Mummies, Malaise and Murder Novel

12:45 – 13:15 REFRESHMENTS

13:15 – 14:00 **Albert ZINK**
Molecular History and Evolution of Tuberculosis –
Evidence from Mummies and Skeletal Remains

14:00 – 14:45 **Charles L. GREENBLATT**
Towards the Molecular Archaeology of the Holyland

14:45 – 15:30 **Lassi ALVESALO**
The expression of human sex chromosome genes in
tooth growth

15:30 – 16:30 DINNER

Tuesday 17 October

Lecture Room no. 1 at the Training and Conference Centre, University of Lodz

9:00 – 9:20

Official Opening Addresses

Rector of the Medical University of Łódź

Rector of the University of Łódź

9:20 – 9:30

Announcements

Population Genetics Chairman: Milford H. Wolpoff

9:30 – 9:55

The Pleistocene horse mtDNA diversity in Sungir, Russia

Ovchinnikov I., Götherström A., Eriksson T., Bader N., Angerbjörn A., Goodwin W., Lidén K.

9:55 – 10:20

Genetic variability of immunogenetic markers in historic populations

Pollmann J., Herrmann B., Hummel S.

10:20 – 10:45

Medieval ancestors of European sturgeon

Gruchota J., Popović D., Stanković A.

10:45 – 11:15

REFRESHMENTS

11:15 – 11:40

Pompeii and Murecine: tales from ancient DNA

M.Cipollaro, S. del Gaudio

11:40 – 12:05

Characterisation of intermediate and null alleles for Y-chromosomal microsatellites commonly used in forensic genetics

Rębała K., Ciesielka M., Koziol P., Szczerkowska Z.

12:05 – 12:30

Was Inca from Ccopan genetically different from his subjects?

Baca M., Sobczak M., Stanković A.

12:30 – 13:30

LUNCH

Preservation of DNA Chairman: Igor V. Ovchinnikov

13:30 – 13:55

Assessing DNA damage of ancient DNA

Matheson C.D., Beaulne C.H.

13:55 – 14:20

Aspartic Acid racemization as a preservation marker: some considerations.

Fernández E., Ortiz J.E., Arroyo E., Torres T., Turbón D.

14:20 – 14:45

Ancient DNA analysis of bones and textiles of prehispanic populations settled in the Palpa Valley/Peru

Renneberg R., Fehren-Schmitz L., Hummel S., Herrmann B.

14:45 – 15:15

REFRESHMENT

15:15 – 16:15

MiniSTR - a new era of DNA analysis

Beata Popowska, Applied Biosystems

18:00 – 21:00

Old Days in Łódź – tour (~16:00)

Grill Party (~18:00 – 21:00)

Wednesday 18 October

9:20 – 9:30 Announcements

Diseases I Chairman: Mark Spigelman

9:30 – 9:55 **Microbiological investigation of a Korean medieval child mummy found in Yangju**
Spigelman M., Donoghue H.D., Grant P., Klein A., Shouval D., Pappo O., Schulman L.M., Kim M.J., Kim J.W., Park S-M., Kim T.H., Park S.S., Bok G.D., Jung C-K., Oh C.S., Lee E., Kim S.B., Shin J.E. and Shin D.H.

9:55 – 10:20 **Molecular identification of Leishmaniasis in ancient Egypt and Upper Nubia**
Zink A.R., Spigelman M., Schraut B., Greenblatt Ch.L., Nerlich A.G., Donoghue H.D.

10:20 – 10:45 **Molecular evidence for leprosy and tuberculosis in a South German Ossuary**
Nerlich A., Marlow S., Zink A.

10:45 – 11:15 REFRESHMENT

11:15 – 11:40 **Considering the past: the immunogenetics of a Canadian First Nation cohort.**
Larcombe L. and Orr P.

11:40 – 12:05 **Presence of $\Delta 32CCR5$ in medieval specimens from Poland**
Zawicki P., Żądzińska E., Jerszyńska B., Jędrychowska-Dańska K., Wrzesińska A., Wrzesiński J., Nadolski J., Witas H.W.

12:05 – 12:30 **Identification of *M. tuberculosis* sensitivity/resistance alleles in medieval inhabitants of central Poland. Preliminary results**
Kołodziejczak M., Jerszyńska B., Jędrychowska-Dańska K., Żądzińska E., Wrzesińska A., Wrzesiński J., Nadolski J., Witas H.W.

12:30 – 13:30 LUNCH

Panel discussion Moderator: Ovchinnikov I.

13:30 – 14:45 **Authenticity and Contamination**

Business Meeting

18:00 – 21:00 Gala Dinner (Bidermann Palace)

Thursday 19 October

9:20 – 9:30 Announcements

Applications Chairman: Matheson C.D.

9:30 – 9:55 **Ancient DNA methodologies: the Iberian horses as a case study**

Morais J., Oom M., Matheson C.D.

9:55 – 10:20 **Isolation of aDNA: procedure semi-destructive for the specimen**

Krzyżażńska A., Jonkisz A., Lebioda A., Markowska J., Dmochowska G., Bartnik B., Dobosz T.

10:20 – 10:45 **Verification of molecular sex identification method with anthropometric data in medieval specimens**

Karasińska M., Żądzińska E., Witas H.W.

10:45 – 11:15 REFRESHMENTS

Diseases II Chairmen: Charles L. Greenblatt

11:15 – 11:40 **Specific and non-specific infestations of archaeological artifacts**

Zylber M.I., Lemma E., Spigelman M., and Greenblatt C.L.

11:40 – 12:05 **Spoligotypes of ancient *Mycobacterium tuberculosis*: development of a data bank**

Lemma E., Lev-Maor G., Kahila Bar-Gal K., Brauner P., Spigelman M., Greenblatt C.L.

12:05 – 12:30 **Searching for *PRNP G/A 129* allele within Polish medieval population**

Kołodziejczak M., Jerszyńska B., Jędrychowska-Dańska K., Żądzińska E., Wrzesińska A., Wrzesiński J., Nadolski J., Witas H.W.

12:30 – 12:55 **Further data on autoimmuno-predisposing alleles in specimens from medieval Poland**

Zawicki P., Wrzesińska A., Wrzesiński J., Jędrychowska-Dańska K., Żądzińska E., Jerszyńska B., Nadolski J., Witas H.W.

12:30 – 13:30 LUNCH and FAREWELL

Delegates and addresses

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Department of Parasitology, Hebrew University –
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**PUBLIC DAY
PRESENTATIONS**

The expression of human sex chromosome genes in tooth growth

Alvesalo Lassi

Department of Oral Development and Orthodontics, Institute of Dentistry,
University of Oulu, Finland

It is generally accepted that dimorphic sex chromosomes evolved from cytogenetically indistinguishable pairs of autosomes. The exact mechanisms through which the homomorphic pair diverged to produce the gene-rich X chromosome and the small, gene-poor Y chromosome are not fully understood. However, most hypotheses agree that the initial stages of differentiation, perhaps 200-300 million years ago, involved suppression of recombination in the sex-determining region. The male specific region on the Y chromosome is then important to the development of a testis. Sexual dimorphism in the growth of bony structures has commonly been attributed to difference in hormonal balance between the sexes. The action of hormones during puberty in particular has been considered important for the expression of this difference e.g. in adult body height. However, it has also been assumed that the X and Y chromosome genes influence final body height. Metric determinations of tooth crown sizes and/or the thickness of enamel and dentin have been performed from dental casts and radiographs of Finnish Hailuoto population and in individuals with sex chromosome anomalies, 45,X, 45,X/46,XX, 46,Xi (Xq), 47,XXX, 46,XY females, 47,XYY, 47,XXY males and males with Y chromosome deletion of the long arm of the Y chromosome (Kvantti-project). The results of these studies have demonstrated effects on growth of the genes on the human X and Y chromosomes. The Y chromosome promotes growth of both tooth enamel and dentin, whereas the effect of the X chromosome on crown growth seems to be restricted to enamel formation. Enamel growth is decisively influenced by cell secretory function and dentin growth by cell proliferation. Quite recent results have indicated that tooth root growth which is influenced by cell proliferations is also affected by X and Y chromosome genes. The location of tooth crown growth promoting gene on the Y chromosome is probably in the proximal non-fluorescent part of the long arm and on the X chromosome in the short arm. It has been suggested that the effect of the Y chromosome is regulatory, at least on amelogenesis, and that the differential effects of the X and Y chromosomes on growth explain the expression of sexual dimorphism in various somatic features such as size, shape, number and maturation of the teeth, and under the assumption of genetic pleiotropy, torus mandibularis, statural growth and sex ratio. It is of great interest that molecular studies have shown that loci for amelogenin, the main protein component of the organic matrix in enamel, are on both the X and Y chromosomes. However, the transcriptional products of the X and Y chromosome genes are both quantitatively and qualitatively different.

