

HAPLOID DNA MARKERS IN FORENSIC GENETICS

Charité, Berlin, Germany
April 22nd - 24th, 2010

Dear friends, colleagues and guests,

The success story of Y-Chromosome markers in forensic, genealogical and anthropological research has many authors and nearly all of them could be met at the six previous Y Chromosome User Workshops in Berlin, Porto, Innsbruck and Ancona. In 2004 we joined our major community venture, the YHRD, with Walther Parson's EMPOP project to form the concept of a workshop called "Haploid DNA Markers in Forensic Genetics". This meeting has since developed into a working and communication space sui generis.

In 2010 it is our pleasure to invite you to the site of the first "outbreak" meeting which took place 1996 in Berlin. The host and the venue is the Charité, Europe's largest university hospital, celebrating this year its 300th anniversary. At the same time we commemorate the 10th anniversary of the online YHRD database instituted and curated at the Institute of Legal Medicine and Forensic Sciences in Berlin. In fact, nowadays YHRD 3.0 is the world's largest curated online population database which has evolved far beyond its original scope. It is a well received scientific project supported and used by practitioners and academics from many countries. Likewise EMPOP developed into the largest open mtDNA database whose concept meets the quality requirements expected for forensic science. Besides its importance as population data repositories providing probabilistic information for court use, both projects play an important role for education, quality assessment and the development of several scientific disciplines – as forensic genetics, population genetics, evolutionary genetics and archaeogenetics.

This year we welcome more than 230 participants from 34 countries. We thank all participants who contribute with their research results to the program and with their inquisitiveness to the discussions.

We wish all colleagues some beautiful days in Berlin!

Sincerely
Lutz Roewer and Walther Parson

We gratefully acknowledge our sponsors - Life Technologies / Applied Biosystems, Qiagen, Promega and Biotype / Qualitytype. We thank the Institutes of Pathology and Legal Medicine of the Charité for their continuous support.



Thursday, April 22nd (Y-Chromosome session I)

- 9h00 - 14h00 **Registration**
- 14h00 - 14h30 **Opening**
Michael Tsokos (Berlin, Germany)
Niels Morling (Copenhagen, Denmark)
Lutz Roewer (Berlin, Germany)
Walther Parson (Innsbruck, Austria)
- 14h30 - 15h00 **Y-chromosome analysis using next-generation sequencing.**
Chris Tyler-Smith (Hinxton, United Kingdom)
- 15h00 - 15h10 **Analysis of Y-SNPs in the HapMap3 project.**
Yali Xue, Qasim Ayub, Sarah Hunt and Chris Tyler-Smith (Hinxton, United Kingdom)
- 15h10 - 15h25 **Contrasting Male Population Substructure Among African Agriculturalists and Hunter-Gatherers.**
Peter de Knijff and Jeroen Pijpe (Leiden, Netherlands)
- 15h25 - 15h35 **Y-Chromosomal variation in Sub-Saharan Africa and the Bantu expansion: insights from biallelic and microsatellite markers.**
Cesare de Filippo, Chiara Barbieri, Mark Stoneking and Brigitte Pakendorf (Leipzig, Germany)
- 15h35 - 15h45 **Early Y-Chromosome lineages in African populations.**
Batini C, Ferri G, Luiselli D, Brisighelli F, Sanchez-Diz P, Rocha J, Jorde L, Brehm A, Montano V, Elwali NE, Spedini G, D'Amato ME, Myres N, Ebbesen P, Destro-Bisol G and Comas D, Christian Capelli (Oxford, United Kingdom)
- 15h45 - 15h55 **Development of SNaPshot minisequencing of 12 Y-SNP loci in Chinese Han population for forensic application.**
Wei Wei, Jing Yan, Haibo Luo and Yiping Hou (Chengdu, China)
- 15h55 - 16h05 **Genetic Portrait of Bangladeshi Population: A Y-Chromosome Perspective.**
Sharif Akhteruzzaman, Shafiu Alam, Ahmad Ferdous, Md. Eunos Ali, Tania Hossain and Md. Mahamud Hasan (Dhaka, Bangladesh)
- 16h05 - 16h20 **Increased resolution within Y-Chromosome haplogroup R1b-M269 sheds light on the Neolithic transition in Europe.**
George Busby, Francesca Brisighelli, Dan Bradley, Leonor Gusmão, Mark Thomas, Bruce Winney, Walter Bodmer, Paula Sánchez-Diz, Eva Ramos-Luis, Marielle Heinrich, Valentina Coia, Federica Trombetta, Sergio Tofanelli, Rafal Ploski, Carla Vecchiotti, Tatijana Zemunik, Igor Rudan, Sena Karachanak, Draga Toncheva, Paolo Anagnostou, Gianmarco Ferri, Cesare Rapone, Tor Hervig, James F. Wilson and Cristian Capelli (Oxford, United Kingdom)
- 16h20 - 16h30 **Mapping Genetic Landscapes of the Modern Admixed Europe based on Forensic Haplotypes with Geographic Information Systems.**
Amalia Diaz-Lacava and Maja Walier (Bonn, Germany)
- 16h30 - 17h00 **Coffee break**

Thursday, April 22nd (MtDNA session I)

- 17h00 - 17h30 **Identifying variants within Eurasian mtDNA haplogroup U8 and their application to studies of human phylogeography.**
Phillip Endicott (Paris, France)
- 17h30 - 17h40 **Benefits of using unbiased, complete mtDNA genomes for uncovering population histories.**
Mark Whitten, Mingkun Li, Cesare de Filippo, Mark Stoneking and Brigitte Pakendorf (Leipzig, Germany)
- 17h40 - 17h50 **Haplogrouping mtDNAs through protein coding region: trouble in paradise?**
Inês Soares, Ana Goioz and António Amorim (Porto, Portugal)
- 17h50 - 18h00 **Rapid parallel capture and sequencing of complete mitochondrial genomes.**
Tomislav Maricic, Mark Whitten and Svante Pääbo (Leipzig, Germany)
- 18h00 - 18h10 **Towards entire mtDNA sequencing in phylogeny, forensic and medical genetics.**
Liane Fendt, Harald Niederstätter and Walther Parson (Innsbruck, Austria)
- 18h10 - 18h20 **Mitochondrial DNA mutations in complete genomes of colorectal cancer patients.**
Katarzyna Skonieczna, Boris Malyarchuk, Arkadiusz Jawień, Andrzej Marszałek and Tomasz Grzybowski (Bydgoszcz, Poland)
- 18h20 - 18h30 **The potential of mtDNA SNP analysis in forensic casework.**
Stephan Köhnemann and Pfeiffer H (Münster, Germany)
- 18h30 - 18h40 **Rare variants in the coding region mtSNPs.**
Joana Bom, Paulo Dario, Teresa Ribeiro and Helena Geadá (Lisbon, Portugal)
- 18h40 - 18h50 **The GHEP-EMPOP collaboration on mtDNA population data - a new resource for forensic casework.**
Lourdes Prieto, Goios A, Zimmermann B, López-Soto M, Catelli L, Barreto RM, Alves C, Rodríguez-Monge A, Fridman C, Alonso A, Rodríguez-Quesada A, Lima G, Cardoso S, Anjos MJ, Whittle M and Parson W (Madrid, Spain)
- 18h50 - 19h05 **Never miss a profile – String-based search using EMPOP2 and application to phylogenetic alignment.**
Alexander Röck, Arne Dür, Stefan Troger, Martin Pircher and Walther Parson (Innsbruck, Austria)

Friday, April 23rd (Biostatistics session)

- 9h00 - 9h30 **A Probability Theoretical View of Genetic Matches.**
Michael Krawczak (Kiel, Germany)
- 9h30 - 9h50 **Evidentiary-STRength of a rare haplotype match.**
Charles Brenner (Berkeley, United States)
- 9h50 - 10h55 **YHRD & EMPOP – How to use empirical data to calculate match probabilities.**
Lutz Roewer (Berlin, Germany)
Sascha Willuweit (Berlin, Germany)
Walther Parson (Innsbruck, Austria)
Alexander Röck (Innsbruck, Austria)
- 10h55 - 11h10 **Use of Artificial Random Mixtures from a Y-STR Database to Estimate Expected Frequencies of Potential DNA Contributors.**
Vincent R. Miller and James R. Bentley (Phoenix, United States)
- 11h10 - 11h25 **Problems with frequency surveying.**
Mikkel Meyer Andersen (Aalborg, Denmark)
- 11h25 - 12h35 **Panel discussion**
Niels Morling (Copenhagen, Denmark)
Michael Krawczak (Kiel, Germany)
Bruce Weir (Seattle, United States)
- 12h35 - 14h00 **Lunch & Poster exhibition**

Friday, April 23rd (MtDNA session II)

- 14h00 - 14h15 **Mixtery - confirmed presence of two different mtDNA genomes in maternally related individuals.**
Sabine Lutz-Bonengel, Sanger S, Niederstatter H, Huber G, Pollak S and Parson W (Freiburg, Germany)
- 14h15 - 14h25 **Phylogeny-specific filters for quasi-median network analysis: sharpening the blade for mtDNA error detection.**
Bettina Zimmermann, Alexander Rock, Tanja Kramer, Peter Schneider and Walther Parson (Innsbruck, Austria)
- 14h25 - 14h35 **Origin and evolution of the mitochondrial DNA lineages in Norway.**
Erika Hagelberg, Maja Krzewinska and Ian Frame (Oslo, Norway)
- 14h35 - 14h45 **Update on the mtDNA phylogeny in Eastern and Western Slavs.**
Boris Malyarchuk, Miroslava Derenko, Urszula Rogalla, Patrycja Dada and Tomasz Grzybowski (Bydgoszcz, Poland)
- 14h45 - 14h55 **Towards better mtDNA population samples by inspecting autosomal STR markers.**
Martin Bodner, Jodi A. Irwin, Michael D. Coble and Walther Parson (Innsbruck, Austria)
- 14h55 - 15h10 **Evaluating length heteroplasmy in the human mitochondrial DNA control region.**
Forster L, Peter Forster, Gurney SM, Spencer M, Huang C, Rohl A and Brinkmann B (Munster, Germany)
- 15h10 - 15h20 **Evaluation of sequence-derived length heteroplasmy interpretation by fragment analysis**
Cordula Berger, Petra Hatzer-Grubwieser and Walther Parson (Innsbruck, Austria)
- 15h20 - 15h30 **Improved method for profiling dog mtDNA and expansion of the Belgian population database.**
Leonie Gijbers and Stijn Desmyter (Amsterdam, Netherlands)
- 15h30 - 16h00 **Coffee break**

Friday, April 23rd (Y-Chromosome session II)

- 16h00 - 16h15 **The genetic structure of Y-Chromosome in 1338 Italians.**
Valerio Onofri, Loredana Buscemi, Adriano Tagliabracci and the GeFI Group (Ancona, Italy)
- 16h15 - 16h30 **Y-Chromosomal Population Substructure in Eastern Tyrol (Austria) Disclosed by Mapping the Linguistic Background of Toponyms.**
Burkhard Berger, Harald Niederstätter, Gerhard Rampl, Daniel Erhard, Isolde Hausner and Walther Parson (Innsbruck, Austria)
- 16h30 - 16h45 **Y-STRs and the history of Western Slavs.**
Marcin Woźniak, Boris Malyarchuk, Miroslava Derenko and Tomasz Grzybowski (Bydgoszcz, Poland)
- 16h45 - 16h55 **Genetic polymorphisms of 17 Y-Chromosomal short tandem repeat loci in Central Croatian population.**
Branka Gršković, Gordan Mršić, Ozren Polašek, Andro Vrdoljak, Siniša Merkaš and Šimun Anđelinović (Zagreb, Croatia)
- 16h55 - 17h05 **Micro-geographic distribution of the Y-Chromosomal variation in the Duchy of Brabant.**
Maarten H.D. Larmuseau, Nancy Vanderheyden, Manon Jacobs, Monique Coomans, Lucie Larno and Ronny Decorte (Leuven, Belgium)
- 17h05 - 17h15 **Genetic stratification of the Sami inferred from biparental and uniparental markers among four subpopulations.**
Ville N. Pimenoff, Palo JU, Tillmar A, Roewer L and Sajantila A (Berlin, Germany)
- 17h15 - 17h25 **Y-STR DNA Analysis of the 7th Century Human Remains from the Burial Site in Ergolding, Germany.**
Daniel Vanek, Saskova L and Hubert Koch (Prague, Czech Republic)
- 17h25 - 17h35 **Seventeen short tandem repeat loci Y-Chromosome haplotypes: Genetic analysis on populations residing in San Andres Island (Afro-caribbeans) and Coffee region (Mestizos) of Colombia.**
Claudia Martin and Sandra Avila (Bogota, Colombia)
- 17h35 - 17h50 **Reduced Gene Flow Among Argentinean Native American Ethnic Groups: Evidences from Y-Chromosome and mtDNA data.**
Daniel Corach and Andrea Sala (Buenos Aires, Argentina)
- 19h30 **Dinner**

Saturday, April 24th (Casework session)

- 10h00 - 10h15 **Lost, Found, and Lost Again: The Strange Case of Everett Ruess.**
Michael Coble, Charles Brenner, and Odile Loreille (Gaithersburg, United States)
- 10h15 - 10h30 **New Horizon for Y-chromosome Applications in Forensic Analyses.**
Kaye N. Ballantyne, Miriam Goedbloed, Rixun Fang, Ying Choi, Manohar R. Furtado and Manfred Kayser (Rotterdam, Netherlands)
- 10h30 - 10h40 **Design and validation of a highly discriminatory 10-locus Y-chromosome STR multiplex system.**
Maria Eugenia D'Amato, Bajic Vladimir B and Davison S (Cape Town, South Africa)
- 10h40 - 10h50 **Utility of Y-STR haplotypes databases in forensic caseworks.**
Federica Alessandrini, Buscemi L, Onofri V, Turchi C and Tagliabracci A (Ancona, Italy)
- 10h50 - 11h05 **Haplotype-specific separation and sequence based typing of HLA genotypes – more than resolving ambiguities?**
Marion Nagy, Patricia Entz, Petra Otremba and Johannes Dapprich (Berlin, Germany)
- 11h05 - 11h15 **Individual specific extraction of DNA from male mixtures – first evaluation studies.**
Jessica Rothe, Lutz Roewer and Marion Nagy (Berlin, Germany)
- 11h15 - 11h30 **Testing for independence of X-chromosomal markers, a cautionary note.**
Andreas O. Tillmar and Petter Mostad (Linköping, Sweden)
- 11h30 - 11h40 **The Fromelles War Graves Project: Identification of skeletal remains from WW1.**
Jim A. Thomson (Teddington, United Kingdom)
- 11h40 - 11h50 **DNA evidence: original DNA test results overturned by a retrial in the Ashikaga case.**
Katsuya Honda, Sugano Y and Nishi T (Tsukuba City, Japan)
- 11h50 - 12h00 **A rare mutation in the primer binding region of the Amelogenin locus in the AmpF/STR® NGM™ PCR Amplification Kit results in dropout of the X but not the Y homologue.**
Julio J. Mulero, Robert E. Lagace and Lori K. Hennessy (Foster City, United States)
- 12h00 - 12h20 **Haploid DNA Workflow: QIAGEN's new portfolio from sample to STR, SNP and Sequencing.**
Christian Starke (Hilden, Germany)
- 12h30 **Coffee & Cigarettes (with Buffet)**

Poster

- P1 **Hierarchical Y-SNP assay to study indigenous populations of South America.**
Maria Geppert, Marion Nagy and Lutz Roewer
- P2 **A rapid tool to detect Y-Q* paragroup variability in Amerindians.**
Ferri G, Alù M, Franceschi Z, Luiselli D, Pettener D, Yang Yao D, Bertoncini S, Tofanelli S, Corradini B, Franceschi C and Beduschi G
- P3 **Analysis of microsatellite markers of the Y-Chromosome of the remnants of quilombos in the State of Alagoas.**
Gustavo R. B. de Souza, Luiz Antonio F. da Silva, Dalmo A. de Azevedo, Alexandro M. L. de Assis and Iede H. E. Ferreira da Silva
- P4 **Haplotype population data for 15 Y-STR loci in Alagoas, northeast Brazil.**
Dalmo A. de Azevedo, Luiz Mauricio da Silva, Luiz Antonio F. da Silva, Leonor Gusmão and Elizeu F. de Carvalho.
- P5 **SRY gene polymorphism: a tool to evaluate natural hybridization in South American primates.**
Andréa M. de Oliveira, Denise Monnerat Nogueira, Ana Maria dos Reis Ferreira, Alcides Pissinatti and Elizeu Fagundes de Carvalho
- P6 **Distribution of Y-Chromosomal haplotypes in the Sherpa of the Khumbu Valley (Nepal).**
Pamela Tozzo, Luciana Caenazzo, Silvano Presciuttini, Elena Ponzano, Irene Amoruso and Gianumberto Caravello
- P7 **Haplotypes for 17 Y-Chromosomal STR loci in a population from eastern Libya (Benghazi region).**
Samir Elmrghni, Ron A Dixon and D. Ross Williams
- P8 **Analysis of 17 Y-Chromosomal STR loci in the Iranian population.**
Nafiseh Amini, Maryam Sharafi, Mahmood Tavallaie and Sirous Zeinali
- P9 **Comparative analysis on 17 Y-STRs between 5 regional Mongolian populations and a Japanese population.**
Toshimichi Yamamoto, Rieko Uchihi, Takashi Yoshimoto and Yoshinao Katsumata
- P10 **Project "Genetics and Surnames" in Czech Republic.**
V.Stenzl, L.Urban, Z.Ječná, J.Hlavová, M.Silerova, V.Urbanova and D.Vanek
- P11 **Mutation rates at Y-STRs: a collaborative study of the GEFI-ISFG Group.**
Valerio Onofri, Loredana Buscemi, Adriano Tagliabracci and the GeFI Group
- P12 **Y-Chromosome STR Polymorphisms in Ethnically Diverse Populations of North-Eastern Europe.**
Giovanna Bellusci, Paola Blasi, Galina Vershubsky, Andrey Kozlov and Andrea Novelletto