Presentation on Monday, October 16 at 14⁴⁵

Towards the Molecular Archaeology of the Holyland

Greenblatt Charles L.

Department of Parasitology
Hebrew University – Hadassah Medical School
Jerusalem, Israel

The history of the Israelite origin and residence in the area of present day Israel stretches back through a number of important archaeological periods; from the Early Bronze Age (3,500 BC), through the Iron Age (1150 – 586 BC), the Hellenistic (300 BC) and the late Roman period (70 – 400 CE). Several major questions dominate the archaeology of the Holyland. They concern the origin of the Israelites, the maintenance of their social structure (that is the priestly class, the kings, prophets and rabbis), and the conquest of the land from the Cannanites. More generally stated, how did these nomads with a record of bad kings, corrupt priests, conquest by major enemies – Egyptians, Assyrians, Arameans (Syrians), Babylonians, Persians, Greeks, and Romans, persist and even become dominant in the land? Molecular biology and aDNA research can lend insights into some of these questions. Central to the successful settlement was plant and animal domestication. Recently we have been able to do the evolutionary tree of the indigenous grape and traced its relationship to those of the Mediterranean basin. Parchments from the Dead Sea Scrolls provide molecular signatures of early goat domesticates. Y chromosome analysis shows relatedness of the Jews to Mesopotamian populations, and on the same chromosome gene clusters define the priestly class's origin as occurring before the separation of the mainstreams of the Jews. We are also gaining a better idea of the diseases of the Biblical period, with a recent finding of a member of the elite class co-infected with leprosy and tuberculosis.

Presentation on Monday, October 16 at 14⁰⁰

150 years of the discovery: from the odd bones to the Neanderthal genome project

Ovchinnikov Igor V.

Dept. of Molecular and Cell Biology and Dept. of Anthropology,
University of Connecticut, Storrs, CT 06269-2131, USA

Mankind likes to celebrate historical milestones even if the anniversary is related to its own origin and evolution. Two dates in the XIX century, 1856 and 1868, mark the discoveries of the Neanderthals and the Cro-Magnons, respectively. Since that time, the origin, species history, and extinction of the Neanderthals as well as the evolutionary relationship between the Neanderthals and modern humans are among the most strenuously debated issues in human evolution. Anthropologists and archaeologists developed several principal theories that explore potential genetic contributions of archaic hominins to the modern humans. Ancient DNA gives us the opportunity for direct dissection of the past.

I will emphasize several cornerstone achievements on the genetic road to the Neanderthal genome. The first and second Neanderthal sequences, published in 1997 and 2000, had equally crucial significance in our understanding of the possibilities and limitations of the analysis of mitochondrial DNA from Neanderthals. Consequent extensive studies of many specimens added just a few Neanderthal sequences; some of these specimens yielded very short fragments with the length less than 10% of the pioneering Neanderthal sequences. It seemed that the genetic analysis of Neanderthals met its logical limits.

New hopes and expectations have recently been reborn when several manufacturers have announced a new generation of DNA sequencing technology based on the emulsion clonal amplification of a DNA library with subsequent pyrosequencing of each DNA template. Since the introduction of the Sanger sequencing method in 1977, only two modern human genomes have been sequenced by the Sanger approach. With advanced new sequencing technology, it is possible to reach a tremendous progress in the sequencing of individual genomes including the restoration of genome of extinct people. This will broaden our understanding of genetics of our ancestors who lived 500,000 years ago and the genetic foundation of different evolutionary fate of the Neanderthals and modern humans.

Presentation on Monday, October 16 at 11⁰⁰

Medicine Mummies, Malaise and Murder

Spigelman Mark

Centre for Infectious Diseases and International Health, Department of Infection, Windeyer Institute of Medical Sciences, UCL, London UK.
Department of Parasitology Kuvin Centre for the Study of Infectious and Tropical Diseases Hebrew University - Hadassah Medical School Jerusalem

Paleopathology and diseases of the past: take a look at how modern day science uses mummies to help us understand modern disease.

This illustrated talk will introduce us to the studies of the large mummy and bony collections from 17th century Hungary and 5th century Sudan: two of the largest mummy studies currently being undertaken in the world.

We will also hear about an investigation of an old murder mystery for the FBI. A suspected murder in a mummy in the Cairo Museum, as well as the search for a vaccine for the 1918 flu epidemic from bodies buried in the arctic and a suspected rape murder of a woman by the Assyrians almost 3000 years ago during their invasion of Palestine. How a body found in Khinon valley in Jerusalem helped solve the riddle of why Hensen's disease disappeared from Europe.

Mark Spigelman is an Australian/Israeli currently dividing his work as a visiting professor in Israel at the Kuvin Centre in the Hebrew University and in London at the Centre for Infectious Diseases and International Health, Department of Infection, Windeyer Institute of Medical Sciences, UCL, Medical School as an archaeologist/ human remains specialist/ anthropologist, researching the history and development of microbial diseases utilising microbiological techniques on ancient human remains. But still remain a Surgeon but one who has started to think about things other than cutting people open.

Currently his work has been filmed for the National Geographic "Tales of the Living Dead" and also Discovery Channel and Channel 3 in the UK.

This is an Promo of a talk I gave on our work at the BM. 40 slides plus a short Video on how we operate and sample from mummies and look for the DNA. The lay press gave it excellent reviews- despite with questions it running 1/2 hr over time the reviewer said it was too short. I do this in 50 mins but need to know how long I have and then cut it down.

Presentation on Monday, October 16 at 12⁰⁰

The Paleontology of Human Evolution - How can DNA Analysis Help?

Wolpoff Milford H.

Department of Anthropology, University of Michigan, Ann Arbor, MI 48109-1092, U.S.A.

The study of human evolution is traditionally a problem within paleontology and archaeology. Many issues have been well resolved, but others remain intractable. The actual fossil data are sparse and not well distributed, and will very probably remain so for the foreseeable future. This means progress in resolving many issues might depend on additional sources of information. Paleoanthropologists have turned to comparative analysis, biomechanical modeling, and archaeological reconstructions of behavior for additional insights, and now DNA analysis is a significant potential source.

There are three unresolved problem areas that various aspects of DNA analysis can address. The most obvious of these is in the identification of genes that have been under positive selection in the human lineage. A second problem area concerns the size of the human population at the time of hominid origins (the phylogenetic split with chimpanzees) and human population size at human origins (this question addresses the mode of speciation), and the interrelated question of when these origins took place. The third problem area concerns the character of the ancient DNA (mtDNA) that has been recovered from human fossils so far, and the insights these mtDNA data provide for recent human evolution and the history of the human species.

Presentation on Monday, October 16 at 10⁰⁰

Molecular History and Evolution of Tuberculosis – Evidence from Mummies and Skeletal Remains

Zink Albert R.

Department of Earth and Environmental Sciences, LMU München
and Institute of Pathology, Academic-Teaching Hospital Munich-Bogenhausen,
Germany

The origin and evolution of the infectious disease tuberculosis and its pathogens, the member of the *Mycobacterium tuberculosis* complex, is still not fully understood. An important effort for a better understanding of the underlying mechanisms of tuberculosis evolution lies within the investigation of skeletal and mummified material dating back up to several thousand of years. In this presentation, we compare molecular data from different time periods of mummified and skeletal material from the Old and New World and describe the current status of ancient mycobacterial DNA-analysis in ancient human remains with particular reference to the genetic evolution of human tuberculosis.

Presentation on Monday, October 16 at 13¹⁵

**ORAL and POSTER
PRESENTATIONS**

Was Inca from Ccopan genetically different from his subjects?

¹Baca M., ²Sobczak M., ^{3,4} Stanković A.

¹ Institute of Genetics and Biotechnology, Warsaw University.

² Center for Precolumbian Studies, Warsaw University.

³ The Antiquity of Southeastern Europe Research Center, Warsaw University.

⁴ Institute of Biochemistry and Biophysics, PAS

Polish archeological team headed by dr M. Ziolkowski discovered in Ccopan site in Peru a rich grave dated for XV century. Most probably, the grave belonged to an Inca of high rank. We have amplified and analyzed the 290 bp mtDNA fragment from the remnants (teeth) found in this grave and from the remnants of 4 individuals buried nearby in much poorer graves. The aim of this study is to verify the hypothesis that Inca leaders were ethnically different from their subjects. We are planning to analyze mtDNA sequences from the greater number of individual and to extend our study on the chromosome Y sequences.

Supported by grant no. UW- BW 1643/R

Oral presentation on Tuesday, October 17 at 12⁰⁵

Pompeii and Murecine: tales from ancient DNA

Cipollaro M., del Gaudio S.

2nd University of Naples, Dept. of Experimental Medicine, Se

Ancient DNA extracted from individuals found in three houses located in Pompeii and Murecine has been studied. Mitochondrial DNA and single genes analysis has been carried out beside histochemical evaluation of bone tissue. Data are shown concerning: a possible pedigree of Polybius house inhabitants, the mitochondrial hypervariable segment I sequences of Murecine remains and a single gene fragment sequence of three equids found in "Casti Amanti" house.

Oral presentation Tuesday, October 17 at 11¹⁵

Population study of four X-chromosomal STR loci from North part of Poland

Cybulska L., Wysocka J., Kapińska E., Rębała K., Szczerkowska Z.

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In forensic science, X-chromosomal STRs bear the potential to efficiently complement the analysis of other genetic markers (autosomal, Y-chromosomal or mitochondrial) and can be a more powerful tool in deficiency cases, especially when a disputed child is a female. Additionally, some special deficiency cases can be solved only through the application of X-linked markers. For example, X-markers are required to exclude paternity where the question is whether two women who were separated as children have the same father.

We have investigated the four X-chromosomal STR loci: human phosphoribosyltransferase (HPRTB), DXS101, DXS7423 and DXS8377. 200 samples of DNA of unrelated persons (males and females) from northern part of Poland were analyzed. DNA was isolated using non-enzymatic method. After amplification, PCR products were separated by means of capillary electrophoresis using Genetic Analyzer 3130. The most common alleles at each locus were sequenced and used as a control ladder to type unknown samples. Testing for Hardy-Weinberg equilibrium (HWE) showed no significant deviation for all loci. Statistical parameters (PD, HET, MEC) showed that examined systems are useful in forensic medicine.

Poster presentation

Aspartic Acid racemization as a preservation marker: some considerations

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The extent of racemization of aspartic acid (Asp) -expressed as D/L ratio- have been used as a marker of biomolecular degradation in ancient remains (Poinar et al. 1996). However, Asp racemization rate is very variable, and depends on biochemical and geochemical factors, mainly the thermal history of the site (Smith et al. 2003). The D/L Asp ratios were obtained in 39 ancient human samples, 4 were taken in bones, 16 in powdered teeth (enamel+cement+dentine) and 19 in dentine. Six samples consisted of total powdered teeth and dentine from two teeth of the same individual. The samples belonged to 8 archaeological sites from Chalcolithic (4500 B.P.), Neolithic (9000 B.P.) and Palaeolithic (18000 B.P.) times. The results showed that there are important differences between the D/L Asp ratios obtained in dentine and total teeth fractions from the same tooth and individual, among dentine samples from the same archaeological site and also among samples of the same age from different -but close- archaeological sites. These results confirm the differences among archaeological sites reported by other authors (Torres et al. 2002) resulting from a different microenvironmental history. The aforementioned differences observed between the different fractions of the same teeth and individual and among samples from the same archaeological site suggest that other factors should also influence the racemization rate. This point out that standardization is urged before employing the Asp racemization ratio as a threshold value for ancient DNA search.

Oral presentation Tuesday, October 17 at 13⁵⁵

Lactose persistence in prehistoric individuals

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Introduction: The dietary habits of ancient populations are often issues of stake. With the domestication of animals like cattle, sheep and goat these habits changed. The question since when milk and its products were used as daily life aliment is of special interest, because the normal condition in mammals is that after the lactation period they are not able to digest lactose. In 95% of the European individuals the state of lactose tolerance maintains, whereas in Africa and Asia 95% are lactose intolerant.

The point of time and the place of development of the lactase persistence are in request. Two different theories of it exist. One is that the lactose tolerance developed in Anatolia in the Neolithic period and would have spread in Europe 8000 years ago. The other theory declares a nomadic tribe (Kurgan culture) as population of origin. Following this theory the lactose tolerance would have spread 4500-3500 years ago.

One method to detect the status of the digesting ability of lactose is to determine the pointmutation C/T (-13910) which is linked to the lactase gene. If a T is realized (homo- or heterozygote) you are able to digest lactose. In this study we investigated this mutation in 38 individuals of the Bronze Age Lichtenstein Cave and in nine Celtic individuals from Manching to get a clue when the lactose tolerance was spread in Europe.

Materials and Methods: The DNA was extracted of femora and mandibles with a silica-based method on the EZ1-extraction robot (Qiagen). For detecting the pointmutaion a dye labelled primer was designed. The upper primer contained a mismatch which permits the digestion with HinfI if thymidin is realized at the position 13910. For proving the authenticity of the results the lactose Primer was coamplified with six autosomal STRs.

Results and Perspective: The lactose genotype could be amplified and authenticated in 27 Individuals of the Lichtenstein Cave. About 60% were lactose tolerant and around 40% were intolerant. Similar results were detected in the nine Celtic individuals: around 50% was lactose intolerant. These high rates of intolerant individuals suggest that even if stock farming was done consume of milk was not a daily habit. These results give a hint that lactose tolerance was not only spread 8000 years ago. For proving this thesis earlier and later populations should be analysed.

Poster presentation

Medieval ancestors of European sturgeon

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Sturgeon belongs to these species of fish which in XX century came to extinction in Polish rivers. Attempts of its reintroduction have been undertaken, however it was not clear which of two surgeon species, *Acipenser sturio* or *A. oxyrinchus* was inhabiting in the past the Wisła and Odra tributaries. The second of these species is still abundant in North American rivers, while the first one can be found only in Garonne River in France. It was believed that the Garonne population represents the true European sturgeon. This believe was challenged by Ludwig et al. (2002) who found that in European waters *A. oxyrinchus* was already present in XIV century.

We have amplified and analysed mtD-loop an/or cytochrome b DNA sequences from 30 fossil samples dated from IV to XX century from various sites in Poland. Only one sample (XIV century) revealed the *A. sturio* haplotype. All others, including one dated for IV century, were found to be *A. oxyrinchus*. Our results indicate that *A. oxyrinchus* colonised Wisła and Odra rivers at least in fourth century and since then it was a prevalent sturgeon species. Therefore restocking of these rivers with *A. oxyrinchus* will not break the conservation genetics rules.

Oral presentation Tuesday, October 17 at 10²⁰

Verification of molecular sex identification method with anthropometric data in medieval specimens

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Problems with sex identification in *subadultus* archaeological specimens lean us toward the work of which results are presented in this paper. aDNA was extracted from specimens excavated on Polish medieval site - Brześć Kujawski (XI-XII century). Ancient DNA was extracted and purified with high sophisticated workstation MagNa Pure Compact (Roche).

N-phenacylthiazolium bromide (PTB) was used to reverse the Maillard reaction. All steps during sample preparations were carried out following the commonly accepted precautions for ancient DNA work, including negative control reactions of extraction and amplification processes. Amplicons obtained for each sample and locus was reproduced at least three times using different source of DNA of the same specimen to confirm initial result. Sex was identified using four different DNA sequences: within amelogenin gene (106/112bp and 330/218bp), *SRY* gene (92bp) and alpha satellite sequences (130/170bp).