- P13 **Comparison of Y-genetic structure of Romany populations in the three regions of Slovakia – Spiš, Gemer and Šariš.**
Eva Petrejčíková, Alexandra Bôžiková, Miroslav Soták, Jarmila Bernasovská, Ivan Bernasovský, Iveta Boroňová, Adriana Sovičová, Dana Gabriková, Soňa Mačeková, Jana Čarnogurská and Petra Švíčková
- P14 **The genetic legacy of Greek colonisation in South Italy and Sicily.**
Brisighelli F, Busby G, Anagnostou P, Luiselli D, Cali' F, Romano V, Shepherd G, Destro-Bisol G and Capelli C
- P15 **Comparative Y-STR and Y-SNP analysis in two Vlachian Romani population groups from Eastern Hungary.**
Andrea Zalán, Judit Béres and Horolma Pamjav
- P16 **Y-Chromosome STR Haplotype Diversity in Three Ethnic Isolated Population from North-Western, Romania.**
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- P17 **The Distribution of Y-Chromosome Haplogroups in Lithuanian Population.**
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- P18 **Y-Chromosomal STR haplotypes in an isolated Population from Germany: the Sorbs.**
Immel UD, Tönjes A, Kovacs P, Stumvoll M and Kleiber M
- P19 **Analysis of two Sardinian isolates (Italy) through Y-Chromosome polymorphisms.**
Calo' C, Vona G, Corrias L, Scudiero CM, Di Fede C, Mameli A and Robledo R
- P20 **Basques and Germans Y-STRs haplotype distribution in Europe.**
Valverde L, Köhnemann S, Cardoso S, Schuerenkamp M, Pfeiffer H and M de Pancorbo M
- P21 **An enrichment of forensic haploid marker databases: mtDNA and Y-chromosome polymorphisms in a population of Romanians.**
Chiara Turchi, Federica Alessandrini, Valerio Onofri, Loredana Buscemi, Walther Parson and Adriano Tagliabracci
- P22 **Y-Chromosome Forensic Database: Haplogroup and Haplotype Sharing in a Multiethnic Population Living in Bologna (Italy).**
L. N. Riccardi, S. Ceccardi, R. Lanzello, D. Luiselli, C. Bini and S. Pelotti
- P23 **Development of Multiplex Y-SNP Identification Assays for Molecular Evolution and Forensic Applications.**
Rixun Fang, Pius Brzoska and Manohar Furtado
- P24 **Two different cases of deletion on the short arm of the human Y-Chromosome.**
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- P25 **An interesting mutation at locus DYS385 in an uncle/nephew pair in a fatherless paternity case.**
Lancia M., Coletti A., Massetti S., Severini S., Dobosz M., Carlini L. and Carnevali E.

- A new tool for population and forensic genetics: 33 X-Chromosome Indels in one multiplex reaction.**
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- An analysis of segregation data for 10 X-chromosome STR loci.**
P27 Suelen Botão, Miguel Gelabert-Besada, Amelia Rodríguez, Ángel Carracedo, Leonor Gusmão and Paula Sánchez-Diz
- Linkage and linkage disequilibrium analysis of X-STRs in Italian informative families.**
P28 Inturri S, Menegon S, Amoroso A, Torre C and Robino C
- Genetic Analysis of US population samples using a X-STR 20-plex PCR assay.**
P29 Chien-Wei Chang and Lori K. Hennessy
- Genetic characterization of a north-western Iberian region using Mentype® Argus X-8 kit.**
P30 Miguel Gelabert-Besada, Raquel Calvo, Manuel García-Magariños, Anna González-Tendero, Maviky Lareu and Paula Sánchez-Diz
- Study of 25 X-chromosome SNPs in the Portuguese.**
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- Rapid Screening for Mitochondrial and Y-Chromosome Haplogroups in Routine DNA Analysis.**
P32 Gala Zuccarelli, Evguenia Alechine, Mariela Caputo, Cecilia Bobillo, Daniel Corach and Andrea Sala
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- In search of the lost Mountain Bushmen of Lesotho.**
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- PhyloTree - an updated human mtDNA tree.**
P35 Mannis van Oven and Manfred Kayser
- MtDNA typing improvements... and alternatives.**
P36 Paulo Dario, Teresa Ribeiro and Helena Geada
- The Portuguese Gypsy community: tracing their routes through maternal genetic lineages.**
P37 Cristina Valente, Alfredo Gusmão, Isabel Mendizabal, Cíntia Alves, Verónica Gomes, Ana Goios, Walther Parson, António Amorim, Luis Alvarez, David Comas, Leonor Gusmão and Maria João Prata
- mtDNA data of 3 Ethnic Groups from Angola.**
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- P39 **The frequencies of mtDNA haplogroups in Czech Republic and a comparison to the other European populations.**
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- P40 **Diversity of mitochondrial DNA of Belarusians and its forensic application.**
A. Kushniarevich, L. Sivitskaya, S. Borovko and O. Davydenko
- P41 **Analysis of mtDNA SNPs genotyped with Affymetrix GeneChip 6.0 array in a population sample from South-Sardinia.**
Piras Ignazio, Corrias Laura, Calò Carla Maria and Vona Giuseppe
- P42 **Validation 'Maxwell®16 for hair shafts' reveals multiple mtDNA profiles in an individual's hair.**
Stijn Desmyter, Sylvie Comblez and Fabrice Noël
- P43 **Mitochondrial DNA control regions sequences and maternal inheritance: a comparison between mother-child pairs.**
N. Cerri, A. Verzeletti, V. Cortellini, A. Cincotta and F. De Ferrari

Abstracts

Haplotype Population Data For 15 Y-STR Loci In Alagoas, Northeast Brazil

Dalmo A. De Azevedo¹, Luiz Mauricio Da Silva², Luiz Antonio F. Da Silva¹, Leonor Gusmão³ and Elizeu F. De Carvalho⁴

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Y-chromosome STRs are largely used in forensic casework, paternity and in population genetics studies. The State of Alagoas is located in northeastern Brazil, and its population is typically admixed. In this study a sample composed by 247 unrelated males from Alagoas was analyzed with the haplotype composed by the markers DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS447, DYS458 and DYS464. By using the minimal haplotype loci 187 lineages were identified (diversity of 0.9943 ± 0.0018) whereas 230 haplotypes were distinguished when DYS447, DYS458 and DYS464 markers were also considered (diversity of 0.9993 ± 0.0005). A comparative analysis of the new generated data to those previously published from different Brazilian populations (Rio de Janeiro, Rio Grande do Sul, Santa Catarina and a population group constituted by individuals from five geopolitical regions), Africa (Mozambique, Equatorial Guinea and Angola), Europe (Portugal) and Amerindians (Terenas and other native groups from Brazil) was carried out. The AMOVA test revealed homogeneity between all the Brazilian populations ($F_{ST}=0.00098$, $P=0.19135 \pm 0.00295$). It was also observed that the population of Alagoas is genetically similar to Portugal, differing significantly from African and Amerindian populations. The results show that this 15 loci haplotype presents a high power of discrimination between male lineages in Alagoas and a homogeneity between the Brazilian populations. The genetic proximity between Alagoas and Portugal populations points out to the fact that the Portuguese were the main source of male lineages nowadays present in Alagoas, in accordance with studies concerning to other Brazilian populations.

The Distribution Of Y-Chromosome Haplogroups In Lithuanian Population

Pauliukevicius A.¹, Caplinskiene M.¹, Baranoviene R.¹, Jankauskiene J.¹, Kukiene J.¹, Savanevskyte K.¹, Bunokiene D.¹, Jureniene A.¹ and Ruzgaite G.²

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The geographic origins of Y-Chromosomes in the Lithuanian population we applied a new Bayesian allele-frequency approach for predicting the Y-haplogroup from a set of Y-STR markers. Data were collected from the blood samples of 194 unrelated males throughout various regions of Lithuania. DNA was extracted using Proteinase K/Chelex 100 procedures. The amplification of 17 Y-STRs was carried out with the AmpFISTR Yfiler™ PCR Amplification Kit according to the supplier's protocol. The PCR products were separated and detected by capillary electrophoresis on the ABI PRISM® 3130 Genetic Analyzer. Results were analyzed using Gene Mapper ID software v.3.2. Prediction of Y-Chromosome haplogroups from Y-STR values was performed by using Whit Athey's Haplogroup Predictor Program ver.5. The Bayesian probability for each Y-haplogroup was estimated. Y-haplogroup diversity and Y-STR diversity within haplogroup was calculated according to Nei, 1987. The Bayesian probability was greater than 79% in all of the samples. We found 13 Y-haplogroups from the total in the studied population. The most prevalent Y-haplogroups R1a and N3 comprised 46.4% and 30.4%, respectively, of all analysed Y-Chromosomes. Y-haplogroups E1b1b, G2a, H, I1a, I2a, I2b1, J2a1b, J2a1h, R1b, Q and T were detected in small numbers (representing range 0.5-6.2%). Comparative analysis results revealed high R1a haplogroup frequencies up to 37% among all the Lithuania's neighboring populations. Haplogroup N3 had a high frequency of up to 30% in Baltic countries. The most common haplogroup of Western Europe R1b was rare in the studied populations (range 4-11%). Conclusions: Lithuanian Y chromosomal gene pool structure is comprised of two main components - R1a and N3 haplogroup which are the result of male migrations. On the basis of Y-haplogroups comparative analysis, this genetic flow is in relation to Lithuania's neighboring populations.

Genetic Portrait Of Bangladeshi Population: A Y-Chromosome Perspective

Sharif Akhteruzzaman¹, Shafiul Alam², Ahmad Ferdous³, Md. Eunus Ali³, Tania Hossain³ and Md. Mahamud Hasan³ (Presented by Sharif Akhteruzzaman)

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Historically, there are four ethnic groups in Bangladesh.: Dravidian, proto-Australian, Mongolian and Bangali. The Bangalis are by far the largest group of all, constituting about 98% of the total population. Here we present the genetic diversity of 17 Y-chromosome STR markers in a Bengali population of Bangladesh who constitute the mainstream population of the country. A total 216 unrelated males were typed for DYS19, DYS389I, DYS389II, DYS390, DYS391, YS392, DYS393, DYS385a/b, DYS438, DYS439, DYS437, DYS448, DYS458, DYS456, DYS635, and Y-GATA-H4 loci. A total of 211 haplotypes were identified, of which 206 were unique. The haplotype diversity was 0.9998, indicating a high potential for differentiating between male individuals in this population. We compared our data of extended minimal haplotype (minimal haplotype + DYS438 and DYS439) and 17-loci haplotype with YHRD database. In case of 17-loci haplotype we found only one hit with Romania, Wallachia and 65.4% haplotype of Bangladeshi population were found to mismatch in YHRD in extended minimal haplotype database. For having extensive illustration of the genetic relation, minimal haplotype of Bangladeshi population was compared via AMOVA with 16 other neighboring populations. This comparative study revealed the close relationship of our population with Gaddi population from Himachal Pradesh, India, followed by Southern Indian (Tamil) populations. The Neighbor Joining tree structured from 1st distance matrix shows that Bangladeshi Bengali population along with Indian Gaddi, Southern Indian, Malaysian Indian and Panjabi Indian population form a conspicuous cluster standing far apart from other asian populations.

Utility Of Y-STR Haplotypes Databases In Forensic Caseworks

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Y-chromosome haplotypes can be very helpful in forensic casework because they give information about population of origin of the DNA collected on a crime scene, although this information must be used with caution in relation to the delicate issue to be judged. In this paper two interesting forensic caseworks in which results of YHRD search were very profitable to address police investigations are discussed.

A Brazilian woman, a prostitute, was found dead in her apartment in a big building, inhabited and/or frequented by hundred of people of different nationality, clandestines, transsexuals, prostitutes and clients. Y-STR analysis was performed to establish a 17 loci haplotype on evidences recovered from the crime scene. Query against the Y-STR Haplotype Reference Database (YHRD) found a match in a population of Russian Federation. This information was immediately given to the police to address investigations, but policemen did not pay attention to our indications. Following days a big number of suspects of different geographic origins were typed and only after some weeks police recovered some biological stains belonging to a Russian man, a friend of a Russian woman living in the flat near the victim. The Y-STR haplotype recovered matched that on the crime scene, but in the meantime the man had escaped.

A man was kidnapped, beaten and released the day after. Collected evidences provided a 17 loci haplotype that matched a profile from Bologna area, contained in our internal database of Italian population (samples not yet submitted to YHRD). One of the men involved in the kidnapping was actually from that area. Y-STR haplotypes database is a very useful tool to retrieve intelligence information on an unknown culprit's identity and address police investigations. Its constant update with new samples allows coverage to increase and information more and more accurate to be retrieved.

Analysis Of 17 Y-Chromosomal STR Loci In The Iranian Population

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One of the smallest human chromosomes is the Y-Chromosome with an average size of 60 Mb. Exchanges is limited to small pseudoautosomal regions of the X-Y pair between X and Y-Chromosomes in the meiosis. The Y-Chromosome in most of its length is male-specific and effectively haploid and is transmitted from father to his son unchanged unless a mutational event takes place.

Y-Chromosome-specific STRs have proved to be an important tool in paternity cases, especially when the alleged father is deceased, as well as forensic and non-forensic fields.

Blood samples were collected from 130 randomly selected, unrelated Iranian males, following procedures that are in accordance with Promega kit and FTA Cards.

All 17 (DYS19, *DYS389I*, *DYS389II*, *DYS390*, *DYS391*, *DYS392*, *DYS393*, *DYS385a/b*, *DYS438*, *DYS439*, *DYS437*, *DYS448*, *DYS458*, *DYS456*, *DYS635*, and Y-GATA-H4) markers were co-amplified using the AmpFISTR Y-filerTM PCR Amplification kit (Applied Biosystems, United States). The amplified products were separated by capillary electrophoresis on ABI Prism 3130 XL Genetic Analyzer. The sample run data were analyzed by GeneMapper IDX Software V. 1.0. Allele frequencies were estimated. Haplotype diversity was calculated by Nei's formula. To determine of other parameters we are using Arlequin software and also the online AMOVA tool from YHRD.org3.0 for y-STR haplotyping.

Allele frequencies in our study are similar to reported allele frequencies in Iranian population on Y-STR haplotype databases. In this study we found some new mutation as off-ladder which most of them are located on *DYS358* marker.

Haplogrouping mtDNAs Through Protein Coding Region: Trouble In Paradise?

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Aiming to test a new approach to sequence comparison using an absolute minimum of evolutionary assumptions, a training set consisting of deposited 104 complete human mtDNA sequences, comprising representatives of all major haplogroups was used.

In order to maximize the mutational signal-to-noise ratio and avoid homoplasies due to recurrent mutation and alignment ambiguities resulting from indels and other rearrangements, each sequence was subsequently processed by STRipping out all non-protein coding regions and concatenating the remaining fragments into a single 11344bp long string, and thus encompassing 68.5% of total mtDNA length. All resulting pseudo sequences revealed to be of equal length and showed no signs of any inversion, corresponding thus as expected to 'naturally' aligned strings.

These were compared using either (a) a numerical approach (where a digit was attributed to each base) or (b) through vectorization, and a genetic distances matrix generated accordingly.

Both methods provided the same results (although at different speeds) and then the matrix used to build a Neighbour-Joining dendrogram.

The clustering resulted to be in agreement with haplogroup classification (<http://www.phyloree.org>) for basal clades as L macrohaplogroup and terminal branches as H, but the fit was generally very poor. In order to check if this outcome was an artefact of our method, the same pseudosequences were used for the construction of a network (<http://www.fluxus-engineering.com/sharenet.htm>), which showed however similar results (good separation of L and H/V sequences, but lack of resolution for the remaining ones).

We conclude that human mtDNA haplogroup definition deserves a closer look, as it seems that the historical load of the hypervariable region may have introduced significant noise in the phylogeny most likely due to mutational recurrence and heterogeneity (other events besides substitutions).

Acknowledgements

IPATIMUP is an Associate Laboratory of the Portuguese Ministry of Science, Technology and Higher Education and is partially supported by FCT, the Portuguese Foundation for Science and Technology, AG and IS are recipients of FCT post-doc (SFRH/BPD/43646/2008) and PhD (SFRH/BD/38171/2007) grants, respectively.

Problems With Frequency Surveying

Mikkel Meyer Andersen (Presented by Mikkel Meyer Andersen)

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Frequency surveying was introduced by Krawczak et al. in 2000 as a method of estimating haplotype frequencies. In this talk some problems with the surveying method are presented.

Seventeen Short Tandem Repeat Loci Y-Chromosome Haplotypes: Genetic Analysis On Populations Residing In San Andres Island (Afro Caribbeans) and Coffee Region (Mestizos) Of Colombia

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In this study, seventeen Y-Chromosomal (DYS19, DYS389 I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439, DYS437, DYS448, DYS456, DYS458, DYS635, YGATA-H4 and DYS385a/b) short tandem repeat (STR) polymorphic systems were typed by PCR multiplex reaction in four Colombian populations: San Andres (short term for San Andres Island), (Afrocaribbeans) in the atlantic ocean Risaralda, Quindio and Caldas (Mestizos) departments belongs to coffee region in the west-central of Colombia. A total of 273 different Y-chromosome haplotypes were observed in the 335 males analyzed, two hundred eleven haplotypes were found to be unique and the haplotype diversity among populations was 0.998. The AMOVA results show that the percentage of variation is mainly within populations (99.95%).

The two most frequent haplotypes was repeated only seven times, three others were repeated four times and only five others were repeated three times. The highest average gene diversity was found in San Andres Island and the lowest in Caldas. This study provides further comparative genetic information with other Colombian populations like Valle, Cauca and Nariño, among others.