We established the scheme of procedure which allows confirming sex of the specimen at one of its steps. The most successful, reliable and reproducible was amplification of 106/112bp amelogenin sequence, probably because of the short enough length of amplicon produced, serving also as internal control for PCR. Only small part of studied samples produced 330 and 218bp fragments, which could evidence high level of DNA degradation. 977bp amelogenin gene fragment was amplified to exclude contemporary contamination. Amplification of *SRY* sequence confirmed female, and alpha satellites as much more sensitive are used when trace amounts of material to amplify is obtained. To verify molecular method we determined 82 medieval *adultus* specimens, which were earlier sex identified anthropometrically (standard measurements of dimorphic parameters of skull, pelvis, mandible, femur, pubic symphysis, teeth diameters analysis). Both methods succeeded in 61 parallel identifications of sex including 27 females, 32 males and 2 unmatched samples. Statistical analysis with ROC Curve confirmed high level of molecular analysis accuracy (AUC = 0.965; 95% CI: 0.918589 to 1; cut off point = 1).

The work was supported by MSHE grant 3P 04C 055 25

Oral presentation Thursday, October 19 at 10²⁰

aDNA preservation patterns within different human skeletal elements and in dependence on different chromosomal locations

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Introduction: DNA degradation is one of the major issues in aDNA analyses. It is assumed that criteria, like the solidity of tissues and therefore the protection of DNA against environmental factors, limit the impact of degradation processes. It is also assumed that there is a connection between DNA preservation and the position of genetic markers on the chromosomes. Therefore, two different aspects of preservation have been investigated: (i) DNA preservation in different skeletal elements. In this context we also examined the effect of copper ions from burial material, which infiltrated the bone during burial time on the stability of DNA. (ii) Preservation resulting from the position of different genetic markers, dependant on their position on larger or smaller chromosomes and on their location near or close to telomers and centromers. The former aspect was examined by using a multiplex PCR, whereas the latter one is studied by use of Real-Time PCR (cf. Westenthanner et al.). *Methods:* To examine the preservation in skeletal elements the number of the authenticated alleles from a multiplex PCR was compared. aDNA was extracted from three historical skeletons and from each skeleton 21 different skeletal elements were examined. One of these skeletons showed stained parts, due to a rosary manufactured from copper, in the folded hands. These parts were examined as well. In addition all samples were also quantified by the use of real time-PCR. To examine the second aspect, we amplified seven markers localised on large and small chromosomes and thereby in vicinity to the centromere and the telomere respectively. For this examination we used aDNA extracts from prehistoric bone material and amplified PCR products of 103 – 112 bp in length; the DNA amplification was carried out using the LightCycler 2.0 System (Roche Applied Science). To compare different DNA preservation we used the Absolute Quantification with External Standards module (LightCycler 2.0 System). All aDNA extractions were carried out with the BioRobot[®] EZ1 (cf. Wenzel et al.). *Results:* The examined skeletal elements showed clear differences in the degradation state and could be classified into three categories: good, middle and low preservation. Best results showed teeth and Partes petrosae. The DNA of the bones, which were stained by metal ions showed better preservation compared to those, which had no visible contact with metal. Although copper ions are known to inhibit PCR, the purification of DNA with the EZ1 enabled the amplification of the respective DNA extract. Currently, we get information regarding the question whether aDNA on small chromosomes is more degraded than on larger ones and whether the location to the centromere is associated with better preservation of aDNA.

Poster presentation

Identification of *M. tuberculosis* sensitivity/resistance candidate alleles in medieval inhabitants of central Poland: Preliminary results

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Tuberculosis remains one of the major infectious diseases in contemporary world populations. It causes infections in almost eight million people annually. It is believed that beside environmental factors, host resistance/susceptibility allele profile is also responsible for development of the tuberculosis. It is suggested that susceptibility to tuberculosis is multifactorial and is associated with genetic factors that directly or indirectly play a role in immune response. Non-HLA polymorphic genes, including *MBL* and *NRAMP1* may play role in immunodeficiency. *MBL*-associated deficiency is probably caused by at least three polymorphisms within *MBL* gene. Regarding *NRAMP1*, its allele responsible for response to *M.tuberculosis* is associated with base substitution at 543 codon of the *NRAMP1* gene.

Molecular analysis was performed on ancient DNA extracted from early medieval teeth. DNA was isolated under commonly accepted aseptic conditions using biorobot MagNa Pure Roche. First pair of primers recognized fragment of *MBL* gene, which is 142 bp in length and covers the most common polymorphic site at 54 codon. Second pair flanked 543 codon of *NRAMP1* gene and was 240 bp in length. Isolation of aDNA fragments was successful in 31 specimens. This study suggests that it is possible to examine different ancient genomes searching for sensitivity/resistance alleles. Future research will be based on examination of the frequency of these point mutations by using restriction endonuclease *BanI* which recognises the sequence G/GYRCC located at codon 54 of *MBL* gene and *Avall* that recognises G/A change at codon 543 of *NRAMP1* gene.

Oral presentation Wednesday, October 18 at 12⁰⁵

Searching for *PRNP* G/A 129 allele within Polish medieval population

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Prion neurodegenerative diseases that afflict both human and animals are caused by pathogenic protease-resistant isoform of the prion protein (PrP) that exhibits characteristic β - sheet conformation. It is considered that the main feature of abnormal prion protein is both propagation of disease-associated isoform of the cellular prion protein and conversion of physiological protein into disease-associated isoform. Prion protein is responsible for long-term memory and is encoded by *PRNP* gene located within short arm of chromosome 20 (20p12). The human *PRNP* gene has two common alleles that encode either methionine or valine at codon 129. 129_{Met} variant of prion protein is considered to be a genetic marker of predisposition to neurodegenerative prion diseases. Polymorphism at codon 129 affects susceptibility to sporadic and iatrogenic forms of a Creutzfeld-Jakob disease among others.

In the present study aDNA was isolated from medieval specimens and analyzed for the presence of methionine/valine codons. Each tooth was separately cleaned before UV irradiation, using dry and wet procedures. DNA was extracted from powdered specimens using biorobot MagNA Pure Compact Roche. Amplification was performed in the laboratory shortly after finishing isolation procedure. Until now we succeeded in cases of 25 specimens. All amplified 129 bp *PRNP* fragments covering sequence of interest, were analyzed with restriction endonuclease *NspI*, which recognizes G/A variants at 385 position. 26 specimens were sensitive for digestion at position +129, +97 and +32. The distribution of the genotype was 54% Met/Val, 26% Val/Val and 20% Met/Met (0.53 : 0.47 Val : Met). Obtained results suggest that the possibility exists to study different medieval DNA, which is the subject of further research.

Oral presentation Thursday, October 19 at 12⁰⁵

Cystic Fibrosis and Hemochromatosis in a Bronze Age population

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Introduction: In present day Central European populations, Cystic Fibrosis (CF) and Hereditary Hemochromatosis (HH) are the most common genetically determined inherited diseases. Both defects are inherited in an autosomal recessive mode. While for CF the rate of homozygosity is about 1:2000, the value is even higher in HH (1:400). Possible reasons for the high incidence are heterozygous advantages. In case of CF, individuals heterozygous for the $\Delta F508$ mutation do not suffer from dehydration in relation to diarrhoea, which may have positively influenced the survival rate for newborn and infant individuals in historic times. In case of HH which arises from SNPs called C282Y and H63D women are less prone to suffer from the consequences of iron loss due to menstrual bleeding and enhanced iron requirements during pregnancy and lactation. Deviating alleles are thought to have accumulated comparatively recently in European populations. *Methods:* The well preserved Bronze Age skeletal remains of the Lichtenstein Cave from the Harz mountains, which proved to be an invaluable genetic archive already in other contexts (e.g. $\Delta 32 ccr5$, Hummel et al. 2005) now enabled to investigate the genetic markers responsible for CF and HH. DNA extracts were processed from bone powder of all 39 individuals with the help of the EZ1 Biorobot (Qiagen) (cf. Poster of Wenzel et al.). The primers for the detection of the 3 bp-deletion $\Delta F508$ on chromosome 7 indicating CF were co-amplified with the STR typing kit *Profiler Plus* (Applied Biosystems). Therefore, each result for $\Delta F508$ is accompanied by a full genetic fingerprint ensuring the authenticity of the amplification result. In case of HH the primers for C282Y and H63D were each co-amplified with an octaplex STR amplification system, again generating genetic fingerprint data. The determination of the SNPs were then carried out through RFLP analysis of the entire amplification product. *Results:* None of the Lichtenstein cave individuals revealed the 3bp deletion at the $\Delta F508$ locus. This result indicates that the increased allele frequencies of $\Delta F508$ in present day populations may indeed be a result of the younger European history. In contrast, the typing of C282Y and H63D for HH indicates that at least the polymorphism for C282Y must be older than the 2000 years assumed since we found 8% of the 3000 years old Lichtenstein cave individuals being heterozygous. However, none of the individuals is suspect to have suffered from HH which requires the compound heterozygous state for both loci. Although we found 36,5% of the individuals being either homozygous (13,5%) or heterozygous (23%) for the mutation in H63D none of these individuals is showing the mutation for C282Y as well. Again, the results prove that the polymorphism H63D is much older than 3000 years.

Poster presentation

Isolation of aDNA: procedure semi-destructive for the specimen

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DNA analysis can be used to widen our knowledge of extinct populations, world and human history. It can sometimes be our only option to resolve particular historical mysteries. The most convenient source for various sampling methods is a museum specimen. Before DNA extraction a piece of bone or tooth is powdered thus causing damage to the specimen. Most museums have, understandably, imposed restrictions on the use of their often irreplaceable collections. Until now, we have been forced to either study or to keep intact old remains. I shall propose a system which will allow us to receive nuclear DNA from teeth, preventing any significant damage to the specimens. The teeth clean and apparently untouched, after rinsing in water and paraffin oil are returned to the museum collection.

Oral presentation Thursday, October 19 at 9⁵⁵

Considering the past: the immunogenetics of a Canadian First Nation cohort.

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The immunogenetic program of an individual, shaped by the population's evolutionary disease history, determines the effectiveness of immune cellular differentiation in eradicating environmental pathogens. Studies suggest that although socio-cultural and economic factors play a role in the prevalence of infectious diseases in Canada's First Nations populations, functional single nucleotide polymorphisms (SNPs) in the First Nations genome may contribute to their susceptibility to tuberculosis. This hypothesis is being explored through the analysis of the immunogenetic profile of contemporary and ancient First Nations individuals and groups in central Canada. This paper presents some preliminary data and outlines a research strategy for evaluating the factors that influence susceptibility or resistance to infectious disease (genetics, socio-cultural, environmental) within an evolutionary perspective.

Oral presentation Wednesday, October 18 at 11¹⁵

Spoligotypes of ancient *Mycobacterium tuberculosis*: development of a data bank

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Spoligotyping, as introduced by the group of van Embden, has become the international standard of tuberculosis strain characterization. Nearly 40,000 strains from 122 countries, distilled into 1,939 shared-types (STs) are annotated in the Fourth International Spoligotyping Database, a global data bank. Less well known is a small group of some 65 ancient samples whose complete or partial profiles have been noted in the literature or in our laboratory. Today's technologies are being "stretched to the limit" in the application of spoligotyping to some ancient samples, but the method seems reliable enough to contribute to an overall understanding of the evolution of the *Mycobacterium tuberculosis* complex. Spoligotyping, it would almost seem, is designed for "ancient DNA" (aDNA) studies due to its great flexibility in amplicon length (1). 43 unique elements characterize the *M. tuberculosis* complex isolates and direct repeat (DR) sequences separate the spacers. The spacers are arrayed on a membrane in their genomic order to which PCR products generated by primers from the DR's are hybridized. The DR primers can span very short fragments of less than 100 base pair (bp). The advantage of the system is that, although aDNA is highly fragmented, the distance between adjacent DR loci is only 35-41 base pair (bp), and each DR locus is 36 bp. Thus, a fragment of 55-60 bp is sufficient to provide a result. Moreover the luminescent detection system which provides a second enzymatic amplification increases the sensitivity of the system. We have utilized "gene scans" to assess the fragmented nature of the spoligotyping amplicons. We will examine the raw data of the spoligotyping patterns and attempt to place them within the context of the global scheme. The general failure to detect patterns consistent with *Mycobacterium bovis* and the similarity of a few of the ancient specimens to those of *M. africanum* are important considerations in understanding the evolution of the *M. tuberculosis* complex.

Oral presentation Thursday, October 19 at 11⁴⁰

Assessing DNA Damage of Ancient DNA

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The identification and characterization of DNA damage is of great importance to archaeological and forensic research and has significant implications for fields of molecular clinical and medical research. Nucleic Acids incur many forms of damage, chemical and physical modifications and fragmentation. Many systems of detection for different forms of damage have been developed and are widely used. Here we present a systematic approach to the identification of damage, which is not limited to one specific form of damage as this can oversimplify a study of the affects of damage on biological processes. This approach has been used to characterize the damage and stability within nucleic acids from various tissues which allows for method improvements, provides indicators for further analysis and can provide a better understanding of the processes and mechanisms for the formation and repair of these different forms of damage. Some of the techniques include, fluorescent-labelled multiplex PCR, Real-Time PCR, TUNEL, immuno-detection and various repair methods. This assessment provides integral information for the reliable study of damaged and degraded DNA.

Oral presentation Tuesday, October 17 at 13³⁰

Ancient DNA methodologies: the Iberian horses as a case study

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A collection of archaeological samples classified as *Equus caballus* from archaeological sites throughout the Iberian Peninsula was analyzed. The age of these samples ranges between the late Pleistocene and the XIV century A.D. The successful analyses of ancient DNA from these samples was not promising due to the depositional conditions and poor preservation in permeable and often acidic soils. After optimizing existing extraction and amplification methods, consistent and replicable amplification was observed. The method optimization procedure required redesigning the primers because the previously published horse specific primer sequences amplified DNA fragments that were too long and all attempts to use them in the Iberian samples failed. A modified Proteinase K extraction with Phenol: Chloroform purification, followed by ethanol precipitation yielded the best results with the redesigned primers for smaller amplicons. We were able to consistently replicate and clone the results from the samples weighing between as little as 0.1g and 0.3g. All the ancient DNA work was conducted at the Paleo-DNA Laboratory, a specifically designed facility for work with ancient DNA. It was built with separate rooms for each step of the process and has strict SOPs and sterilization procedures to ensure reliable ancient DNA extraction and analysis. There are three important outcomes of this research. First, the methodology was optimized to overcome the highly fragmented DNA recovered from previously thought of as highly unsuitable samples from poorly preserved remains. Secondly, the samples successfully analysed are from a geographic region that presents strong evidence of the continuous presence of forms of *Equus caballus* which may have played an important role in the domestication as well as the distribution and composition of the modern horse breeds. Finally, due to the minimal amounts of material required, we may have the ability to analyze samples with minimal destruction and encourage more samples lending by museums and archaeologists, otherwise reluctant to authorize the use of archaeological material for molecular analysis, so far known to be highly destructive.

Oral presentation Thursday, October 19 at 9³⁰

Mitochondrial Haplogroups and Longevity – Examinations on historical skeleton material in different age groups

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Introduction: There are worldwide existing studies on mitochondrial DNA haplogroups and its connection to longevity. Individuals of different age groups are examined. The haplogroups of older persons (e.g. Vitality 90+) are compared to those of younger age groups. It stands out, that the haplogroup J and a polymorphic site at mt9055 are more frequent in senile persons.

In this project such a study is first realized on skeletal material. In contrary to recent studies this examination has the advantage that the control group of 20-40 year old persons is being set up of individuals already deceased. Since it is out of question that persons of recent control-groups could reach a high age somehow, and could be seen as actual control groups.

A further methodological partial aim is, to establish a DNA-fragment obviously longer than 400bp in ancientDNA-analysis.

Material and Methods: 24 individuals of a skeleton collective with known age and sex are analyzed. Every skeleton was part of a morphological examination. These results were used to identify the skeletons with a church-register and a grave-map of the cemetery. To establish the haplogroup, a DNA-fragment of the mitochondrial HVR I-region with 480bp length is sequenced. In addition a haplogroup defining polymorphic site in the HVR II-region is detected by restriction fragment length polymorphism (RFLP). Also, the polymorphic site mt9055A associated with longevity is analyzed by RFLP.

Results: In 23 out of 24 individuals it was possible to amplify and to sequence the HVR I-fragment in at least one extract. The haplogroup J is not being detected in the age group of senile individuals, but in a member of the control group. None of the sequences of the examined skeletons reveals mt9055A. No recent control for this polymorphic restriction site has being found, either. The tendencies offered by recent studies are not being confirmed in this project.