New Horizon For Y-Chromosome Applications In Forensic Analyses

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Available Y-STR panels of up to 17 markers currently applied to forensic questions are useful for male lineage differentiation, but do not provide maximal lineage resolution. More importantly, they usually fail to separate paternally related males, reflecting a major drawback in the current application of Y-chromosome analysis in forensics, as conclusions can only be made for a group of male relatives, rather than a single male. Aiming to find Y-STR loci for increasing male lineage resolution and to establish male relative differentiation, 187 Y-STRs were genotyped in nearly 2000 father-son pairs, with Bayesian based mutation rates estimated. We identified 13 Y-STRs (RM Y-STRs) with exceptionally high mutation rates (above 1×10^{-2} per locus per generation). Empirical evaluation of the RM Y-STR set to differentiate male relatives was performed by genotyping 250 members of 80 male pedigrees that were separated by 1 to 20 generations. The RM Y-STR set differentiated over 65% of male relatives compared to 13% with Yfiler, even with 4 fewer loci in the RM Y-STR set. The large amount of sequence data generated to confirm the mutations allowed an investigation of the molecular features of Y-STRs that influence the mutability. Repeat number had the largest effect, although the sequence motif, repeat complexity, number of variable repeats within the locus, and the copy number also affected the mutation rates. Hence, with the new set of RM-YSTRs we provide the first way to move towards Y-chromosome based male individual identification. The set of RM-Y-STRs is envisioned to be applied to all forensic cases where conventional Y-STR sets have delivered a matching profile, in order to evaluate the potential involvement of male relatives of the suspects.

*The following colleagues provided DNA-confirmed family / pedigree samples to this study: Lotte Henke, Jürgen Henke, Lutz Roewer, Rafal Ploski, Tadeusz Dobosz, Rüdiger Lessig, Micaela Poetsch, Nicole von Wurmb-Schwark, Ronny Decorte, Hans Knoblauch, and Peter de Knijff. We additionally thank Onno Schaap, Mark Vermeulen, Kate van Duijn, Oscar Lao, Andreas Wollstein, and Silke Brauer for technical or statistical support, and additionally Damla Arslantunali, Ines Correia Rosa, Paul Dekkers, Sevgi Deniz, Cissi Jonsson-Glans, Tomas Petten, Arwin Ralf, Diana van den Heuvel, Anita van den Heuvel and Melike Yüksel for technical assistance.

Y-Chromosome STR Polymorphisms In Ethnically Diverse Populations Of North Eastern Europe

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By virtue of the broad geographic continuity, the genetic signature of bidirectional movements between Asia and Europe can be detected in populations of North-Eastern Europe and Western Siberia. We used 16 Y-Chromosomal STRs to measure population affinities among ethnically diverse population samples now residing in the area of the former Soviet Union. A total of 636 unrelated males (24 samples) from geographically and ethnically-defined populations of Russia, Belarus, Azerbaijan and Georgia were analysed. Some of the populations represent more or less distinct isolates.

Reduced haplotype diversity (<0.95) was found in Udmurts, Northern Komi, Talish, Yakut, and Bashkirs. Modal haplotypes differed in each of these populations, reaching frequencies ranging from 17.6 to 57%. The corresponding predicted haplogroups were R1(xR1a), R1a and N1c.

Our results show that microsatellites alone can have the power of detecting Asian contributions to the gene-pool of populations now residing in Europe. Within Europe, a greater heterogeneity among populations sharing the same language than between populations sharing the same ethnic affiliation was found. Marked population differentiation was detected in some Altaic speakers. Simpatry eroded inter-ethnic differentiation. No regular decline of genetic similarity with geography was evident. These data allow to directly test previous hypotheses on the peopling of Russia, by considering a broader spectrum of potential diversity. Overall, the clinal variation reported in previous works and genetic variation associated with ethnicity represent two layers of the overall diversity in the genetic landscape of the European portion of Russia. These findings prompt for the accurate characterization of Russian ethnicities in view of the appropriate representation in forensic databases.

Y-Chromosome STR Haplotype Diversity In Three Ethnic Isolated Population From North Western, Romania

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In this study, 12 Y-STR loci (DYS391, DYS389I, DYS439, DYS389II, DYS438, DYS437, DYS19, DYS392, DYS393, DYS390, and DYS385a/b) included in the Power Plex® Y system were genotyped in individuals from three ethnic isolated population (Tileagd, Sinteiu, Palota) of Bihor county (Northwestern Romania) and compared with a control population from the general population of the same county.

Allele frequency distributions, gene diversity, haplotype diversity and discrimination capacity were estimated. The most isolated population is Tileagd, gypsy individuals (haplotype diversity = 0,2134 vs. control group - 0,9931; $p < 0,0001$), confirmed by the average gene diversity (0,0546 vs. 0,6342; $p < 0,0001$) and the discrimination capacity (8,57 vs. 82,2; $p < 0,0001$).

Y-Chromosome haplogroups were predicted, confirming the supposed origin of individuals from Tileagd isolate (romani people, haplotypes being included in the YHRD Eurasian-Indo-Iranian metapopulation database) and the known origin of people from Sinteiu isolate (Slovakians, haplotypes included in YHRD Eurasian-European-Eastern European metapopulation database) and from Palota isolate (Germans, haplotypes included in YHRD Eurasian-European-Western European metapopulation database).

Population pairwise comparison showed that the furthest apart genetically is the Tileagd isolate from all the other population groups ($\Phi_{ST} = 0,624$ Tileagd-Palota, $p < 0,0001$; $\Phi_{ST} = 0,635$ Tileagd-Sinteiu, $p < 0,0001$; $\Phi_{ST} = 0,370$ Tileagd-Oradea, $p < 0,0001$; $\Phi_{ST} = 0,624$ Tileagd-Palota, $p < 0,0001$; $\Phi_{ST} = 0,073$ Sinteiu-Palota, $p = 0,01$; $\Phi_{ST} = 0,125$ Sinteiu-Oradea, $p < 0,001$; $\Phi_{ST} = 0,059$ Palota-Oradea, $p < 0,01$).

Y-Chromosomal Population Substructure In Eastern Tyrol (Austria) Disclosed By Mapping The Linguistic Background Of Toponyms

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From the linguistic (onomastic) point of view, East Tyrol is an exceptional area in Austria and even in Europe. Although exclusively German-speaking since the Late Middle Ages, the three big language groups of Europe, the Romans, the Slavs and the Germans settled here contemporaneously for several centuries and therefore left a great amount of toponyms (e.g. names of mountain pastures or mountains). The information on the colonisation of East Tyrol that can be derived from the distribution of these place-names coincides highly with the rare early historic facts that are known about this area and enables an accurate spatial mapping of the areas of settlement of the three language groups. The question is, if this historic and linguistic information of past population structures can be used to dissect genetic variations derived from Y-Chromosomal data of the present population from different regions of East Tyrol.

Blood samples from a total of 270 healthy men living in Eastern Tyrol (Austria) were collected and analyzed for 17 Y-Chromosomal STR-loci using the AmpFISTR Yfiler PCR amplification kit (Applied Biosystems, AB) and for 19 Y-SNPs applying a multiplex PCR followed by a single base primer-extension multiplex (SNaPshot, AB). 142 samples of unrelated men were selected under the 3 generation of residence criterion for further population genetic analysis based on the linguistic data.

Evaluation Of Sequence Derived Length Heteroplasmy Interpretation By Fragment Analysis

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Length heteroplasmy (LH) describes the co-occurrence of at least two mitochondrial (mt) DNA sequences in a sample that differs in the number of nucleobases which is often difficult to interpret as the sequence patterns are then overlaid and appear blurred. LH is usually observed in homopolymeric tracts and has also been found in the CA-repeat of the control region. Forensic science usually does not capture LH when interpreting evidence (exclusion scenarios) and reporting mtDNA haplotypes in a case or population study. This information however can be important when identical haplotypes (except for length variant regions) are to be interpreted in the context of a study. Since quantitative information (on the mixture of LH variants) is not included in the difference-coded annotation of a haplotype (with respect to the revised Cambridge Reference Sequence, rCRS) detectable LH variants cannot be described properly.

It has therefore become routine practice to call the dominant type (major molecule) of the LH variants present in a sample and in the majority of cases a clear single dominant variant can be identified. However, there are examples where this interpretation is challenging as equally quantitative LH variants may be present that result in identical signal heights. To investigate these cases in particular we designed fragment analysis assays for five known LH regions in the mtDNA control region to determine the ratio of the LH variants by fluorescence based fragment sizing. The PCR assays were optimized using samples containing high quality DNA and then tested on haplotypes comprising difficult LH regions. In the majority of cases we found agreement between the results of the sequence and fragment analyses. The study also confirmed that the determination of the dominant sequence variant is depending on the applied primer combinations.

Towards Better mtDNA Population Samples By Inspecting Autosomal STR Markers

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Mitochondrial DNA with its features of a circular genome, strictly maternal inheritance and high copy number is an intriguing marker applied in population, medical and forensic genetics and phylogeography. Reliable data are required for all these applications. Quality control measures have been introduced to ensure highest standards in sequence data generation, validation and a posteriori inspection. A phylogenetic alignment strategy has been widely accepted as a prerequisite for data comparability and database searches, for forensic purposes, a reconstruction of human migrations and a correct interpretation of mtDNA mutations in medical genetics.

There is continuing effort in enhancing the number of worldwide population samples in order to contribute to a better understanding of human mtDNA variation and dispersal routes. This often means to rely on convenience samples collected for other purposes that do not meet all quality requirements for mtDNA data sets. Here, we introduce an additional quality control means that deals with one aspect of this limitation: by combining autosomal STR marker with mtDNA information, it helps to avoid the bias introduced by related individuals included in the same (small) sample. BY-STR analysis of individuals sharing their mitochondrial haplotype, pedigree construction and subsequent calculation of likelihood ratios based on the allele frequencies found in the population, closely maternally related individuals can be identified and excluded. However, we also want to discuss scenarios that allow related individuals in the same set. An ideal population sample would be representative for its population: this new approach represents another contribution towards this goal.

This work was supported by the Austrian Science Fund FWF, project L397.

An Analysis Of Segregation Data For 10 X-Chromosome STR Loci

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Autosomal STRs are currently the most used markers in parentage testing and also in human genetic identification. However, analysis of complex kinship cases must be supported by information from other genetic markers as Y-STRs and mtDNA. Recently, X-chromosome markers have been introduced to complement the usual forensic toolkit. However, segregation data for X-STRs are scarce yet. An X-STRs decaplex was genotyped in a total of 112 father-daughter-grandson trios in order to evaluate mutation rate and meiotic recombination frequency for these markers. In 1,950 allele transfers, two single step mutations were found at DXS7132 and DXS6789. The obtained average mutation rate across the 10 loci was 1.03×10^{-3} . Recombination frequencies were also estimated for all the markers, excluding those cases in which meioses were no informative.

Evidentiary Strength Of A Rare Haplotype Match

Charles Brenner (Presented by Charles Brenner)

UC Berkeley School of Public Health, CA, United States (DNA-VIEW)

When a rare haplotype is shared between suspect and crime scene, how strong is the evidence linking the two? The relevant number is the conditional probability of an innocent person to match the crime scene profile, given available data including the crime scene profile, population data, and scientific knowledge.

The traditional methods of evaluating the strength of DNA evidence include several institutional misconceptions. First and most fundamental is to confuse probability (a summary of available data) with population frequency (which is not available). Next, the normal statistical assumption that sample frequency reasonably estimates population frequency ignores among other things scientific knowledge and fails badly for rare traits. Third is forgetting to condition on the crime scene observation. Hence the traditional paradigm in forensic practice is to confuse sample frequency with population frequency and in turn confuse that with probability. The consequence is very roundabout and unsound reasoning.

More direct methods are not difficult. The simplest is based on the proportion singletons – of haplotypes that occur just once – in a sample, which I call κ (kappa). For present-day Y-STR typing, the most common situation by far is that the crime scene haplotype is previously unseen in a sample of size $n - 1$. In that case the matching probability for an innocent suspect is $(1 - \kappa)/n$.

I shall also discuss and contrast other approaches that have been suggested.

Increased Resolution Within Y-Chromosome Haplogroup R1b M269 Sheds Light On The Neolithic Transition In Europe

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Early studies on classical polymorphisms have largely been vindicated by the growing tome of information on the genetic structure of European populations, with mtDNA, Y-Chromosome and autosomal markers all combining to give a fundamental pattern of migration from the East. The processes behind this pattern are however, less clear, particularly with regard to uniparental markers. Much debate still rages about how best to use Y and mtDNA to date particular historical movements, or indeed if it is appropriate at all. For example, whilst some progress has been made recently in calibrating the mtDNA clock, the selection of a mutation rate with which to date the Y-Chromosome is contentious, as the two most favoured values can give dates that differ by a factor of three.

In order to address this we have investigated the sub-lineages of the common European haplogroup R1b-M269. This haplogroup has been shown to be clinal in Europe, and more recently has been posited to be the result of the Neolithic expansion from the Near East. Here, we use newly characterised SNPs downstream of M269 to produce a refined picture of the haplogroup in Europe, and further show that the diversity of this lineage cannot be entirely attributed to Neolithic migration out of Anatolia. We use simple coalescent simulations to estimate an absolute lower bound for the age of the sub-haplogroups. Rather than originating with the farmers from the East, we suggest that the sub-structure of R1b-M269 visible in Europe today, and thus the great majority of European paternal ancestry, is the result of the interaction between the Neolithic wave of expansion and populations of early Europeans already present in the path of the wave.

Analysis Of Two Sardinian Isolates (Italy) Through Y-Chromosome Polymorphisms

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In this paper we analyzed two Sardinian isolates (Italy): the linguistic isolate of Carloforte and the geographic isolate of Benetutti through Y-STRs and Y-haplogroups with the aim to evaluate the degree of isolation and the different influence of cultural and geographic boundaries on isolation. Individuals from Carloforte and Benetutti were all selected with the method of surnames. We also selected two comparison samples: one from Sulcis Iglesiente and one from Northern Sardinia, the nearest regions to Carloforte and Benetutti, respectively. DNA was extracted from peripheral blood or buccal swab and amplified with the AmpFISTR Yfiler PCR amplification kit in a Geneamp®PCR System 9700 thermal cycler. Separation and detection of Y-STR 17plex PCR products were accomplished with the ABI Prism 3100 Genetic Analyzer sixteen-capillary array system. Analysis of the data was performed using GeneScan v. 3.1. Haplogroups were inferred by haplogroup predictor.

Through the analysis of STRs, both the isolates appear significantly differentiated from the neighboring areas. The analysis of haplogroup reveals a peculiar distribution: Carloforte population is characterized by the absence of haplogroup I2a, present in all the other areas. This haplogroup is identified by the mutation M26, characteristic of Sardinia, and absent in the rest of Italy. The shape of the Median Joining Network suggests a heterogeneity of the founder population for both isolates. Comparison with other Italian populations, carried out with genetic tree, confirms the genetic peculiarity of Sardinian populations. Carloforte, despite being on a separate branch, is in the same cluster of Italian populations, while Benetutti, closer to the other two Sardinian populations, is collocated on a separate branch as an outlier.

In conclusion, from the first analysis, it seems that cultural and linguistic barriers have the same influence of the geographic boundaries on isolation and as a consequence on the genetic structure of a population.

Early Y-Chromosome Lineages In African Populations

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Y-Chromosome variation has provided a number of insights within the genetic history of *Homo sapiens* (Jobling and Tyler-Smith, 2003). Focusing on sub-Saharan Africa, demographic events associated with the dispersion of languages and pastoralism have been targeted, ranging from the dispersion of the Bantu languages to the evolutionary relationships of click-speaking groups (Beleza et al., 2005; Berniell-Lee et al., 2009; Destro-Bisol et al, 2004; Wood et al, 2005; Tishkoff et al, 2007; Henn et al, 2009; Cruciani et al., 2009). The first two branches of the Y-Chromosome genealogy, namely haplogroup A and B, are African specific, with average continental frequencies of 14-34%, reaching up to 60% in groups of foragers (Underhill et al, 2000; Cruciani et al, 2002; Tishkoff et al, 2007). Despite the potential of such lineages in understanding the ancient peopling of the continent, an exhaustive investigation of their distribution and variation is currently missing. Here we show that the systematic dissection of these lineages can open new perspective on the early African history of *Homo sapiens*, with particular attention to areas of the continent where human fossil remains and archaeological data are scanty.

Mitochondrial DNA Control Regions Sequences and Maternal Inheritance: A Comparison Between Mother Child Pairs

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MtDNA analysis is of central importance for forensic identity testing as well for studies of human evolution. Due to maternal inheritance, lack of recombination, high number of copies for cell, high mutation rate and then high polymorphic density, the mtDNA hypervariable regions (HV) are well suited for forensic identification using a maternal relative as reference sample.

Nowadays, mtDNA analysis has found different application, above all in cases where DNA is only present in small amounts or where it is badly degraded, in human identification and in maternal testing.

In order to determine the frequency and the type of mutation between two generations, we investigated uniparental mtDNA inheritance in 50 mother-child pairs through the analysis of the HV1 and HV2 sequences. Preliminary results show that most maternal relatives share identical mtDNA sequences, and neomutation rate seems to be very low from one generation to the another. These data confirm the importance and the utility of mtDNA, allowing comparison of family members who share a common matrilinear ancestry and providing the basis for identification and maternal relationship both in biological evidence and in living individuals.

Genetic Analysis Of US Population Samples Using A X-STR 20 Plex PCR Assay

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Due to hemizyosity and a lack of recombination of the X-Chromosome in males, X-STR typing has been demonstrated in forensic practice to be a powerful tool for complex cases of kinship testing. We have configured a 20-plex X-STR genotyping assay to amplify simultaneously DXS101, DXS6789, DXS6797, DXS6800, DXS6807, DXS6810, DXS7132, DXS7133, DXS7423, DXS7424, DXS8377, DXS8378, DXS981, DXS9895, DXS9898, DXS9902, GATA165B12, GATA172D05, GATA31E08, and HPRTB in a single PCR reaction. The use of 5-dye technology and non-nucleotide linkers on primers ensures no overlap between marker ranges during capillary electrophoresis analysis and genotyping can be performed using the Applied Biosystems 3130/3130xl Genetic Analyzers. This X-STR multiplex PCR amplification assay has been tested to produce reliable and accurate X-STR genotyping data, the results of which have been confirmed by sequence analysis. Allele and haplotype frequency, genetic diversity, and forensic efficiency parameters are reported based on a large selection of US population samples together with an evaluation of the advantages afforded by increasing multiplex complexity in resolving common haplotypes. Information obtained from this study point to the utility of such a multiplex for use in complex kinship investigations.

Lost, Found, and Lost Again: The Strange Case Of Everett Ruess

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Everett Ruess (b. 1914) was a poet and artist who navigated the deserts of the American southwest in the 1930s. During his brief life he befriended artist Ansel Adams and photographer Dorothea Lange. He was also among a select few Western Europeans welcomed into Native American settlements. In 1934 Everett left to explore the Escalante region of southern Utah and was never seen again. His burros and camp was ultimately found at Davis Gulch in the Escalante Canyons. Many have speculated that he died from falling off a cliff, or perhaps drowned crossing the Colorado River.

In 2008, a set of remains found near Comb Ridge (approximately 60 miles from Davis Gulch) were purportedly those of Everett Ruess. An elderly Navajo man told a story to his granddaughter that as a young man, he saw a Caucasian man murdered by two Ute Indians (ref). Feeling sorry for the man, he buried the remains in a rock crevice to prevent the destruction of the remains by coyotes. The grandson of the elderly man eventually found the burial site and the remains were analyzed by an anthropologist. The conclusion of the anthropological analysis was that the skeleton was most likely a Caucasian male in his 20s. Facial superimposition of a photo of Everett Ruess to the upper maxillary teeth gave even more evidence to the putative identification. DNA analysis of the remains to the four surviving nieces and nephews of Everett Ruess (using an analysis of 600,000 genomic SNPs) concluded that the skeletal remains shared 25% of the DNA of the relatives, as one would expect with an avuncular relationship.

Based upon the totality of the evidence, the remains were given to the surviving family members of Everett Ruess in May of 2009 for burial. In June of 2009, Utah's state anthropologist came forward to question the findings based upon the wearing pattern of the teeth in the Comb Ridge skull, suggesting that the individual was likely Native American persisting on a corn diet. He also questioned the lack of evidence for dental work consistent with the archived files of Everett Ruess from the University of Southern California Dental School. Given the doubts raised in this case, the Ruess family contacted AFDIL to perform an additional DNA analysis of the remains. In this presentation, we will present the mtDNA and Y-STR results along with our analysis that the Comb Ridge remains are most likely those of a Native American, probably a Navajo individual, and not Everett Ruess.

Reduced Gene Flow Among Argentinean Native American Ethnic Groups: Evidences From Y-Chromosome and mtDNA Data.

Daniel Corach and Andrea Sala (Presented by Daniel Corach)

Servicio de Huellas Digitales Genéticas, Cátedra de Genética, Biología Molecular Facultad de Farmacia y Bioquímica, Buenos Aires University, Argentina

Argentinean population is the result of diverse admixture events. The original population suffered a severe process of replacement as can be observed within the urban areas. However, some aboriginal groups still persist and about thirty ethnicities inhabit the territory, they represent a small proportion of the overall population (1%). In order to determine the extent gene flow between well represented ethnicities a set of 401 unrelated individuals belonging to Guaraní (N=121), Wichi (N=48), Toba (N=64), Pilagá (N=56) and Mapuche (N=112) were selected for mitochondrial hypervariable Regions I and II (mtDNA HVRI and II) sequencing. In addition males donors (N=256) were typed by means of M242, M269, M3Q SNPS as well as by Y-STRs using the minimal haplotype proposed by YHRD. A high proportion (>95%) of maternal lineages belonged to one of the four Native American haplogroups (A2, B2, C1 and D1) and a slightly smaller proportion (>85%) of Q3a1a were observed within the males. Very few mtDNA haplotypes were shared between the diverse tribes and when it occurred the ethnicities belonged to the same linguistic branch, mainly the Mataco-Guaycurú speakers. Mataco is the former name of Wichi tribe and Guaycurú speakers include Pilaga and Toba. Tobas and Guaraní shared only two haplotypes belonging to haplogroup B2. mtDNA haplotypes detected within the Mapuche were not detected within the other groups with only one exception with Guaraní, but this individual was confirmed as a recent immigrant to the Patagonia where Mapuche inhabits. The similar pattern is detected when male lineages are analyzed. Only within Mataco-Guaycurú tribes some few haplotypes were shared. These observations are in line with the effects of language as an efficient cultural barrier for gene flow to occur.

MtDNA Data Of 3 Ethnic Groups From Angola

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Mitochondrial DNA is an excellent tool for population studies and forensic genetics due to its high copy number per cell, maternal inheritance and high mutation rate of evolution. Discrimination power of mtDNA is due to the polymorphic nature of its hypervariable control region. MtDNA haplogroups are important to clarify the history and demographic past of a population since they can reflect phylogenetic relationship between populations. Angola population has origin from Occidental and Southern Bantu people that came from the great lakes region. Linguistic ethnic groups Bakongo, Kimbundo and Ovimbundo represent $\frac{3}{4}$ of the Angolan population and the remaining are represented by others Bantu groups with the same language, social and cultural patterns. The aims of this work were to obtain the mtDNA variability, classify the haplotypes into haplogroups, and infer the phylogenetic relationship of the Bakongo, Kimbundo and Ovimbundo ethnic groups from Angola.

Blood samples were obtained from 30 individuals, 10 of each ethnic group from Angola. DNA was extracted using Chelex®100 method. PCR amplification performed with primers L15971/ H017/ L16450/ H599 and chemistry from QIAGEN Multiplex PCR Master Mix. PCR products were purified with ExoSap-IT. Cycle sequencing was performed using the ABI Prism BigDye Terminator v.3.1 Cycle Sequence Kit. BetterBuffer has been incorporated into the sequencing procedure. Before DNA analysis a simple bead purification method (XTerminator/SAM) was made. Sequences were analysed in the sequencer 3130 – Genetic Analyser. Obtained haplotypes were compared with the Cambridge Reference Sequence and typed following the nomenclature recommendations of the IUPAC. Haplogroups were determined on the mtDNAmanager. Statistical analysis of the control region and genetic affinities among the populations were computed using Arlequin v.3.0 software.

Preliminary results showed great variability with high frequency of unique haplotypes and significant values of nucleotide and sequence diversity parameters. All mtDNA sequences were including into specific African mtDNA haplogroup.

Design and Validation Of A Highly Discriminatory 10 Locus Y-Chromosome STR Multiplex System.

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The Y-chromosome STRs (short tandem repeat markers) are routinely utilized in the resolution of forensic casework related to sexual assault. For this, the forensic community has adopted a set of eleven (core) Y-STR that is incorporated in all commercial diagnostic systems. The most widely used commercial Y-STRs identification extended the number of STRs to 12 (Powerplex) and 17 (AmpF Φ STR Φ Y-filerTM).

Our studies of Y-STRs polymorphisms in the South African population identified low levels of diversity and discrimination capacity for many commercial markers, determining a limited applicability of these systems to the local population groups. To overcome this shortcoming, we designed a Y-STR 10-plex system consisting of DYS710, DYS385, DYS447, DYS504, DYS449, DYS626, DYS644, DYS612, DYS481 and DYS518. The markers were selected from a dataset of 283 individuals with African, European and Asian ancestry genotyped at 45 Y-STRs, applying an optimization based selection procedure to achieve the highest possible DC with the minimal number of markers. Overall the 10-plex DC = 0.9591, whereas AmpF Φ STR Φ Y-filerTM DC = 0.9145. The 10-plex was satisfactorily subjected to developmental validation tests following the SWGDAM guidelines showing its applicability to forensic casework: 1.6 microg female DNA did not yield amplification, full male profiles were obtained with (1) 100 pg male DNA, (2) 0.5 ng male DNA in 1000-fold excess female DNA (3) 150 pg male DNA in male:male mixtures in 1:20 ratio; environmentally exposed samples and non-probative casework gave satisfactory results. Reproducibility, precision and accuracy were tested using both ABI 3130XL and ABI 377. Some of these markers also show potential for their application to genealogical and demographical studies.

MtDNA Typing Improvements... and Alternatives

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For several years, mitochondrial DNA (mtDNA) analysis is performed by sequencing of the two hypervariable regions - HVI and HVII in many forensic laboratories. However, there have been improvements in methods and technologies that permit the outcome of bigger sequence reads with superior quality in less time. This comes from the use of better designed primers, as 017H reverse primer, which are located further apart and allow for bigger amplicons, instead of those obtained in the beginning of mtDNA sequencing technique; also comes from the use of membrane-base sized-exclusion filter plates systems to clean the labeling reaction versus the use of the time-consuming ethanol precipitation technique or even the very good but also very laborious column gel filtration procedures. In this work we have compared reference samples studied during the last 3 years. These samples were analyzed using different primers and two different PCR clean-up techniques. MtDNA was sequenced with BigDye® Terminator v3.1 Cycle sequencing Kit (Applied Biosystems). The results shows that small changes in methodology resulted in the achievement of sequence reads about 200bp longer than the ones obtained before and with better quality, for instance, allowing for a complete HVI sequence read. We even observed that some haplotypes would have been wrongly typed - six 16519C haplotypes would have been characterized as Cambridge Reference Sequence (CRS) if the 017H reverse primer was not used, which allows a complete HVI sequence read. The mtDNA sequencing technique for forensic purposes used by the majority of Forensic Laboratories can still be improved by small changes in methodology allowing for a better haplotype characterization.

Validation 'maxwell®16 For Hair Shafts' Reveals Multiple mtDNA Profiles In An Individual's Hair

Stijn Desmyter, Sylvie Comblez and Fabrice Noël

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The discontinuance of the Centricon® (Millipore) filter device urged us to reconsider our established organic DNA extraction procedure. Vivacon® (Sartorius stedim) and Microcon® (Millipore) are alternative filter devices, while the Maxwell®16 (Promega) automate can replace the whole manual process. The extraction efficiency was evaluated by the PCR yield of a 242 bp mtDNA fragment visualized on a GelRed™ stained agarose gel. Four extraction series of 2 cm hair shaft fragments from eight individuals revealed that Vivacon® removed the least inhibitors from dyed hairs. Only for Maxwell®16 none of the undiluted hair extracts showed inhibition. Together with the easy handling, this makes the Maxwell®16 the preferred method. Nevertheless, the yield variability is higher for Maxwell®16 than for Microcon®. Hair shaft decolouration has a negative influence on the DNA yield, sometimes resulting in the absence of a PCR amplicon.

The amplicons (Microcon® and Maxwell®16) were double stranded sequenced to confirm each donor's buccal swab mtDNA profile. For five of the eight test panel members a mixed base position was observed in one of the eight hair sequences. The sixth individual showed for two hairs two additional profiles which were different at a single position from the reference sample. In an additional series of 14 hair shaft fragments a fourth profile was observed which differed also at a single position from the reference. The full profiling (position 16024-576) of the 22 hair fragments revealed no further differences between the four profiles. The proven presence of multiple profiles in a single person's hair that differ mutually at two positions causes implications for the mtDNA evidence interpretation.

Mapping Genetic Landscapes Of The Modern Admixed Europe Based On Forensic Haplotypes With Geographic Information Systems.

Amalia Diaz Lacava, Maja Walier (Presented by Amalia Diaz Lacava)

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We present a procedure to map the predominant genetic landscapes of an admixed geographic region, primarily designed for forensic Y-STR haplotypes. We analyzed a data set of 7 forensic Y-STR, genotyped for 33,010 samples, collected in 249 locations in Europe, Western Asia and North Africa. The data set comprises 4,176 distinct haplotypes. Geographic analysis was performed with the Open Source geographic information system GRASS-GIS 6.4 (Geographic Resources Analysis Support System, <http://grass.itc.it>). We grouped distinct haplotypes into 20 clusters. Sample frequency per cluster and per location was calculated. Making use of geostatistical methods, we spatially interpolated frequency values across the study area, separately for each cluster. We obtained one interpolated surface of Europe per cluster. Juxtaposing the 20 interpolated surfaces, we pointwisely searched across the total study area for the maximum cluster frequency and the label of the respective cluster. Combining these two data sources, we obtained a concise map dividing the study area in six large regions and further smaller regions. A region delimits an area accounting for higher genetic similarity in regard to Y-STR haplotypes. This map shows a clear differentiation between Northern, Western and Eastern Europe. North Africa builds a separate region, southeastern Europe and Western Asia get divided in several regions. This result is coincident with previous analysis of genetic frequencies, validating our approach. We repeated this procedure for the second maxima. The summary map showing the second maximal cluster frequency across the study area divides Western Europe into four regions. 3-dimensional views allow visual comparison of cluster-frequency differences across the study area. Our genetic geostatistical approach is capable of summarizing in two accurate maps the geographic distribution and relative frequency of most predominant groups of the extant male European population, examined on the basis of forensic Y-STR haplotypes.

Haplotypes For 17 Y-Chromosomal STR Loci In A Population From Eastern Libya (Benghazi Region).

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The seventeen Y-STR loci included in the AmpFISTR® Yfiler™ PCR Amplification kit were used to type a sample population of 104 males from eastern Libya (Benghazi region). Of 104 observed haplotypes, 100 were unique (96.2 %) and 4 shared the same profile. The 17 loci gave a discriminating power of 0.9984. DYS438 showed the highest diversity as a single-locus marker ($h = 0.93$). Allelic frequencies and gene diversities for each Y-STR locus were determined. The high haplotype diversity and discrimination capacity (0.962) demonstrate the utility of these loci for human identification in forensic applications. Comparative analysis with Y-STR datasets of relevant populations and submission of the haplotypes to the Y-STR Haplotype Reference Database (YHRD) was undertaken. Enlightening conclusions can be drawn from these comparisons. Keywords: Forensic; Y-STR; Libya ; Benghazi

Identifying Variants Within Eurasian mtDNA Haplogroup U8 and Their Application To Studies Of Human Phylogeography.

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Human mtDNA is routinely used to discriminate between individuals using polymorphisms found in the first and second hypervariable portions of the control region, but homoplasy can lead to an underestimation of haplotypes without the provision of additional data. The extent of this problem varies between mtDNA haplogroups, but is particularly acute within U8, which constitutes up to 10% of some European populations. New high-resolution data were produced for U8 individuals from Eurasia and North Africa, using a variety of methodologies, and the ability to correctly assign haplotypes based on phylogenetic criteria assessed. The results were then compared to U8 data from the literature and key mtDNA genomes sequenced to improve the level of resolution within the haplogroup as a whole. The improved phylogenetic resolution of U8 haplotypes provides potential insights into prehistoric migrations within Eurasia, and highlights the need for compatible standards of mtDNA typing in both forensic and population genetic laboratories.

The Genetic Legacy Of Greek Colonisation In South Italy and Sicily

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From the 8th century B.C., the west Mediterranean witnessed a colonization enterprise that originated in the Greek area and had its centre of gravity in Eastern Sicily and Southern Italy, an area later to be broadly known as Magna Graecia. The scale of the demographic impact of the first colonizers and their genetic legacy is still a matter of debate. Archaeologists, historians and demographers have proposed different degrees of Greek contribution, with scenarios swinging between a colonisation process based on small groups of males admixing with autochthonous groups to substantial migrations from Greece and a Hellenic origin for a significant part of the ancient Italian-based population. Preliminary genetic data analysis suggests a closer affinity of Southern Italian samples to Anatolia than Greece (Capelli et al, 2007), but the samples available did not cover areas of interest.

The aim of the study is to tackle the question of the male Greek legacy in Sicily and Southern Italy aimed at integrating and complementing current archaeological evidences. We collected 184 samples from Greece (98, Eubea Island; 86, Corinth region), 134 from Southern Italy (56, Metaponto; 32, Cosenza; 46, Catanzaro) and 263 from Eastern Sicily. Male lineages have been investigated by a combination of slow and fast evolving markers (40 SNPs and 20 STRs), to provide a resolution ranging from populations to single lineages. SNP markers have been selected to be informative in Southern Europe and to integrate available data on Italy and Anatolia. We present preliminary data to evaluate the Greek genetic legacy in populations currently living in these areas and estimate the original demographic impact of the Greek colonisation.

Development Of Multiplex Y-SNP Identification Assays For Molecular Evolution and Forensic Applications

Rixun Fang, Pius Brzoska and Manohar Furtado

Applied Biosystems/Life Technologies, Foster City, United States

Single nucleotide polymorphisms (SNPs) on non-recombining region of Y-Chromosome have been widely used in many different fields, such as molecular anthropology, evolutionary biology, medical genetics, and genealogy. In recent years, studies have shown that Y-SNP can also be a valuable tool for forensic analysis, such as paternity testing and crime investigations. The aim of this study was to develop a robust system that can simultaneously genotype multiple Y-SNPs and be used for both molecular evolutionary and forensic applications.

We have developed a SNP detection platform named GenPlex™ System that meets the need of multiple Y-SNP detection. This system involves a multiplexed PCR amplification of genomic target regions and detection of SNPs in these amplicons using an oligonucleotide ligation assay (OLA). In PCR, regions containing specific SNP loci are first amplified by multiplex PCR. SNPs in the amplified regions are then detected by OLA reaction using multiple sets of locus and allele specific ligation probes. The ligated products containing universal ZipCode™ oligonucleotide sequences are then hybridized to corresponding fluorescent ZipChute® reagents, which contain sequences complementary to the ZipCode™ oligonucleotide sequences and unique mobility moieties. The ZipChute® reagents are subsequently eluted, separated by capillary electrophoresis, and genotyped by association with target SNPs using the GeneMapper® Software. In our previous studies, we have demonstrated that this system is accurate, sensitive, and easy to use. In total, 50 SNPs can be analyzed simultaneously in a single reaction.

In order to demonstrate the usability of this technology on Y-SNP detection, three multiplex panels were designed using information from the Y-SNP consortium parsimony tree. The first panel contains markers for distinguishing the major haplogroups. Other two panels were used to determine the subclades of the individual. The result from the method development, on-going optimization studies, and evaluation studies will be discussed.