These results are restricted to a low number of analyzed skeletons and to the population specificity of mitochondrial DNA-markers. A wider examination may supply more distinct results.

Poster presentation

Molecular evidence for leprosy and tuberculosis in a South German Ossuary

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There is historical evidence that leprosy spread in Europe from the Roman period until the Middle Ages, but then strongly declined in infection rates. This has previously been attributed to upcoming tuberculous infection and a hypothesized cross-immunity between the two mycobacterial diseases. However, a recent publication questioned this by showing co-infection with both diseases in various populations between the first and the sixteenth century.

In the present study, we therefore analyzed long bones of a skeletal population of at least 2547 individuals dating between the 15th and 19th century that had been housed in an ossuary in a small South German town (Rain/Lech). Statistical analysis indicates that almost half of the total population of that town was covered by the study population. Following a careful paleopathological analysis including radiology of selected samples, skulls and long bones with evidence for chronic inflammatory reaction these were subjected to a molecular analysis for the identification of mycobacteria specific for tuberculosis or leprosy.

In the series we had identified 59 specimens with inflammatory osseous reaction. Out of this material aDNA was retrieved from 24 samples (45%) with 10 cases showing aDNA of the *M. tuberculosis* complex and 5 cases with *M. leprae* aDNA. In only one case a co-infection with both was seen.

This is the first methodical paleopathological and molecular study analyzing tuberculosis and leprosy by investigation of mycobacteria specific for tuberculosis and leprosy. Thereby, we provide evidence for significant infection by both infectious diseases in a Middle Age to modern period population; however, the rate of co-infection in this population was surprisingly low so that this observation does not confirm the previously described high co-infection rate.

Oral presentation Wednesday, October 18 at 10²⁰

The Pleistocene Horse mtDNA Diversity in Sungir, Russia

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The recent data on modern horse mtDNA (Vilà et al., 2001; Jansen et al., 2002) have been interpreted as an evidence of multi-regional domestication of horses. This interpretation is based on two assumptions. First, in case of the restricted origin, domesticated horses should include only a few founding lineages. Second, high mtDNA diversity of modern horses and relatively low mtDNA diversity of the Pleistocene horses from Alaska support the model of multi-regional domestication. However, making this conclusion, the authors of the multi-regional model did not analyze the mtDNA diversity from the remains of the first domesticated horses from the epicenter(s) of the horse domestication in the Eurasian steppe belt and the Eurasian Pleistocene horses after the last glaciation. Without this, it is very prematurely to make any final conclusion about the model of horse domestication.

The archeozoological and proper archaeological sources indicate that the primary major epicenter of horse domestication was in the middle Volga and south Ural region during the period of the Neolithic and Eneolithic steppe cultures (Dergachev, personal communication). As the first step to fill gaps in the molecular genetic study of domesticated horses, we analyzed jaw fragments of the Pleistocene wild horse from the Sungir archaeological site that is located near Moscow and not far from the middle Volga. The mtDNA analysis was carried out using the teeth of three horses from the most recent cultural layer. One of the Sungir specimens was radiocarbon dated to $18,255 \pm 310$ years before present (Ua-14511). The DNA extraction was successful in three teeth, and the mtDNA HVR1 sequences were isolated from position 15,480 or 15,489 to 15,702. The three Sungir horse sequences are dispersed among the modern horse sequences on the phylogenetic tree demonstrating high mtDNA diversity in one very limited site. The sequences are within three clades embracing approximately half of known modern horse lineages. Combined data on the average horse population density and Ne indicate that the territory of the Volga-Ural epicenter would be a sufficient area to maintain a population that encompasses all modern horse mtDNA diversity. Thus, it is possible to suppose that much of the horse mtDNA lineages may indeed have entered early during domestication in the limited geographic area.

Oral presentation Tuesday, October 17 at 9³⁰

Genetic Variability of Immunogenetic Markers in Historic Populations

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Introduction: Inherited immunogenetic variations raise questions on their functional and evolutionary significance. Models of explanation draw a connection to morbidity and mortality in epidemic infectious diseases. Numerous genetic polymorphisms have been detected, which influence the function of the immune response. They are associated with an increased susceptibility to diseases and tumors. Informations on distribution patterns of polymorphisms in genetic archives can provide clues, if allelic frequencies of markers with significance for tumours have changed in the course of time and if these markers can be seen in association with epidemic infectious diseases, most prominently Cholera and the Black Death.

Particular immunogenetic markers are investigated because of their tumorigenic significance (toll-like receptors (TLR), cytokines, heat-shock proteins). For the beginning the focus is on allele frequencies and possible factors of natural selection for two main components of immune response: TLR 2 and 4 and Interleukin 6 and 10. All exhibit single-nucleotide polymorphisms which are in discussion for an impact on susceptibility to infectious diseases.

Material and Methods: Skeletal collectives were selected by their paleoepidemiological relevance. A mass-grave collective from the 14th century was dated to a plague-epidemic, a second collective from the same burial site was dated to a preceding famine and serves as contemporary control. For pre-epidemic samples the 2900 years old Bronze-Age population from the Lichtenstein cave (Harz mountains) was selected. Furthermore a collective from Goslar (18th century) will be used as post-plague sample. A last collective comprises victims of a cholera-epidemic in Alia, Sicily (19th century).

This far we investigated SNPs in the coding region of TLR2 and 4 as well as an IL6 promoter SNP by PCR and RFLP-analysis. Further analysis is planned with a single-base extension reaction (SBE or "minisequencing") which is being adapted. For means of authentication of results the genotyping of the SNPs is integrated into a STR multiplex amplification system for genetic fingerprinting.

Results: Genotyping of the Bronze Age population for IL6-SNP -174 has been already completed. The results show no significant difference in distribution from our modern German population. The TLR-SNPs have been typed for the Lübeck and Bronze Age collectives. First results show divergence in allelic frequencies for the medieval plague samples, but the impact of DNA-degradation needs to be ruled out by further experiments.

Oral Presentation Tuesday, October 17 at 9⁵⁵

Ancient DNA analysis of bones and textiles of prehispanic populations settled in the Palpa Valley/Peru

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Introduction: The Palpa-Valley is located in the southern costal line of Peru. The colonisation history could be proved for the past 3000 years. In this research, samples of three consecutive archaeological periods are analysed, these periods are: the Paracas (800-200BC), the Nasca (200BC-600AD) and the Huari (600-800 AD). The aim is to achieve answers to cultural requests, like settlement continuity or change, genealogical coherences, trading and colonisation routes with the use of different methods of aDNA analysis.

Analysis of human mitochondrial DNA in a plurality of individuals of the three periods will give answers about the genetic variability of populations and lead to clarification about continuity in these populations. The analysis of chromosomal DNA will give results about genealogy. Camelid bones and textiles will be assigned to one of the four South American camelid species and the mitochondrial and chromosomal diversity will be determined. We also want to show that it is possible to extract wearers' trace-DNA from textiles.

Material and methods: Approximately 200 human and 150 camelid bones will be analysed. The DNA is extracted by a silica based method on the extraction robot EZ1 (Qiagen). The DNA of the textiles (50 samples) is extracted by a hair extraction protocol (Pfeiffer et al. 2004).

The analysis of human mitochondrial HVSI is made by direct sequencing and also by a PCR-RFLP method which was optimized for aDNA. These two independent approaches will lead to valid results.

The species identification is made by a PCR-RFLP analysis of a 277bp fragment of the cytochrome b. For every camelid species a typical pattern in the analysis of three polymorphisms is realized. The genetic variability is determined by sequencing the fragment of the cytb and a new designed Multiplex-PCR of known camelid microsatellites.

First results and perspectives: Until now for 25 human samples the examined HVSI region could be determined. In two camelid bones and in six textiles the species determination was successful so far. In the animal samples which have results in the analysis of the cytb, the Multiplex-PCR was successful too. At this point of research no statement to results in variability of the populations can be made. The extracts of textiles are sometimes inhibited so the extraction method has to be optimized.

We have observed that degradation is high in some samples, so another focus will be taken on more usable extraction and amplification methods for these samples. Furthermore, new systems for the analysis of camelid DNA are in development.