Towards Entire mtDNA Sequencing In Phylogeny, Forensic and Medical Genetics

Liane Fendt, Harald Niederstätter and Walther Parson (Presented by Liane Fendt)

Institute of Legal Medicine, Innsbruck Medical University, Austria

MtDNA analysis is performed in many disciplines addressing various questions. Forensic genetics targets mtDNA when nuclear DNA lacks quality or quantity. Population genetics aims on evolution and dynamics of populations by using this matrilineal marker, while medical genetics focuses on diseases connected to an impaired energy metabolism triggered by mitochondrial dysfunction. The feature in common for all of these investigations is that genotyping is performed with chain termination sequencing, which provides reliable and accurate data, albeit at relatively low throughput and high expenses for chemistry. Mainly due to these instances the sequencing efforts have been largely limited to the analysis of the mtDNA hypervariable segments (HVS) 1 and 2 or even the entire mitochondrial control region. Moreover, the medical community lacks large scale studies targeting the coding part of the mitochondrial genome. However, a batch of new sequencing technologies is emerging: the second generation PCR based technologies as well as the third generation sequencing methods promise a reduction of costs with massive parallelisation of sequencing reactions on single mtDNA molecules. Here, we outline the state of the art in mtDNA typing and the potential of the new technologies for the near future: entire mt genome data is anticipated to become easily available, facilitating large scale mtDNA disease and population genetic studies. Likely, some of these methods will make their way into forensic genetics within the next few years, bolstering the establishment of full mtDNA databases being indispensable in the endeavour to utilize the full potential of mtDNA testing.

Evaluating Length Heteroplasmy In The Human Mitochondrial DNA Control Region.

Forster L, Forster P, Gurney Sm, Spencer M, Huang C, Röhl A and Brinkmann B (Presented by Peter Forster)

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We present allelic data for three known and one new C-tract in the human mitochondrial DNA (mtDNA) control region, and we measure intergenerational mutation rates at such C-tracts. In detail, in a sample of 1,172 mtDNA sequences, we demonstrate the existence of an instability threshold of eight consecutive cytosines, at and above which the phenomenon of length heteroplasmy arises. To determine mutation rates, we draw on mtDNA sequences in up to four generations of 248 pedigrees for families living in high or low-radiation environmental conditions. The high-radiation sample gives the most conservative (fastest) mutation rate likely to be encountered in any forensic context. We find that the C-tract mutation rate is up to 6% per generation, and we observe an excess of cytosine gains over losses. Case studies and guidelines for evaluating mtDNA heteroplasmy are provided.

A Rapid Tool To Detect Y Q* Paragroup Variability In Amerindians

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The Y-Q* paragroup is believed to have arisen in Central Asia from 15 to 22 Kya. Nowadays, it is found scattered at low frequency throughout the Eurasian continent as well as in Northern Africa and Polynesia but the highest peaks are reached in indigenous populations from Siberia and the Americas.

We first describe a novel SNaPshot multiplex assay developed to genotyping 8 major subclades within the Q* paragroup, with special attention to M3 downstream mutations. It has been used to investigate Q* variability in an admixed (the Hispano-American Criollos, N=105) and a native (Whici, N=127) population from the Gran Chaco region (South America). The frequency of the Amerindian-specific Q1a3a-M3 subclade was 0.331 % in the Criollos and 0.866 % in the Whici. No further Q* or Q1a3a subclade was found. When coupled with genotyping tests for European-specific haplogroups (Onofri et al 2006) the Q* multiplex assay covered the whole spectrum of the Y-SNP variability in both groups, thus representing a rapid and reliable tool to estimate the proportion of Amerindian ancestry in present Americans.

Rare Variants In The Coding Region mtSNPs

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Genotyping of Single Nucleotide Polymorphisms (SNPs) of the mtDNA coding regions has been suggested as a way to increase the power of discrimination between individuals. MtDNA polymorphisms compiled in databases allow for SNP selection to study specific population groups.

For forensic purposes, two multiplex assays were developed for mtDNA haplogroup typing of the two most common populations in our casework - European and African ancestry populations. SNPs were selected for their ability to discriminate among these two major ancestral lineages by the coding region of the mitochondrial genome.

In order to study the most relevant H (H1 to H7) and non-H (I, J, T, K, L1/I2, L3, M, N, U, V, W, X) haplogroups, SNaPshot™ (Applied Biosystems) methodology was used to genotype seventeen SNPs in 64 samples from Portuguese individuals.

Some of the SNPs presented high variability in the coding region such as nucleotide position (np) 8251 shared by W and also X/I haplogroups. In addition, when studying both H and non-H multiplex, some SNPs were found shared defining rare variants. For example, sub-haplogroup H7a can be obtained through a substitution at np 1719 used to define X/I haplogroups; H10a was obtained by a substitution at np 4216 used to define J/T haplogroups. On the other hand, one sample exhibited a drop-out (null allele) of the np 10398 peak, giving rise to a U*/K haplogroup. Sequencing of this region was not performed to determine the cause of this null allele, but the sample was reextracted and reamplified.

Although the small number of samples studied, rare variants were obtained with these two multiplex, which implies that the constant evolution of coding region SNPs defining sub-haplogroups is a permanent challenge for forensic geneticists.

Genetic Characterization Of A North Western Iberian Region Using Mentype® Argus X 8 Kit

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A total of 163 individuals (83 females and 80 males) from Galicia, a region located in north-western Spain, were genotyped for eight X-STRs by the use of Mentype® Argus X-8 kit. No significant frequency differences were found between males and females. Observed allele frequencies in female population samples showed no deviations from Hardy Weinberg equilibrium. The parameters for forensic evaluation were also calculated for each X-STR in this population sample, being DXS10135 the most polymorphic marker, followed by DXS10101. High combined values of power of discrimination were obtained for both female (1 in 1.8×10^{12}) and male samples (1 in 5×10^6) as well as a high power of exclusion in father/mother/daughter trios (99.9999%), father/daughter duos (99.9983%) and in half sisters with the same father (99.6412%). Furthermore, haplotype frequencies in Galicia were compared with other available population data. This study represents the first X-STR database for Galician population until present.

Hierarchical Y-SNP Assay To Study Indigenous Populations Of South America

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Studying indigenous tribes of Ecuador revealed that there is a lack of a Y-SNP assay to examine the substructure of South American sample sets. In most studies dealing with South American samples only the most common Y-SNP M3 of haplogroup Q was analyzed, because this is known to define a founder group in South America. Studies of ancestral SNPs of Q-M3 or the typing of subclades to confirm the results have often been neglected. For this reason we decided to develop a SNaPshot assay, which allows first for a hierarchical testing of all main haplogroups occurring in indigenous sample sets and second for a detailed analysis of haplogroup Q. We selected 16 SNPs from the YCC SNP tree and established two multiplexes. The first multiplex includes 12 Y-SNPs defining the major haplogroups (M42, M207, M242, M168, M3, M145, M174, M213, RPS4Y711, M45, P170, M9). The second multiplex contains Y-SNPs of haplogroup Q, especially of the subclade Q-M3 (M19, M194, P292, M3, M199).

Within our Ecuadorian sample, haplogroup Q-M3 (xM19, M194, P292, M199) was predominant, but we also found haplogroup E and R, which can be attributed to recent admixture. Moreover, we found four out of 77 samples, which were tested to be haplogroup C. This haplogroup is not known to be the result of recent admixture and has been found one time before in the Amerindian tribe Wayuu (1). Since haplogroup C occurs in North America, we assume that these C-samples are ancient as well. Therefore, we established a third multiplex, which allows a further subtyping of haplogroup C, mainly of the subclade C3 defined by the Y-SNP M217 (M407, M48, P53.1, M217, P62, RPS4Y711, M93, M86, P39). Altogether, these three multiplexes cover the most frequent haplogroups in South America and allow for an evaluation of the Y-Chromosomal diversity of Amerindian sample sets.

(1) T.M. Karafet, S.L. Zegura, O. Posukh, L. Osipova, A. Bergen, J. Long, D. Goldman, W. Klitz, S. Harihara, P. de Knijff, V. Wiebe, R.C. Griffiths, A.R. Templeton, M.F. Hammer, Ancestral Asian source(s) of new world Y-chromosome founder haplotypes, *Am J Hum Genet.* 64 (1999) 817-831.

Improved Method For Profiling Dog mtDNA and Expansion Of The Belgian Population Database

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The mtDNA profiling of the first series of dog samples for the Belgian population database occasionally produced poor sequence results. An aspecific band was observed on agarose gel after amplification of the whole control region in a single PCR. This aspecific product is suspected to be a numt and to cause the poor sequence results.

A new primer set for amplification increased the specificity and the aspecific band was no longer present. Furthermore, a higher annealing temperature, shorter cycle durations and a higher number of cycles during PCR improved specificity and yield. New primers were also introduced in the eight sequencing reactions, which resulted in a better double stranded coverage of the control region. Using the new method, samples that gave poor sequencing results before were reanalysed and heteroplasmy could be detected in HV1 at position 15931. This way three sequences could be added and two haplotypes were withdrawn from the original database.

The Belgian dog mitochondrial DNA database was expanded with samples of unrelated dogs, resulting in a total of 209 sequences. Two new polymorphic sites and 12 new haplotypes were observed after analysing the 89 additional samples. In total, 45 polymorphic sites were identified in HV1 and 14 in HV2, resulting in 58 haplotypes of which 35 occurred once (heteroplasmy disregarded). The most common haplotype represents about 16% of the individuals. Positions 15632, 15652 and 15931 show heteroplasmy and position 15639 was found to be a quaternary site. 69.4% of the sequences belong to clade A, 24.9% to clade B and 5.7% to clade C. No representatives of clade D, E or F were observed. The exclusion capacity is 0.92.

Genetic Polymorphisms Of 17 Y-Chromosomal Short Tandem Repeat Loci In Central Croatian Population

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In forensics, Y-chromosome short tandem repeat (STR) haplotyping is used in human identification, paternity testing and sexual assault cases where Y-STRs provide a male-specific DNA profile. The reference Croatian Y-STR database is important for forensic purposes, especially because there is a lack of information about the genetic structure of Y-chromosome in some Croatian regions. The aim of this study was to describe the genetic structure of Y-chromosome in Central Croatian population. We carried out a statistical analysis of the data from previously performed genetic analysis collected during the routine forensic work by the Forensic Science Centre "Ivan Vučetić". A total of 220 unrelated healthy men from Central Croatia were selected for the purpose of this study. Genomic DNA was extracted using Chelex procedure from FTA cards. Y-Chromosomal STRs were determined using the AmpFISTR Yfiler PCR amplification kit. The haplotype frequencies were determined by direct counting and analyzed using Arlequin 3.1 and AMOVA analysis calculated with YHRD online analysis tool. A total of 212 haplotypes were identified, 204 of which were unique. Total haplotype diversity was 0.993. Locus diversity varied from 0.325 for DYS392 to 0.786 for DYS385. Discrimination capacity was 92.7%. Allele frequencies diversity was 0.6149. Intermediate alleles 17.2, 18.2 and 19.2 were found at DYS458 locus. The analysis of Y-chromosome variability among publicly available population samples from the YHRD.org website was based on a minimal European haplotype set. AMOVA analysis indicated that Central Croatian population was the closest to population sample from Croatian capital Zagreb and some population samples from Bosnia and Herzegovina with no paternal contribution from the other neighboring countries such as Slovenia and Austria. Further studies are needed in order to fulfill Croatian Y-STR database and to use this data in routine forensic work and to get information about genetic similarity between the neighboring populations.

Origin and Evolution Of The Mitochondrial DNA Lineages In Norway

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The Viking settlement of the North Atlantic region had a great impact on the history of Europe, and continues to have an important place in the popular imagination. Recent studies on uniparental genetic markers have shown that, contrary to the “raping and pillaging” image of the Vikings, much of the Norse settlement of the accessible territories such as Orkney, Shetland and coastal Scotland was by family groups of males and females. To increase our understanding of the genetic history of the peoples of the North Atlantic, we are performing an extensive study of genetic variation in Norway, encompassing both mtDNA and Y-chromosome polymorphisms. We present the preliminary analysis of sequence variation in coding and non-coding regions of the mitochondrial genome of 660 present-day Norwegians, and discuss the putative origin and affinities of the principal maternal lineages in Norway. In addition, we report several novel variants in tRNAThr, the most polymorphic of the mitochondrial tRNA genes.

Dna Evidence: Original DNA Test Results Overturned By A Retrial In The Ashikaga Case

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The DNA re-examination was conducted for the first time in Japan by the Tokyo High Court in December 2008, the result proving remarkably different from the original test done by the National Research Institute of Police Science.

The first DNA typing in 1991, only a single D1S80 (VNTR) locus was examined. Results found repeats 16-26 of DNA taken from a girl's clothes to match that of a murder suspect, Mr. Sugaya. By 1993, we had already reported incidences of mistyping due to use of polyacrylamide gel electrophoresis without using allelic ladder marker (Int J Leg Med 106:111-114,1993).

This led to the appraiser revising the typing results to repeats 18-30. However, the evidence was still held to confirm the identity of the suspect, Mr. Sugaya being sentenced to life imprisonment by the Supreme Court in 2000.

Nevertheless, based on the lower accuracy of DNA analysis in the 1990s, Mr. Sugaya's legal counsel continued thereafter to petition the court for a DNA re-test. The Tokyo High Court eventually accepted the request in November 2008, with separate tests being carried out by two forensic scientists, one recommended by the prosecution and another by the defense counsel.

Under recommendation by the defense counsel, we examined the D1S80 locus, eight Y-STRs and mtDNA regions. Results showed the D1S80 type of semen from a shirt to be 18-24, and that of Mr. Sugaya to be 18-29; five of the eight Y-STR loci, and two mtDNA nucleotide positions were also remarkably different from that of culprit. Furthermore, six of the Y-STR loci were found to be identical to a DNA profile done by the appraiser of prosecution, indicating the DNA profile of the true murderer. Finally, our results were admitted as reliable DNA evidence in the retrial court on November 24, establishing Mr. Sugaya's innocence almost beyond doubt.

Development Of SNaPshot Minisequencing Of 12 Y-SNP Loci In Chinese Han Population For Forensic Application

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The single nucleotide polymorphisms on Y-Chromosome (Y-SNP) have been applied in many fields including evolutionary biology, forensics, medical genetics, and genealogical reconstruction. In forensic genetics, it was used not only for typing of degradation DNA, but also for predicting the geographic origin of the donor of a crime scene sample and analysis of mixed biological stains in sexual assault cases. The number of Y-SNP is increasing in the global population. However, only few Y-SNP loci were reported in Chinese population. Forensic genetics urgently needs to seek more Y-SNP loci in the Chinese population. In this study, we tried to find out more new Y-Chromosome binary markers using a high-throughput SNP genotyping technique. Two new Y-SNP loci were observed in Chinese Han population by us. To Development of SNaPshot minisequencing for forensic applications, we combined our Y-SNP loci with 10 Y-SNP loci that had been reported to be polymorphic in Chinese population from more than 600 binary markers compiled in the phylogenetic tree published by the Y-Chromosome Consortium and from the studies previously published. All of the 12 Y-SNP genotyping were carried out by SNaPshot minisequencing and capillary electrophoresis. This is an efficient and rapid test for typing 12 Y-SNP loci in one PCR and minisequencing multiplex reaction. A total of 100 individuals from a Chinese Han population sample were analyzed with this strategy. Our results showed all of 12 Y-SNP loci selected were polymorphic and a total of 22 different haplotypes were observed in the studied Chinese Han population sample. Our result implied that SNaPshot minisequencing with 12 Y-SNP loci selected were useful for forensic purpose in Chinese Han population.

Analysis Of mtDNA-SNPs Genotyped With Affymetrix Gene Chip 6.0 Array In A Population Sample From South Sardinia

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We present preliminary results about 97 SNPs of mtDNA, genotyped in a population sample for South Sardinia (Trexenta and Sulcis regions, N = 92) with Affymetrix GeneChip 6.0 array.

Ninety-six SNPs are positioned in different 18 genes of the coding region, one SNPs is placed in a non-coding region (NC3). The three genes with the greatest number of SNPs are: CYB (16.5%), RNR2 and ND5 (11.1%) and 36 SNPs are polymorphic. Genes with highest number of polymorphic SNPs are: CYB and ND5 (16.7%), RNR2 and ND4 (13.9%). 56 of the 61 monomorphic SNPs are shared with Caucasians (HapMap), whereas 5 SNPs are specific for our sample.

We detected 20 different haplotypes, with frequencies ranging from 19.6% to 1.1%. Eight haplotypes are unique. The Network Median Joining does not show an ancestral haplotype; the results do not change when we compute a separate network for each subpopulation. Moreover, the two regions sampled do not have characteristic haplotypes and seem not distinct. Conversely, a network computed with HVR-I sequences for two samples of same Sardinian sub-populations from a previous our work, indicates different results. Here, we observe one ancestral haplotype with an high frequency, common in both subpopulations.

This results showed that these set of markers are weakly informatives for population analyzed, to some extent for elevated number of monomorphic markers (62.9%), probably due to choice of SNPs for Affymetrix Chip Array, based on 270 HapMap individuals. In a founder population as Sardinia, a loss of informative markers is expected, as already observed for autosomal SNPs. Moreover, the location in the coding region of mtDNA involve the non - neutral nature of this markers. It is feasible an integration of these data with HVR-I sequences, to increase informative power, with the purpose to make use of this markers for forensic and population studies.

A Probability Theoretical View Of Genetic Matches

Michael Krawczak (Presented by Michael Krawczak)

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One of the key issues of forensic biostatistics is the assessment of the evidential value of a genetic match between a trace and a suspect. I will formalise this topic in a probability theory framework, highlighting in particular the (sometimes challenged) duality of probability and frequency. Since match probabilities are thus undoubtedly related to allele and haplotype frequencies in specific (real or hypothesised) groups of individuals, any sensible estimation of the former must take the population and evolutionary genetic aspects of the markers in question into account. I will therefore briefly recall which predictions are made by population genetic theory about allele type and allele frequency distributions, and how these distributions may be assessed empirically. An account will also be given to the consequences of violations of the highly idealised assumptions underlying the above considerations, particular in relation to population structuring. My presentation is intended to set the scene for the contributions by other experts, and to stimulate the subsequent discussion. Hopefully, it will also serve to promote a consensus view on how the evidential value of genetic matches should be assessed by biostatistics means, particularly in the case of haploid markers.

Diversity Of Mitochondrial DNA Of Belarusians and Its Forensic Application

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Wide use of mitochondrial DNA (mtDNA) analysis in forensic genetics has necessitated the creation of special databases which consist of the information about mtDNA haplotypes in respect of particular population or geographical region. It has become evident that both the representativeness of the databases and the accuracy of mtDNA sequencing are vital since they can influence on final expert report.

292 mtDNAs of Belarusians were analyzed based on sequence of HVSI region between positions 16020–16400 followed by RFLP analysis of 44 diagnostic positions of coding region. Samples descend from ethnic Belarusians - representatives of six ethnic and geographical regions of Belarus which were formed based on historical, ethnic and geographical data. Biological material (peripheral blood) was obtained after informed consent from healthy adult males who identified themselves as Belarusians during at least three generations. Since ethnic Belarusians form a majority and cover more than 60% of total number of inhabitants there is obvious need to make stress on the ethnic portion of total population while organizing the database of mtDNA of Belarus population for forensic use.

Pool of mtDNA haplogroups in Belarus is typical for western Eurasian composition of mtDNA haplogroups. The biggest part (88%) of it is formed by haplogroups which descend from macrogroup R: H, U, J, T, V, HV and smallest one shared by branches of macrogroups N (9%) and Asian specific M (2%). The haplogroups distribution among six regions of Belarus has stochastic mode. The maximum diversity of mtDNA lineages was shown for southern region of Belarus.

Recent study is one of the first attempts to cover Belarusian “white spot” on European forensic mitochondrial map at least partially. We plan to study other mtDNA polymorphic regions of Belarusians and to complete data with Y-chromosome polymorphisms.

The Potential Of mtDNA-SNP Analysis In Forensic Case-work

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The mtDNA SNP analysis via SNaPshot minisequencing for old and degraded samples is presented by six forensic cases, examined at the Institute of Legal Medicine in Münster from 2008 to 2009.

The age of the samples in those cases ranged from 2 months to 1400 years. Different tissues were investigated and analysed by 32 mtDNA SNPs in one multiplex PCR and one subsequent multiplex SNaPshot minisequencing reaction.

Although mtDNA SNP analysis is less informative than the analysis of nuclear DNA SNPs or nuclear DNA STRs, this technique has a good exclusion potential. Furthermore, mtDNA SNP analysis may provide reliable results where STR-analysis or Sanger sequencing failed. It is as well a very good screening method for forensic cases with many sample materials like human hairs.

Basques and Germans Y-STRs Haplotype Distribution In Europe

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The Y-STRs DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385, DYS438 and DYS439 loci have been analyzed in three European populations: Autochthonous Basques (n=182), residents in the Basque Country (n=196) and Germans (n=132).

Genetic diversities (h) were calculated using the Arlequin software (ver 3.1). The diversity value for Basques (0.9849) can be considered low when compared to the ones obtained for Basque Residents (0.9949) and for Germans (0.9983).

The intrapopulation power of discrimination of this set of Y-STRs loci for Basque population was 0.9385, less than for Residents (0.9835) and Germans (0.9885), according to the low diversity observed in this native population.

In order to analyze the Y-STR haplotype distribution in Europe, we made haplotype grouping. Firstly, we selected the most frequent haplotypes observed in the populations here studied and also in 12 European populations compiled from the literature. Secondly, we grouped these frequent haplotypes with its one-step mutational neighbours (plus or minus one repeat unit). Thus we determined 3 representing groups (in descending order of frequency): Western European Group native from Atlantic Region (14/13/29/24/11/13/13/11,14/12/12), Northern European Group (16/13/29/25/10/11/13/11,14/11/11), and Eastern European Group (14/12/28/23). The autochthonous Basques and the residents in the Basque Country belong almost completely to the Western European Group (69 and 53% respectively), without representation of the described groups from the north and the east of Europe. On the other hand, some German samples belong to Northern European Group (10.6%) and others to the Eastern Group (13.6%), although most of them belong to the Western Group (30.3%).

In conclusion, these results are in concordance with haplotypic frequencies gradients described in previous studies.

Project “Genetics and Surnames” In Czech Republic

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The aim of this presentation is to inform the auditorium about the results of the research project “Genetics and surnames” and the existence of the specialized Czech genealogic DNA database. The authors will highlight the main features, goals and directions of the project, as well as the connection of the resulting database to the similar projects abroad. The biological material of selected donors is tested for 26 Y-STR markers (DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385 a/b, DYS393, DYS391, DYS439, DYS635, DYS392, Y GATA H4, DYS437, DYS438, DYS448, DYS446, DYS444, DYS388, DYS481, DYS426, DYS459, DYS457, DYS455, DYS454, DYS453, DYS452, DYS451, DYS450, DYS449, DYS448, DYS447, DYS446, DYS445, DYS444, DYS443, DYS442, DYS441, DYS440, DYS439, DYS438, DYS437, DYS436, DYS435, DYS434, DYS433, DYS432, DYS431, DYS430, DYS429, DYS428, DYS427, DYS426, DYS425, DYS424, DYS423, DYS422, DYS421, DYS420, DYS419, DYS418, DYS417, DYS416, DYS415, DYS414, DYS413, DYS412, DYS411, DYS410, DYS409, DYS408, DYS407, DYS406, DYS405, DYS404, DYS403, DYS402, DYS401, DYS400, DYS399, DYS398, DYS397, DYS396, DYS395, DYS394, DYS393, DYS392, DYS391, DYS390, DYS389I, DYS389II, DYS388, DYS387, DYS386, DYS385 a/b, DYS384, DYS383, DYS382, DYS381, DYS380, DYS379, DYS378, DYS377, DYS376, DYS375, DYS374, DYS373, DYS372, DYS371, DYS370, DYS369, DYS368, DYS367, 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Micro Geographic Distribution Of The Y-Chromosomal Variation In The Duchy Of Brabant

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A future issue in human phylogeography is surveying the distribution of genetic variation on a micro-geographic scale to identify key determinants of population structure in humans. An interesting region to investigate regional distribution of genetic variation is Belgium mainly due to the presence of a language border without physical barriers, the Romance-Germanic language border. To survey the origin and the evolutionary significance of this border, a micro-scaled biogeographical analysis will be performed with data from haploid markers and whole-genomic approaches. Here we want to present results of a first phase of this project, a study of the Y-Chromosomal (Y-chr) variation in the Duchy of Brabant, a historical region in the Low Countries containing three contemporary Belgian provinces and the Dutch province of North Brabant. 477 males from five different regions within the Duchy of Brabant were selected based on their genealogical data (pedigree at least before 1800). For all participants, the finest level of substructuring are defined according to the latest published Y-chr phylogenetic tree, as well the Y-haplotypes are determined based on 37 Y-STR loci. The observed Y-chr variation is subdivided within eight Y-haplogroups and within 32 Y-subhaplogroups, whereby 70% of all participants belonged to only four subhaplogroups: R1b1b2a1 (R-U106), R1b1b2a2* (R-P312*), R1b1b2a2g (R-U152) and I1* (I-M253*). Significant micro-geographical differentiation within Brabant was observed between the Dutch province of North Brabant vs. the Flemish provinces based on the differences in (sub)haplogroup frequencies but not based on Y-haplotypes within the main subhaplogroups. A clear gradient was found with higher frequencies of R1b1b2 chromosomes in the northern vs. southern regions. The data will be extended by sampling campaigns of all regions in Belgium as well of the Grand Duchy of Luxemburg and French Flanders (France). This project will give more insights into regional distribution of genetic variation along language borders and will define new hypotheses on the role of cultural characteristics in human genetics.

Mixtery: Confirmed Presence Of Two Different mtDNA Genomes In Maternally Related Individuals

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The mode of mitochondrial DNA (mtDNA) inheritance is usually associated with the maternal lineage. There has been only one reported exception to strictly maternal inheritance of mtDNA in humans that has so far not been refuted. Schwartz and Vissing (2002) observed a mixture of two mtDNA haplotypes in the muscle tissue of a patient with mitochondrial myopathy.

Now, we have observed (accidentally) two distinct mtDNA haplotypes within a female individual. What was first believed to originate from contamination during sample collection or in the laboratory process later turned out as pristine mtDNA mixtures of hg V and hg U4c1 haplotypes and was confirmed in two independent laboratories by multiple analyses of repeatedly taken tissue samples.

Further analysis of blood, saliva and hair samples reveal the mixture in a total of seven maternally related family members, consisting of two sisters and her five children. A further sister and her son only show hg V sequences.

Autosomal Short Tandem Repeat typing of the family members confirmed relatedness but did not reveal any mixture at nuclear DNA level.

SRY Gene Polymorphism: A Tool To Evaluate Natural Hybridization In South American Primates

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Besides habitat destruction, natural hybridization is the most important threat to marmoset primates *Callithrix aurita*, endemic to Brazil's southeast region. The *Callithrix* genus comprises six taxa, originally parapatric. *C. penicillata* and *C. jacchus*, the species with the most extensive distribution, has been introduced in this geographic region approximately 100 years ago. Nowadays, they have been appointed as the likely responsible for hybridization with the threatened species *C. aurita* although it has not been confirmed by genetic analysis. Previous studies have shown genetic similarity between the six species but a nine base pairs deletion in SRY gene distinguishes *C. aurita* from the others. Y- chromosomes from three male marmosets considered as hybrids according to their phenotypes and wild species individuals were compared using a pair of primers designed to amplify a fragment of 200 bp including the deletion region. The results showed that the hybrid individuals have the SRY gene deletion. This study have shown for the first time that the hybrids are fathered by *C. aurita* in a natural hybridization. The set of primers developed in this study can be used in conservation management plans since the removal of invasive species and hybrids is cogitated by IBAMA (Brazilian Environmental and Renewable Resources Institute). Subsequent studies of mitochondrial DNA sequences will allow identification of the maternal species involved in these individual's hybridization.

An Interesting Mutation At Locus DYS385 In An Uncle / Nephew Pair In A Fatherless Paternity Case

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The Y-Chromosomal short tandem repeat polymorphisms (Y-STRs) included in the AmpFISTR® Yfiler® amplification kit (Applied Biosystems) are currently used for forensic and evolutionary applications, therefore a consistent knowledge on mutation properties is necessary for correct data interpretation.

Recently, in a fatherless paternity case we found an interesting mutation at locus DYS385 in an uncle/nephew pair.

The paternity test was carried out typing the son, his mother and his father's brother; the father was unavailable because he died some years ago in South America where is currently buried.

The results of AmpFISTR® Identifiler® and AmpFISTR® Yfiler® profiling were statistically analyzed using Probabilistic Expert Systems (PES) FINEX and Familias, that allow to obtain a high paternity probability. However an interesting mutation at locus DYS385 was observed: the uncle's profile at that locus was 15-16 opposing the nephew's profile was 14-16.

Profiles were confirmed using Powerplex Y (Promega), therefore a sequence of the locus was performed.

The Frequencies Of mtDNA Haplogroups In Czech Republic and A Comparison To The Other European Populations

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The aim of this study was to compare the results of full HVRI and HVRII mtDNA sequences of 120+ individuals with the previously published Czech population data obtained using PCR-RFLP and population data for other European populations. The results indicate that the full control region sequencing can bring more precise population information that is useful for the comparison with other data sets and also for the forensic identification purposes.

Paternal and Maternal Lineages In Guinea Bissau Population

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The analysis of mitochondrial DNA (mtDNA) and Y-Single Nucleotide Polymorphisms (Y-SNPs) can be helpful in some forensic scenarios, since they allow defining haplogroups showing a high geographic specificity, providing information about the maternal or paternal ancestry of an individual or evidence under investigation. MtDNA and Y-chromosome information can also be useful in order to evaluate population substructure in admixed populations, which is essential in the definition of relevant forensic databases. The aim of this work was to study the origin of paternal (Y-SNPs) and maternal (mtDNA) lineages of Guinea-Bissau population, inferred by phylogeographic analyses of the observed haplogroups.

To calculate the frequency distribution of the male lineages in Guinea-Bissau, 37 unrelated males were typed, using a PCR-SNaPshot multiplex method, including 13 Y-SNPs (P2, M154, M293, M81, M85, M78, M35, M96, V6, M191, M33, M123 and M2). The most frequent haplogroup observed in our samples was E1b1a (xE1b1a4,7)-M2, with a frequency of 68%, followed by E1a-M33 with a frequency of 19%. Haplogroups A and R1b1 comprised 13% of the chromosomes.

Polymorphisms of the two hypervariable mtDNA regions were analyzed in 79 unrelated individuals from Guinea-Bissau, using the method referred by Wilson et al. (1995). Sequences were obtained with dRhodamine Terminator Kits (Applied Biosystem) and detected with ABI 3100 Avant. Haplogroups were classified based on the different polymorphic positions of these regions using the software manager-forensic mtDNA database. The most frequent haplogroups were L2a1 (13%), L1b1 (11%), L3d (10%), L3e4 (9%), L1c1 (8%), L2c (8%) and L3b (8%).

Our results, like other recent studies, confirm a low variability of paternal lineages in the studied population of Guinea-Bissau, with a typically West African profile, marked by a high frequency of haplogroup E1b1a(xE1b1a4,7)-M2. The maternal lineages (67%) also demonstrate a West African profile, belonging to specific sub-clusters of L1-L3 haplogroups with sub-Saharan origin.

Rapid Parallel Capture and Sequencing Of Complete Mitochondrial Genomes

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Mitochondrial DNA is a valuable resource for identity testing. We describe a novel approach for rapid and economical retrieval of complete mitochondrial genome sequences from complex samples mixtures. The method is suitable for degraded DNA and can be highly parallelized. It achieves enrichment of mitochondrial DNA from DNA sequencing libraries by hybridization to bait DNA produced by PCR and attached to a solid phase. To demonstrate the performance of the method we captured 46 complete mitochondrial genomes and sequenced them on a single lane of an Illumina GAII instrument.

In Search Of The Lost Mountain Bushmen Of Lesotho

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Oral histories and archaeological studies have previously been used to explore Lesotho history, yet Lesotho populations have not previously been studied from a genetic perspective, and therefore have the potential to throw light onto the currently understudied human genetic history of southern African. Lesotho is of particular interest as it was one of the last places to become settled by the so called 'Bantu expansion', when Bantu-speaking farmers arrived in the country from western Africa in about AD 1600. There are no longer any people present in Lesotho who self-identify as San, yet through admixture with the Bantu farmers, it is possible that some of the contemporary population are descendants of the San hunter-gatherers. In this study, the mitochondrial hypervariable region 1 and SNPs and STRs on the Y-Chromosome have been analysed in 379 unrelated males from Lesotho, and in 73 Nharo (hunter-gatherers), and 50 Herero (Bantus) from Botswana. These results have been compared with published results from other populations across Sub-Saharan Africa. Preliminary results suggest that Lesotho populations have a higher than average proportion of the San-associated L0d mtDNA haplogroup when compared to other Bantu populations. In contrast, the A3b1* Y-Chromosome haplogroup seems to be present at a level comparable with other southern African Bantu populations, suggesting a potentially sex-biased barrier affecting gene-flow between the Bantu and San populations. The results also show that despite the geographical distance between Lesotho and Botswana, there are shared haplotypes in both the mtDNA, and Y-Chromosome STRs between both countries, suggesting past admixture between the populations. Work is currently being carried out to try and determine whether this admixture is ancient or more recent.

Use Of Artificial Random Mixtures From A Y-STR Database To Estimate Expected Frequencies Of Potential DNA Contributors

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Y-STR mixture analyses are confounded by the statistical limitations of Y-STR analysis. The methods that have been developed to date do not adequately resolve the problems encountered with the partial profiles and allelic dropout commonly encountered with forensic samples. However, without statistics, the weight and significance of the evidence cannot be properly assessed. Here we describe the development of a method that uses artificial mixtures of two, three or four random individuals from a database of over 2500 individuals. This allows us to determine the expected frequency of partial or entire matches of an individual's Y-STR profile to the random mixtures and simultaneously provides a normalized method to compensate for possible allelic drop-out. The results of this research indicates that while a match of over 90 percent of the loci in a mixture may seem intuitively compelling, it can be very misleading. For instance, using 1000 random mixtures from the database, the profile may match 15 of 16 Y-STR loci from only a few percent to over 45 percent of the random mixtures, depending on the potential contributor's individual Y-STR profile. The research also indicated individuals from African American and to a lesser extent Southwestern Hispanic populations, exhibited a bimodal distribution. We attribute this to admixture, estimated to be over 26% and 13% in African Americans and Hispanic populations, respectively. Not surprisingly, it was also observed that there appears to be a correlation between the haplogroup frequency and the distribution. For instance, a profile from a common haplogroup such as R1b more often matches random mixtures at 14 to 16 loci than a profile from a rarer haplogroup, such as G2a. As expected matches of 14 to 16 loci occur more frequently when profiles were compared to 4-person random mixtures than either 2-person or 3-person random mixtures.