Oral presentation Tuesday, October 17 at 14²⁰

Characterisation of intermediate and null alleles for Y-chromosomal microsatellites commonly used in forensic genetics

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Due to presence of the largest non-recombining region in the whole human genome, a unique inheritance pattern and specificity to males, the Y chromosome has been widely studied by researchers interested in human evolution and forensic geneticists. The latter's interest has focused mainly on Y-chromosomal short tandem repeat (Y-STR) loci, which due to sensitivity of the PCR method and small amplicon sizes and are used commonly in analysis of small quantities of DNA, including highly degraded DNA. A set of Y-STR systems called a minimal haplotype has been widely accepted by the forensic community and genotyping results for different human populations throughout the world have been collected in publicly available databases.

During casework analysis and population studies, intermediate and null alleles at three Y-STR markers belonging to the most popular set of Y-STR loci analysed in forensic laboratories have been identified. Microvariants were characterised by sequencing. Lack of amplification of DYS19 null allele with the use of an alternative, non-overlapping primer pair excluded polymorphism at primer-binding sites and confirmed deletion of the whole locus. DYS385 microvariant possessed a 2 bp insertion/deletion in the tandem repeat array and was designated as DYS385*10.2. In case of DYS392 intermediate allele, electrophoretic mobility shift was shown to be caused by single nucleotide polymorphism (SNP), which was found in the 5' flanking region, while repetitive units remained unchanged. Due to relatively large distance of the SNP site from the tandem repeat array, different Y-STR commercial kits designate this allele either as a microvariant or as a regular allele.

Oral presentation Tuesday, October 17 at 11⁴⁰

Microbiological investigation of a Korean medieval child mummy found in Yangju

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We present preliminary findings on a child mummy from the Korean Peninsula. The child was interred in a pine coffin fully encased in a lime-soil mixture that remained unbroken during the intervening 500 years. There was a high level of DNA preservation that reflected the extraordinary soft tissue preservation. We have commenced a PCR-based search for the DNA of bacterial and viral microorganisms in the tissues of this mummy.

Initial endoscopy revealed nodules on the child's' gut, raising the possibility of miliary TB as a possible cause of death.

Hepatitis B (HBV) virus was also sought since it is currently endemic in 12% of the Korean population and we had mummified liver tissue available both for DNA search and for histological examination. DNA from the liver was also assayed for a more ubiquitous virus, the Transfusion Transmitted virus (TTV). Initial results indicate the presence of MTB DNA. As well as genotype C, HBV DNA. The Hepatitis work was confirmed in three independent virology laboratories. The search for TTV is ongoing.

Histological studies of the liver were of interest, as they appear to suggest we are dealing with a carrier rather than a person with active disease.

Implications for future genomic studies of these microorganisms and associated diseases will be discussed.

Oral presentation Wednesday, October 18 at 9³⁰

Parameter optimization of sample pretreatment and extraction of aDNA with the BioRobot EZ1

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Introduction: The extraction of DNA from hard tissues is the essential step for the possibility of genetic analyzes of historical populations. Studies show that a lot of different extraction methods have been tested but sample pretreatment was only of little interest. So in this study the impact of variation of defined parameters of sample pretreatment on DNA yield from hard tissues is investigated.

Material and methods: Bone material was used from the Lichtenstein cave dated to the Bronze Age. After sampling, bone powder was homogenized in order to enable a series of experiments of aliquots with identical properties. Genotype of the individual (reference genotype) was known from former investigations. Determination of recovery of DNA for every single test series was defined by the amount of detectable alleles identical to the reference genotype after amplification of extracts. Parameter that have been tested during sample pretreatment are: composition of incubation medium, time of incubation (6-72h), temperature of incubation (20-56°C) and centrifugation force for the final centrifugation step of sample pretreatment (2000-12000rpm). Extraction was performed with the BioRobot EZ1 (Qiagen) based on a magnetic beads separation that enables an automated extraction with cartridges of prefilled reagents. Finally, three variable parameters of the extraction process were tested.

Results: Results revealed the following improved protocol for sample pretreatment and extraction, which yielded the highest amount of detectable alleles identical to the reference genotype after amplification of extracts: incubation of 0.1g bone material was carried out with 500µl 0.5M EDTA (pH 8.0) for 18 hours at 37°C, lysis of cells with 10µl of Proteinase K (600mAU/ml) for one hour and centrifugation for 2min and 6000rpm. Extraction with the BioRobot EZ1 was carried out with 200µl of supernatant of the pretreatment, "trace protocol"-software and 100µl elution volume.

With this improved protocol an average of 7 of 14 alleles could be detected, so compared to the original protocol which features minor deviations in parameter values, number of detectable alleles could almost be doubled.

Poster presentation

Quantification of ancient and low-copy DNA by Real-Time PCR

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Ancient DNA and Real-Time PCR: Quantitative Real-Time PCR is an approved method to determine the DNA content of samples with high accuracy. By using fluorescent reagents it is detected how many cycles it takes, until a defined DNA quantity is reached. By comparing the result with known standards an initial template number can be assigned to the unknown sample. All presented experiments are carried out in the LightCycler® 2.0 System (Roche Applied Science), a device, which is hypothetically able to even quantify one single starting template. In our applications a 103bp sized nuclear DNA fragment is amplified over 60 cycles and detected by fluorescent dye SYBR Green I. We use this technique to quantify low-copy DNA extracts from archaeological bones, but the technique cannot be simply adapted to this sample type. To quantify ancient DNA correctly we have developed a procedure, which takes into account the three challenges that arise in the context of low-copy DNA extracts from archaeological bones. *Poisson statistics in dependence of template number:* Poisson statistics describe that the accuracy of quantification results depends on the measured quantity. So the quantification results of very low copy numbers can be imprecise. The statistical spread of low one-digit template numbers leads to a more than twofold difference between two identical aliquots of the same sample. So it must be considered that the lower a sample's DNA copy number is, the more measurements must be made to obtain the accurate template number. *Formation of unspecific PCR products at high cycle numbers:* Low DNA starting copies require a high PCR cycle number until the detection threshold is reached. During PCR unspecific products like primer dimers may cross the detection threshold in the same cycle range as the specific product. Unspecific products may lead to incorrect quantifications due to the use of the nonspecific DNA binding dye SYBR Green for detection. This is why primer design and PCR conditions must be optimized in order to avoid the generation of unspecific products. *Reaction efficiency and inhibition:* DNA from bone samples is often impurified with PCR inhibitors. These substances decrease the amplification efficiency, which prohibits a quantification since the amplification efficiency of the unknown sample and the standard must be identical. To detect the presence of PCR inhibitors in an unknown sample, we spike a known and high template number with an aliquot of the sample. If inhibitors in the sample influence PCR efficiency, the PCR becomes is slowed down compared to an unspiked template. In addition to the detection of presenting inhibitors, this assay measures the degree of inhibition. With knowledge of the degree of inhibition it is possible to determine the proper template concentration of a sample even from inhibited PCR reactions.

Poster presentation

Presence of $\Delta 32CCR5$ in medieval specimens from Poland

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A 32 bp deletion within *CMKBR5* gene that encodes chemokine receptor CCR5 aroused particular interest, as its homozygous carriers were found to be resistant to HIV-1 infection, and in heterozygotes the onset of AIDS was slower. HIV virus itself is present in contemporary humans for far too short to select the mutated allele. Therefore pathogens such as *Yersinia pestis* (the supposed Black Death bacillus) or *Variola major* (smallpox virus) were suspected. Although recently the probable date of the mutation event was moved back to a few millennia before present by many authors, and a growing number of publications suggests that no selective pressure is required to account for the present-day frequency, there is still no agreement as to where and exactly when the mutation appeared and what forced European populations to retain it at the relatively high frequency.

With the advances of ancient DNA studies, we decided to establish whether $\Delta 32CCR5$ was present in medieval Poland and if so, to compare its frequency with that characteristic of contemporary population of Poland.

Three archaeological burial sites from central Poland were exploited in this study: Dziekanowice, Daniłowo and Stary Brześć Kujawski, dated back to 10th – 14th century. Bone material, mostly teeth were powdered in an agate mortar and subjected to DNA isolation and purification in Roche's MagNA Pure Compact Nucleic Acid Purification System. In the course of one day after the isolation, aDNA was amplified, using primers that amplified 130 or 98 bp fragments (wild and mutated alleles respectively). PCR products were visualised on polyacrylamide gel and sequenced. All work was done with special care required in aDNA analysis and only results confirmed in at least two independent procedures, where no contamination was found in mock isolation and PCR controls were considered.

55 individuals were successfully typed thus far, and the mutated allele was found to be present in medieval population of central Poland with 6.64% frequency (it is 10.9% on average in present-day Poland. This confirms reports that $\Delta 32CCR5$ is much older than the initially calculated 700 years before present, and that medieval pandemics of plagues may have had little (if any) effect on its frequency.

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Oral presentation Wednesday, October 18 at 11⁴⁰

Further data on autoimmuno-predisposing alleles in specimens from medieval Poland

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Incidence of autoimmune diseases rises dramatically during the last decades. There is no simple answer to what exactly is responsible for this increase. Research data indicate that both genetic predisposition and environmental influence may equally contribute to autoimmunity. Amongst the genes that were found to be associated with intolerance of own antigens, the most influential are *HLA* (human leukocyte antigens), and to a lesser degree: *CTLA-4* (cytotoxic T lymphocyte associated molecule 4) and *INS* (insuline gene) (in type I diabetes).

In order to examine whether a change in alleles frequencies may be responsible for the observed boom of autoimmune disorders incidence we decided to take advantage of ancient DNA methods and study polymorphisms of three genes involved in autoimmunity (*HLA DQB1*⁵⁷, *CTLA4* +49A/T and *INS* -23HphI), in medieval cohorts of central and northern Poland and compare their frequencies with those of contemporary population.

Bone samples, teeth in majority, were collected on archaeological sites in Stary Brześć Kujawski, Daniłowo and Gdańsk dated back to 10th-14th century. Samples were ground to powder in an agate mortar and subjected to DNA isolation, either by silica method described by Krings *et al* (1997) or with the use of Roche's MagNA Pure Compact Nucleic Acid Purification System. In the course of one day following the isolation procedure, aDNA was amplified using primers designed to obtain sequences of interest in fragments of 100bp (*HLA DQB1*⁵⁷), 116 (*CTLA4* +49A/T) and 104 (*INS* -23HphI). PCR products were subsequently visualised on polyacrylamide gel and/or sequenced. Special care was given in order not to contaminate the samples at any of the stages, and only those results were considered true that could be confirmed by analysis of at least two samples from the same individual.

Results indicate that with 43% carriers (n=98) of the protective Asp allele of *HLA DQB1*⁵⁷ locus (presently 74%) and 34% frequency (n=53) of *CTLA4* +49A/T GG predisposing genotype (7.6% presently), and with comparable to contemporary population frequencies of the *INS* alleles, the medieval population was in fact more genetically predisposed to autoimmunity than present-day inhabitants of Poland. This may suggest that factors of other than genetic nature are responsible for the growing incidence of autoimmune disorders, observed since the beginning of the second half of the 20th century.

This work was supported by MSHE grant no. 3P05E 055 25.

Poster presentation Thursday, October 19 at 12³⁰

Molecular identification of Leishmaniasis in ancient Egypt and Upper Nubia

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In this study we analysed 91 bone tissue samples from ancient Egyptian mummies and 70 bone marrow samples from naturally mummified human remains from Upper Nubia. The Egyptian material derived from different sites and time periods, ranging from the Pre- to Early Dynastic site of Abydos (n=7; 3500-2800 BC), a Middle Kingdom tomb in Thebes West (42; 2050-1650) to different tomb complexes in Thebes West, which were built and used between the Middle and New Kingdom until the Late Period (42; c. 2050BC – 500BC). The Nubian samples were taken from two early Christian burial sites at Kulubnarti, between the 2nd and 3rd cataracts of the Nile in Northern Sudan, dated from 550-750 AD and c.750-1500 AD. All samples were tested for the presence of *Leishmania spp.* DNA and further characterised by direct sequencing. PCR was performed with primers targeting a 120 bp fragment of a conserved region of the minicircle molecule of kinetoplast mitochondrial DNA (kDNA) of the parasite. In 4 out of the 91 and 9 out of the 70 Nubian samples the 120 bp fragment of the kDNA could be successfully amplified and unambiguously related to *Leishmania donovani* species following sequencing. All positive samples originated from the Middle Kingdom tomb, while no molecular evidence for the presence of ancient *Leishmania* DNA was found in the Pre- to Early Dynastic and the New Kingdom to Late Period specimens. Our study indicates, that leishmaniasis was introduced in Egypt at this time due to close trade contacts and associated travel with Nubia during the Middle Kingdom.

Oral presentation Wednesday, October 18 at 9⁵⁵

Specific and non-specific infestations of archaeological artifacts

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Archaeological remains, both human and otherwise may show evidence of both specific pre-mortem and non-specific post-mortem infestations. In this report, we shall summarise what is known of the first category of specific pathogens and some of the lessons learned from this research. Especially relevant is the work of Taubenberger's group in elucidating the sequence of the 1918 influenza virus. Our own work on tuberculosis and leprosy will also be reviewed as it affects the understanding of the evolution of tuberculosis, but also on the persistence of pathogens in the environment. Not only is the environment a factor, but the nature of the organism's envelope and G+C content of its DNA may contribute. Two mummy groups; the Nubian and Hungarian mummies will be used to illustrate attempts to culture these agents. Of special importance in these examples is the fact that the former were interred in soil and the latter were maintained in pine coffins. A relevant consideration is the "curse of the mummies". Other important archaeological artifacts such as the Dead Sea Scrolls and amber are also witness to these infestations and understanding these biological agents is an important aspect of taphonomy and degradation of our biocultural heritage.

Oral presentation Thursday, October 19 at 11¹⁵

Index of authors

Adler M. 26, 43
Alvesalo L. 12
Angerbjörn A. 37
Arroyo E. 22
Baca M., 19
Bader N. 37
Bartnik B. 30
Beaulne C.H. 33
Bok G.D. 41
Brauner P. 32
Ciesielka M. 40
Cipollaro M. 20
Cybulska L. 21
del Gaudio S. 20
Dmochowska G. 30
Dobosz T. 30
Donoghue H.D. 41, 46
Eriksson T. 37
Fehren-Schmitz L. 39, 43
Fernández E. 22
Fulge M. 23
Goodwin W. 37
Grant P. 41
Greenblatt Ch.L. 13, 32, 46, 47,
Gruchota J. 24
Götherström A. 37
Herrmann B. 23, 26, 35, 38, 39, 42, 43
Hummel S. 23, 26, 29, 35, 38, 39, 42, 43
Jerszyńska B. 27, 28, 44, 45
Jędrychowska-Dańska 27, 28, 44, 45
Jonkisz A. 30
Jung C-K. 41
Kahila Bar-Gal K. 32
Kapińska E. 21
Karasińska M. 25
Kim J.W. 41
Kim M.J. 41
Kim S.B. 41
Shin J.E. 41
Kim T.H. 41

Klein A. 41
Kleindorp R. 26
Kołodziejczak M. 27, 28
Kozioł P. 40
Krause S. 29
Krzyżańska A. 30
Larcombe L. and Orr P. 31
Lebioda A. 30
Lee E. 41
Lemma E. 32, 47
Lev-Maor G. 32
Lidén K. 37
Markowska J. 30
Marlow S. 36
Matheson C.D. 33, 34
Morais J. 34
Müller C. 35
Nadolski J. 27, 28, 44, 45
Nerlich A. 36, 46
Oh C.S. 41
Oom M. 34
Ortiz J.E. 22
Ovchinnikov I.V. 14, 37
Pappo O. 41
Park S.S. 41
Park S-M. 41
Pollmann J. 38
Popović D. 24
Rębała K. 21, 40
Renneberg R. 23, 39
Scholten A. 29
Schraut B. 46
Schulman L.M. 41
Shin D.H. 41
Shouval D. 41
Sobczak M. 19
Spigelman M. 15, 32, 41, 46, 47
Stanković A. 19, 24
Szczerkowska Z. 21, 40
Torres T. 22
Turbón D. 22
Wenzel M. 42

Westenthanner M. 26, 43
Witas H.W 25, 27, 28, 44, 45
Wolpoff M.H. 16
Wrzesińska A. 27, 28, 44, 45
Wrzesiński J. 27, 28, 44, 45
Wysocka J. 21
Żądzińska E 25, 27, 28, 44, 45
Zawicki P. 44, 45
Zink A.R 17, 36, 46
Zylber M.I. 47

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