A Rare Mutation In The Primer Binding Region Of The Amelogenin Locus In The Amp F/str® NGM™ PCR Amplification Kit Results In Dropout Of The X But Not The Y Homologue

Julio J. Mulero, Robert E. Lagace and Lori K. Hennessy (Presented by Julio Mulero)

Applied Biosystems, Foster City, United States

The Amelogenin locus is frequently used in forensic genetics as a gender-identification marker as a single primer pair can be used to amplify both the X and Y-Chromosome homologues. The NGM™ kit features a new Amelogenin primer sequence with decreased cross reactivity to non-human DNA compared to the primer sequences used in previous AmpF/STR® kits. Characterization of the Amelogenin primer used in the NGM™ kit was performed using 1224 samples (349 Caucasians, 350 African Americans, 390 Hispanic, 135 Koreans) and revealed two instances of X-Chromosome allele dropout not seen with the Amelogenin primer sequences used in the SGM Plus® or Identifiler® kits. Sequence characterization of these two Caucasian males revealed an identical C to T mutation at the 3' base of one of the NGM™ kit primers resulting in failure to amplify the X homologue of Amelogenin. This mutation has not been reported previously. The gender of the two individuals was confirmed by the presence of full Y-STR profiles with the male-specific Yfiler® kit. This rare mutation does not preclude accurate male gender identification because the Y-Chromosome homologue is correctly amplified.

Y-Chromosome Forensic Database: Haplogroup and Haplotype Sharing In A Multiethnic Population Living In Bologna (Italy)

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Haplotyping of human Y-Chromosome is widely employed in forensic practice and a large quality-assessed database is the prerequisite for providing useful informations in criminal cases, paternal relationship and mass disaster events. Freely accessible YHRD (Y-Chromosome Haplotype Reference Database), designed to store Y-chromosome haplotypes from global population, responds to this requirement reflecting also the highly fragmented Y-Chromosomal landscape. Such database permits to estimate the frequency of a Y-haplotype match by counting method allowing also, through metapopulation criteria, inference on the population of origin based on Y-STRs profile of an unknown biological evidence. However, a different population structure nowadays characterizes most of urban areas in Italy, like Bologna in Emilia Romagna region, due to recent immigration from different continents. Considering that the rate of immigration in Emilia Romagna region is one of the highest in Italy (8,6%) and with a growth rate of 10% every year, the local population is transforming in a multiethnic and multiracial one. Qualitatively, the real difference respect to other European countries is the very fragmented origin of immigrant populations. To analyze the Y-chromosome genetic profile of individuals from different ancestries living in Bologna a total of 86 male were typed for 17 Y-Chromosome STRs loci and haplogroups were assigned by single-nucleotide-polymorphisms (SNPs) analysis. Haplotype diversity was determined (99,8%) and results were compared with data from local native populations, previously typed, to evaluate haplogroup and haplotype sharing from a forensic viewpoint.

Haplotype Specific Separation and Sequence Based Typing Of Hla Genotypes – More Than Resolving Ambiguities?

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Haplotype specific extraction, HSE, allows the specific collection of individual alleles by separating diploid samples into their haploid components. The separation step is performed with magnetic beads based on allele-group specific sequence binding sites. The separated DNA samples can be directly typed with downstream applications like sequence based (SB) typing.

Consequently, very long sequence regions of separated alleles are available around the extraction binding site for further typing strategies. With SNP- and STR-studies on the short arm on chromosome 6 we were able to detect more than 120-kb long, haploid, double-stranded DNA samples.

Here we show how HSE was performed to separate samples with locus specific ambiguities in human leukocyte antigens, which could not be resolved by means of sequence-specific oligonucleotide (SSO) typing, sequence specific primer (SSP) typing, and generic SB typing for either sample. After haplotype-specific separation of the respective allele pairs, novel polymorphisms in the HLA-alleles were clearly detected by SB typing. Furthermore deficiency cases in DNA advices are strongly increased in the last years. HLA-typing is with its high discriminatory power of special importance in these cases where the alleged father or grandparents are not available for analysis. We show how HSE was performed to solve paternity in complex kinship cases.

The Genetic Structure Of Y-Chromosome in 1338 Italians.

Valerio Onofri, Loredana Buscemi, Adriano Tagliabracci and The GEFI-Group (Presented by Valerio Onofri)

Section of Legal Medicine, Università Politecnica delle Marche, Ancona, Italy.

The forensic use of Y-Chromosome markers, both STRs and SNPs, is constantly increasing since they could be a tool for implementing informativeness in human identification (e.g. mass disasters), paternity tests, interpretation of mixed male–female samples, and for inferring of geographic origin of a male individual. The study, from fifteen Italian GeFI-ISFG Group participants, consisted on 1338 regional recruited male subjects from different geographic areas of the Italian peninsula, analyzed with both SNPs and STRs in order to collect haplotype/haplogroup frequencies.

The sample was 75% typed by 17 AB Yfiler loci and the remaining by 11 Promega Poweplex Y-System loci. Eighteen markers were selected that allow to explore the basal branches of the phylogenetic tree encompassing all the major clades A-T: M91, M181, M216, M174, M96, M35, M89, M282, M201, M52, M170, M304, M267, M172, M9, M214, M45, M173. Genotyping was accomplished by two multiplex PCRs and subsequent single base extension.

SNPs and STRs data were combined in a single database, that will give the opportunity to obtain a large marker data for each submitted query. The Italian dataset shows a remarkable haplotype diversity and the most frequent haplotype is the same already observed in Europeans R1b chromosomes.

This project represents the first large Italian database for both SNPs and STRs. The possibility of inferring the geographical origin of an unknown male lineage represents the main usefulness for forensic applications. Spatial frequency distribution of Italian haplogroup-related lineages showed different pattern for the observed clades, and analysis of genetic variability was performed. Forensic implications of this project were discussed.

The whole dataset is going to be submitted to the newborn YHRD-YSNPs Records (<http://www.yhrd.org/Research/Y>). Forensic value of this project will be more evident when other local/country databases will be available and comparable with the Italian one.

Mutation Rates At Y-STRs: A Collaborative Study Of The GEFI-Group.

Valerio Onofri, Loredana Buscemi, Adriano Tagliabracci and The GEFI-Group.

Section of Legal Medicine, Università Politecnica delle Marche, Ancona-Italy.

The knowledge on Y-STR mutation rates needs to be considered in the paternity probabilities, especially in deficiency cases of disputed paternity involving male offspring where the alleged father is not available for DNA analysis but is replaced by someone of the paternal lineage. Furthermore, the mutation rate represents a precious tool to estimate the local and temporal origin of a given Y-SNP based haplogroup.

In order to improve the data on Y-STR mutations at the loci mostly used in forensic analysis, a collaborative study was carried out by the Italian ISFG Working Group, following recommendations of the ISFG DNA Commission.

The sample consisted of 915 male subjects, father/son pairs from paternity cases and 23 cases of multi-generational pedigrees collected in 15 different forensic laboratories from Italy. The biological relationship of all father/son pairs was previously confirmed by using autosomal microsatellites. The laboratories used AmpFISTR YFiler kit (AB) and PowerPlex Y System (Promega). The observed mutations or relevant allele variants were analyzed by a second laboratory, and confirmed by sequence analysis.

24 mutations were observed among all of the allele transfers in the sample (23 single step and 1 double step), and up to two mutations in the same father/son pair were found in three cases. Locus amplification was observed in DYS19 and DYS385 loci.

The observed locus-specific mutation rate ranged between 0 of DYS391, DYS392, DYS437, DYS448, GATA H4.1 and $6,961 \times 10^{-3}$ (95% CI $1,4-20,0 \times 10^{-3}$) of DYS19 locus. The average mutation rate in this study was $2,955 \times 10^{-3}$ (95% CI $1,8-4,6 \times 10^{-3}$). The mean age of fathers with mutations (33,22 years) quite similar than that of the fathers without mutations (35,67 years).

A New Tool For Population and Forensic Genetics: 33 X-Chromosome Indels In One Multiplex Reaction

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Having extensively explored autosomal and uniparental genetic markers, forensic and population geneticists are now dedicating a special attention to X-Chromosome. Its peculiar mode of transmission has impact on recombination and Linkage Disequilibrium (LD) patterns, thus making the X-Chromosome interesting for population genetic studies and especially informative in specific kinship investigations involving female offspring. As a consequence a variety of assays has been described using X-STRs, X-SNPs and, more recently, X-Chromosome Insertion/Deletion Polymorphisms (X-Indels). In this study we further explore the multiplexing potential of Indels showing short allele length variation by developing a genotyping assay able to characterize 33 X-Indels in a single PCR reaction. A short amplicon strategy was adopted (≤ 150 bp) aiming to improve the analysis of degraded DNA samples. The selected markers were reported to show high degree of polymorphism in major population groups ($MAF \geq 0.20$ in Africans and Europeans, according to dbSNP and Marshfield Diallelic Insertion/Deletion Polymorphisms database at www.marshfieldclinic.org/mgs). Based on the study of 100 sub-Saharan African individuals (from Angola and Mozambique) and 300 Portuguese individuals (100 father/mother/daughter trio constellations), we present preliminary data regarding allele frequencies, Hardy-Weinberg equilibrium, LD analyses and mutation rate estimates. We also assess the statistical parameters of forensic efficiency of the X-Indel set.

Study Of 25 X-Chromosome SNPs In The Portuguese

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The screening of mtDNA and Y-chromosome diversity in the Portuguese population has already yielded additional insights about migration of various groups of people that inhabited the region over different time periods. In the South, the high frequency of typical sub-Saharan and Mediterranean lineages may be explained by the influx of African slaves between the 15th and 19th centuries and by ancient contacts with Mediterranean populations like Greeks, Phoenicians and Carthaginians, who established important trading networks in the region. Unlike mtDNA or Y-chromosome analyses that inform about the history of female and male lineages, respectively, the X-chromosome allows the simultaneous study of both genders, and can also be an important tool in individual identifications, unraveling some of the challenges presented in population genetics, forensics and kinship testing. Currently, few data on X-chromosome single nucleotide polymorphisms (X-SNPs) have been published.

In this work, we studied 25 X-SNPs in population samples from North, Central and Southern Portugal (n=305) to evaluate the diversity pattern of the Portuguese population. Data were also compared with previous results from Mediterranean populations to assess their genetic affinities. All the markers were polymorphic in the Portuguese populations studied and the minimal allele frequencies ranged between a minimum value of 14.6% - X159 in the South - and almost 50% in several markers in several populations. The average gene diversities were very similar in all studied populations, and no single haplotype was shared among individuals within or between populations. Pairwise linkage disequilibrium analysis revealed no significant linkage disequilibrium between markers after correction for multiple analyses. The results confirmed a general genetic homogeneity in populations in the Mediterranean area as previously reported. The Portuguese sub-populations do not have significant structure concerning the 25 markers analyzed, thus not differing from what is known about the Mediterranean context.

Comparison Of Y Genetic Structure Of Romany Populations In The Three Regions Of Slovakia – Spiš, Gemer and Šariš

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Twelve Y-Chromosomal microsatellite loci included in the Powerplex® Y multiplex kit were analysed in the Romany populations from three Slovak regions: Spiš (n=85), Gemer (n=64) and Šariš (n=103). The haplotype diversities were ranging from 0.9485 to 0.9609. The regions studied share identical or closely related haplotypes in the paternally inherited system. The analysis of the pairwise F_{ST} values among Romany populations from different Slovak regions did not revealed any significant differences between them. Our results point out that Romanies belonged to endogamous and relatively small founder population group, which developed in a relative reproductive isolated group surrounded by the Slovak Caucasian population.

Genetic Stratification Of The Sami Inferred From Biparental and Uniparental Markers Among Four Subpopulations

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We estimated the genetic diversity and population affinities of four Sami subpopulations (n=275) using autosomal, Y-Chromosomal and mtDNA markers. These indigenous Sami subpopulations inhabit the northern Fennoscandia in Sweden, Finland (Inari and Skolt Sami district) and Kola peninsula of Russia. The uniqueness of the Sami genetic variation e.g. compared to their neighboring populations has been well assessed using classical protein polymorphisms, Y-Chromosome, mtDNA and several autosomal SNP and HLA markers. However, this is the first comprehensive study using three different neutral marker systems analyzed within several Sami subpopulations. Our data reveals significant autosomal differentiation of Sami subpopulations both from each other and from their neighbours (15 populations, n=1021), which is supported by the analysis of uniparental marker data (Y-chromosome data; 49 populations, n=7115 / mtDNA data; 68 populations, n=5400).

Moreover, the autosomal genetic structure analysis found two significant clusters among all analyzed North Eurasian populations, in which the Swedish Sami cluster separately from the cluster of all other Sami and non-Sami populations. This stratification of Sami subpopulations may have important value not only for forensic purposes but also for further association studies of common complex traits.

The Ghep Empop Collaboration On mtDNA Population Data A New Resource For Forensic Casework

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The importance of mtDNA analysis has grown in recent years and nowadays it has become in an essential technique in many forensic laboratories. Due to its maternal mode of inheritance, representative population data are needed to evaluate the evidence of matching mtDNA haplotypes in forensic casework. Laboratories are usually more involved in forensic mtDNA testing by establishing dataset(s) of their local population(s), which are important for the individual application but limit general use if they are not made publicly available. To combine these efforts and generate widely accessible databases the individual datasets need to be harmonized in a number of aspects including the systematic evaluation of the data with respect to transcriptional and systematic error, the phylogenetic alignment of the mtDNA haplotypes and the adaptation of the sequencing ranges. Such an effort has been successfully accomplished by the Italian Ge.F.I-Group where a total of 395 mtDNA haplotypes generated from 8 forensic laboratories were assembled and scrutinized with respect to the above mentioned conditions [Turchi et al. 2008].

Here we report the results of a collaborative study involving 12 laboratories from the Spanish and Portuguese Speaking Working Group of the International Society for Forensic Genetics (GHEP-ISFG) in which a total of 1868 haplotypes from Iberia (Basque Country, Andalucía, 2 general Spanish populations, 2 North and 1 Central Portugal populations), and Latin-America (2 populations from Sao Paulo, 1 general Brazilian population, 1 general Argentinean population and 1 population from Costa Rica) were collected and harmonized under defined EMPOP criteria [Parson and Dür 2007, Bandelt and Parson, 2008]. The observed error rate after data evaluation was 5,4%. The majority were clerical errors, demonstrating that the documentation process is still the main source of error, especially when performed manually [Parson et al., 2004].

This GHEP-EMPOP collaboration has significantly improved the quality of the individual mtDNA datasets and adds a valuable resource to the population data represented in the EMPOP database (www.empop.org).

Analysis Of Microsatellite Markers Of The Y-Chromosome Of The Remnants Of Quilombos In The State Of Alagoas

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It is estimated that four million Africans were brought to Brazil by the slave traffic between the half of sixteenth century to nineteenth century. During the period of slavery, runaway slaves formed villages called quilombos. In Brazil there are remnants of quilombo, which are rural communities whose histories and tradition identify with the old quilombos. A sample of a total of 211 men's from nine quilombo remnants communities in the state of Alagoas (Jacu, Cajá, Paus Pretos, Palmeira dos Negros, Carrasco, Silus, Povoado Cruz, Muquem and Poços do Lunga) was analyzed on Y-Chromosome by the STRs markers: DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS438 and DYS439. The statistical analysis was performed using the program Arlequin v. 3.11. The AMOVA test showed that 75.86% of the total variance observed occurred in the intrapopulation level, confirming the existence of heterogeneity among these communities ($F_{ST}=0.24145$, $P=0.00000\pm 0.00000$). Our data were compared with those of Brazilian populations of Miscigenated, Europeans, Amerindians and Africans individuals, and RST statistics used to calculate genetic distances between pairs of populations. Only a single community, Palmeira dos Negros, doesn't showed significant genetic difference in the relation to the Brazilian populations and one European (Netherlands). Moreover, the data found corroborate to others historical sources, which indicates that the quilombos remnants had not been established exclusively by afro descendants occurring a mixing between people from other origins during the formation of those communities.

Update On The mtDNA Phylogeny In Eastern and Western Slavs

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To resolve the phylogeny of certain mitochondrial DNA (mtDNA) haplogroups in Eastern Europe and estimate their evolutionary age, a total of 186 samples representing mitochondrial haplogroups U4, U5, HV*, and R1 were selected for complete mitochondrial genome sequencing from a collection of about 2400 control-region sequences sampled in Eastern (Russians, Belorussians, Ukrainians) and Western (Poles, Czechs and Slovaks) Slavs. On the basis of whole-genome resolution, we fully characterized a number of haplogroups (HV3, HV4, U4a1, U4a2, U4a3, U4b, U4c, U4d, and R1a) that were previously described only partially. Also, complete mtDNA data from eastern European populations allow us to refine the hg U5 phylogeny. Haplogroups HV3, HV4, and U4a1 could be traced back to the pre-Neolithic times (12.0-19.0 ky) in Eastern Europe. Meanwhile, some mtDNA subgroups observed in Slavs (such as U4a2a, U4a2*, HV3a, R1a1) are definitely younger, being dated between 6.4-8.2 ky. Some U5a2 subclusters, such as U5a2a and U5a2b1, which are present mainly in central and eastern European populations, also show similar evolutionary ages of 8.0 ky. This probably reflects distribution of the Chalcolithic and Early Bronze Age Corded Ware European cultures, as it has been suggested recently on the basis of phylogeographic distribution of Y-chromosome R1a1a1-M458 subcluster characterized by similar expansion time. We also present here a short update on Eastern Eurasian and African-specific components in a mtDNA pool of Slavic populations.

Individual Specific Extraction Of DNA From Male Mixtures – First Evaluation Studies

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In forensic work the interpretation of DNA profiles becomes complicated, when samples contain more than one contributor, because individual markers for identification are amplified simultaneously and result in mixed profiles. Therefore the International Society of Forensic Genetics (ISFG) and the SWGDAM Mixture Interpretation Committee released guidelines and recommendations for the interpretation of DNA mixtures by statistical calculation. Nevertheless identification of the individual contributor still remains difficult and requires expertise. To overcome this problem we present here the Haplotype-specific extraction (HSE) as a more straight forward method to analyse a DNA mixture. HSE has been developed to clarify ambiguous HLA alleles by separation of diploid samples into their haploid components and therefore facilitate HLA typing [1, 2]. In order to adapt HSE for separation of male DNA mixtures in forensic analysis we started to establish new protocols and strategies, where first results showed an improved enrichment of male DNA of one contributor. Here we will present the evaluation of a new optimized buffer composition by testing different concentrations of its components. The AmpF Φ STR ® Yfiler was used as downstream application for analysis.

1. Dapprich, J., et al., SNP-specific extraction of haplotype-resolved targeted genomic regions. *Nucleic Acids Res*, 2008. 36(15): p. e94.
2. Nagy, M., et al., Haplotype-specific extraction: a universal method to resolve ambiguous genotypes and detect new alleles - demonstrated on HLA-B. *Tissue Antigens*, 2007. 69(2): p. 176-80.

Never Miss A Profile – String Based Search Using EMPOP2 and Application To Phylogenetic Alignment

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The huge increase in mtDNA population data that is available from world-wide populations calls for standardization and computer-assisted storage and representation of the data. The mtDNA database EMPOP has been developed for collection and searchable presentation of mtDNA data. Here we present release 2 of EMPOP which establishes a major revision of the first release. EMPOP2 comes with 5000 additional haplotypes mainly from Asia filling the gap in this region. Integration of geographic origin, metapopulation affiliation of the data, and a tool for checking the difference encoding of mtDNA data relative to the rCRS provide further benefits of the second release. The main revision is a new search engine that works independently of the positional annotation of profiles and relieves users from the burden of correct alignment. Comparison of query and database profiles is performed by converting both profiles to FASTA strings. The search provides means for selective treatment of length variants and allows ignoring indels in homopolymeric tracts and in the AC repeat region. The results of string search are compared to the results of positional search, and we explain how the search results can be used for phylogenetic alignment.

Linkage and Linkage Disequilibrium Analysis Of X-STRs In Italian Informative Families

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X-chromosomal short tandem repeat (STR) loci provide an extremely useful tool in paternity testing, especially in deficiency cases with female offspring. Moreover, X-STR haplotype analysis allows to detect kinship between alleged relatives in large and incomplete pedigrees. Likelihood ratio calculations in relationship testing with X-STRs require a precise knowledge not only of allele and haplotype frequencies, but also of the genetic linkage and linkage disequilibrium (LD) status among markers. Twenty X-STRs were typed in 80 informative families of Italian descent, composed by mother and two or more sons, for a total of 93 meioses. The analyzed X-STR panel included six clusters of closely linked markers (each spanning < 3 cM): DXS10135-DXS10148-DXS8378 (Xp22); DXS7132-DXS10074-DXS10079 (Xq12); DXS6801-DXS6809-DXS6789 (Xq21); DXS7424-DXS101 (Xq22); DXS10103-HPRTB-DXS10101 (Xq26); DXS8377-DXS10134-DXS7423-DXS10146 (Xq28).

Recombination fractions between pairs of markers calculated by pedigree analysis were compared with those obtained converting physical distances by means of Haldane's mapping function. The observed differences confirm that the occurrence of recombination is not even along the X-Chromosome and that the conventional subdivision of X-STRs in four groups of completely unlinked markers cannot be regarded as true.

Evidence of significant LD was found between markers DXS6801 and DXS6809 ($p=0.018$). The effect on likelihood calculations of inferring haplotype frequencies from allele distributions rather than haplotype count in the relevant population was evaluated.

Mitochondrial DNA Mutations In Complete Genomes Of Colorectal Cancer Patients

Katarzyna Skonieczna^{1,2}, Boris Malyarchuk³, Arkadiusz Jawień⁴, Andrzej Marszałek^{5,6} and Tomasz Grzybowski¹ (Presented by Katarzyna Skonieczna)

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To investigate the variability of mitochondrial DNA in cancer we have examined the population of Polish patients diagnosed with colorectal cancer. 40 complete mitochondrial genomes of tumour and matched non-tumour tissues were determined for 20 patients. All haplotypes were assigned into the known mtDNA haplogroups. Among analyzed patients 1 was classified into haplogroup W, 2 into haplogroup V, 3 into haplogroup U, 7 into clade JT and 7 into haplogroup H. 18 heteroplasmic, somatic mutations were detected in 11 patients. 17 out of 18 somatic mutations were observed in cancer cells, whereas 1 was detected in non-tumour sample. Among the diagnosed somatic mutations, 17 were heteroplasmic substitutions and 1 was heteroplasmic insertion. Heteroplasmic substitutions in cancer cells seem to hit preferentially evolutionary stable (but not haplogroup-diagnostic) positions in the coding region. Indeed, 61.1% of all somatic mutations in a heteroplasmic state appeared in our cancer specimens in positions classified as invariable in complete genome databases. The results of this study suggest that somatic mutational spectra in colorectal cancer specimens is different from that observed in populations of normal cells.

Haploid DNA Workflow: Qiagen´s New Portfolio From Sample To STR, SNP and Sequencing

Starke, C (Presented by Christian Starke)

QIAGEN GmbH, Hilden, Germany

Since 1st April 2010, QIAGEN is able to offer its new portfolio for human identification that features the most comprehensive and versatile coverage of current STR standards, including the new extended European Standard Set (ESS), also fulfilling CODIS, DAD, and ISSOL requirements. The new portfolio complements the high-quality manual and automated DNA extraction and sample management solutions - thus offering a complete workflow solution in the field of Human Identity & Forensics.

The talk outlines the advantages for forensic case-work and paternity testing with focus on:

QIAGEN´s new Investigator Argus X-12 PCR Amplification Kit dedicated for most challenging reconstruction of family trees and complex paternity cases. Investigator Argus Y-12QS PCR Amplification Kit that complies with the "extended Minimal Haplotype (MH) standard" and additionally contains an internal PCR control (Quality Sensor) - providing useful information on efficiency of the PCR and on the presence of PCR inhibitors. QIAGEN´s REPLI-g Mitochondrial DNA Kit leads to highly uniform whole genome amplification from human mitochondria and overcomes the need for time-consuming isolation of mtDNA, thus giving informative results from samples with degraded nuclear DNA.

QIAGEN´s new RotorGeneQ, a rotor-based RealTime Cycler, enables true HRM technology and allows to screen for SNPs. Furthermore, QIAGEN´s PyroMark Q24 uses Pyrosequencing technology for real-time, sequence-based detection and is highly suited for the analysis of SNPs, insertion/deletions, and STRs.

The Fromelles War Graves Project: Identification Of Skeletal Remains From WW1

JA Thomson, (Presented by Jim Thomson)

LGC Forensics, Teddington, United Kingdom

In 2008, a number of mass graves dating from the First World War were identified at Fromelles in northern France. These were believed to contain the remains of Australian and British soldiers killed in the Battle of Fromelles in 1916 and buried behind German lines. The graves were excavated in May 2009 and 250 bodies were recovered. The Commonwealth War Graves Commission was asked to oversee the recovery, and if possible, identification of the remains and to create a new cemetery at Fromelles for their reburial.

LGC Forensics was appointed to carry out DNA analysis on the remains, and on reference samples from living potential relatives.

Y-STR analysis (17 loci) and mitochondrial sequencing (HVI and HVII) was carried out on all 250 remains. Teeth were the primary sample type, although bone and other tissues were also used. All the remains yielded usable DNA results.

By March 2010, over 800 reference samples from relatives of missing soldiers who had died at Fromelles had been analysed for Y-STR or mtDNA as appropriate. Where possible, relatives from both the maternal and paternal lineages were tested to enable comparison of both Y-Chr and mitochondrial data. Searches of reference against body results were completed to identify possible matches. Match probabilities for the Y-STR and mitochondrial haplotypes were estimated using the YHRD and EMPOP databases.

The DNA results were considered alongside anthropological evidence, artefacts found on the remains and historical records. On 17 March 2010, the CWGC project team announced that 75 of the bodies could be formally identified.

All 250 bodies have been reburied in the new cemetery. A commemorative event will be held in July 2010 to mark the reinterment and the naming of 75 of the graves.

The project will continue to receive samples from possible relatives until 2014 and it is hoped that further identification will be made.

Testing For Independence Of X-Chromosomal Markers, A Cautionary Note

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X-chromosomal markers have become more frequently used in forensic genetics during the recent years, particularly in relationship testing. Using multiple loci located on the same chromosome implies that there could be association between them. The discussion below will deal with linkage disequilibrium (LD) and the detection of it. In order to test for existence of LD between any pair of markers, the use of Fisher's exact test is preferably the method of choice. However, the power of an exact test is very limited unless the database is very large. This means that the test will not always detect a dependency even if there exist one. A large number of population studies on X-chromosomal markers have been published during the past years with the common feature of relatively small sample sizes. We argue that for X-STR markers larger sample sizes must be used in order to be able to make proper interpretations concerning the LD situation. Regarding independence testing, a non-significant p-value is not evidence that there is linkage equilibrium (LE). Unfortunately, it is not unusual to see over-interpreted conclusions regarding this in the literature, saying that since no significant p-value did come out from the test, it can be concluded that the markers are in LE. We performed a simulation-based test to show the difference in power as a function of the database size. We used eight markers on the X-chromosome that, for a large Swedish population sample (n=718), display LD. We showed that using sample sizes less than 200 will most often not detect the LD, assuming the degree of association found in the Swedish dataset. Furthermore, we use simulation to study the impact on likelihood ratio computations of using haplotype frequencies versus allele frequencies assuming LE.

Distribution Of Y-Chromosomal Haplotypes In The Sherpa Of The Khumbu Valley (Nepal).

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Due to their high degree of polymorphism Y-Chromosome STRs (Y-STRs) are an highly informative tool both in the forensic field and in population genetics studies. The Y-Chromosome is paternally inherited and since the non-recombining region of the Y (NRY) lacks of homologous recombination during meiosis, it is passed down from father to son virtually unchanged, except for new mutational events. Our preliminary work is part of a wider research aimed at defining the true kinship and possibly the ancient migration history of the Sherpa population dwelling nowadays in the Himalayan region of the Khumbu, in Nepal. Our first approach has been that of establishing the actual correspondence between each Ru, the traditional Sherpa patrilinear inherited clan, and Y-STR haplotypes, identified through the analysis of 17 short tandem repeats loci in a population sample of 25 male Khumbu Sherpas, using the AmpFISTR Yfiler™ PCR amplification kit. In society that use patrilinear family denominations, Sherpa society is organized in patrilinear clans, or Ru: inheritance of Ru and Y-Chromosome transmission thus follow a common paternal lineage. On the basis of the Y-haplotypes obtained a Ru genealogical tree was made.

An Enrichment Of Forensic Haploid Marker Databases: mtDNA and Y-Chromosome Polymorphisms In A Population Of Romanians

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High quality mtDNA sequence variations databases and Y-chromosome databases are essential to better estimate haplotype frequencies and their distribution across populations. This study provides additional information for the application of haploid markers in forensic cases.

90 healthy Romanian donors were subjected to sequence analysis of the mtDNA control region (CR). Three overlapping PCR fragments, spanning the entire control region were sequenced according to forensic standards. To assure high data quality double-stranded sequences were collected and independent evaluation of electropherograms was performed. A phylogenetic approach for a posteriori analysis of the mtDNA types was applied and sequences were aligned according to the mitochondrial phylogeny.

The Romanian samples were also typed for 17 Y-Chromosome STRs and additional Y-SNPs to describe the phylogenetic background of the male lineages. The selected Y-SNPs allowed the detection of basal branches of the phylogenetic tree encompassing all major clades from A through T. A high resolution level database was created, including both haplotype and haplogroup for each sample.

Two Different Cases Of Deletion On The Short Arm Of The Human Y-Chromosome.

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Human amelogenin gene is located on both X (AMELX, on Xp22.1-22.3) and the Y (AMELY, on Yp11.2) chromosomes. PCR amplification of AMELX and AMELY performed with the most common set of primers produces fragments of 106 and 112 bp, respectively. The different size of X and Y homologues yield amelogenin a widely used marker for sex typing in forensic applications and prenatal diagnosis.

Deletions in Yp11.2 region, involving AMELY have been found in different ethnic populations.

In this study we describe two AMELY null cases concerning two unrelated Italian males, one of which was already described in our previous study using fewer marker than present. PCR amplification using AmpFISTR®Identifiler® PCR amplification kit and AmpFISTR®Yfiler™ amplification kit (Applied Biosystems) showed the lack of AMELY and DYS458 markers. Moreover the presence of all other typed markers and SRY gene in both samples, induced to conclude that a deletion was occurred in a portion of the short arm of Y-Chromosome. 23 Y-specific Short Tagged Sequences (STSs) were then chosen to delineate the deletion length that was estimated as 3.35 Mb and 1.19 Mb in the two samples. These and previous results suggest that the sex-typing test based on amelogenin should not be consider infallible and we suggest the use of additional Y-Chromosome markers for unambiguous gender identification.

Y-Chromosomal STR Haplotypes In An Isolated Population From Germany: The Sorbs.

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Y-Chromosome markers are increasingly used to investigate human population histories, being considered to be sensitive systems for detecting the population movements. Furthermore, Y-Chromosomal microsatellites (STRs) have been established in forensic practice for several years. However, an in-depth evaluation of their population genetic properties requires a large number of haplotypes from different populations.

Sorbs, also known as Wends, are of Slavonic origin and settled in a region in Eastern Germany close to Poland and the Czech Republic, the Upper and Lower Lusatia.

At the end of the migration period (600 - 650 AD) West Slavonic tribes moved to the lands between Baltic Sea and Erzgebirge. The two tribes that settled in the Lusatia area (Lausitz), the Lusizi and Milceni, some time between the 7th and 9th century were referred to as Sorbs. For centuries the Sorbs were able to maintain and even develop their national culture mainly due to the high level of religious observance, cultivation of their tradition and strong families. Living protected in compact communities, they still represent a quite distinct population in Germany. Today, the Sorbic speaking, catholic minority still lives in rural Upper Lusatia (Oberlausitz) in Eastern Saxony and there are approximately 15000 full-blooded Sorbs.

We analysed the Y-chromosome with Y-Chromosomal STRs (AmpFISTR YFiler) in this isolated population sample from Germany. Males (n=124) selected for this study were classified by the birthplace of the paternal grandfather.

DNA was extracted from unrelated male blood samples according to standard procedures (Qiagen DNA Blood Midi Kit, Valencia, United States). Amplifications were performed using AmpFISTR YFiler (ABI). The PCR products were analyzed by capillary electrophoresis using the ABI 310® Genetic Analyzer (Applied Biosystems).

First haplotypes as well as data on the haplotype sharing of the Sorb population with other European populations will be presented.

The Portuguese Gypsy Community: Tracing Their Routes Through Maternal Genetic Lineages

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The principal goal of this work was to assess the pattern of diversity in maternal lineages from Portuguese Gypsies. The complete control region of mitochondrial DNA (mtDNA) was analyzed in 138 unrelated Gypsies. Gene diversity within the Gypsies was much lower (84.6%) than in the Portuguese non-Gypsies (97.6%). In the Gypsies the number of different mtDNA haplotypes only summed up 31 out of 138, which comparatively to the host population, 112 distinct haplotypes out of 118, revealed the reduced diversity in the Gypsies.

Accordingly, significant differences were found between the mtDNA profiles in the two samples. The haplogroups contributing more for such differences were U3, J1b3, J1c1 and M5, which accounted for 74.6% of lineages in the Gypsies compared to 4.8% in non-Gypsies. These haplogroups are also very common among other Gypsy groups. Furthermore, since M5 is very well represented in India, it testifies the common origin in the Indian sub-continent of all Roma.

A previous study addressing Y-chromosome diversity in the Portuguese Gypsies revealed higher levels of diversity than in mtDNA. Comparison of mtDNA and Y-chromosome pools, reveals a sex asymmetric migration flow between Gypsy and non-Gypsy populations. Seemingly, the influx of non-Gypsy maternal lineages into the Gypsy population was weaker than the influx of paternal ones.

Y-STR DNA Analysis Of The 7th Century Human Remains From The Burial Site In Ergolding, Germany.

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The main goals of this study were to develop novel DNA extraction and typing procedure that would enable to perform DNA identification of 7th century human remains, to set the familiar relationship between the individuals, to estimate the Y-chromosome haplogroup and to compare the Y-chromosome haplotype with the contemporary populations. Early-medieval burial-place in Ergolding (Bavaria, Germany) was archaeologically examined in years 1997-2002 by the Bavarian State Department of Monuments and Sights, Germany and they recovered more than 440 graves. The human remains of six early adult males examined using DNA analysis in this study were found in the central grave number 244. We clearly demonstrated that the modern methods and procedures of forensic DNA analysis can be successfully used also in the area of archaeogenetics to determine not only the familiar relationship but also to predict the place of the geographic origin of the unknown skeletal remains. Application of forensic genetics into the archaeology can bring new information and help in interpreting of the findings. The number of successfully typed autosomal and Y-STR loci from ancient specimen is one of the most complex announced so far for aged samples.

Benefits Of Using Unbiased, Complete mtDNA Genomes For Uncovering Population Histories

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The number of complete mtDNA sequences deposited in Genbank has grown rapidly in the past few years. However, the majority of these were chosen based on haplogroups of interest or by only using samples with unique, distinctive HVR1 sequences. Using these criteria for selecting samples to sequence can confound efforts to uncover demographic histories. An assumption of the latter selection process is that if HVR1 sequences are identical between individuals then their complete mtDNA genomes should also be identical. If this assumption is incorrect, an ascertainment bias could be introduced.

To investigate this problem of bias, we used a novel sequencing method that combines the preparation of indexed libraries from genomic DNA with hybridization enrichment of mtDNA for sequencing on the Illumina Genome Analyzer IIx. We sequenced complete mtDNA genomes from over 400 samples from five ethnolinguistic groups in Siberia without any sub-selection. By comparing these complete mtDNA sequences to previously published HVR1 sequences, we were able to verify the accuracy of this sequencing method.

By examining basic summary statistics and using a Bayesian coalescent approach to estimate effective population size changes through time, we show that selecting samples for complete mtDNA sequencing based on HVR1 identity does introduce a bias. The differences in summary statistics between using all sequences in our data set and biased subsamples based on HVR1 identity are significant and the Bayesian skyline plots show markedly different demographic histories. Additionally, analyses based on complete mtDNA genomes show pronounced differences from those based solely on HVR1. Therefore, we recommend sequencing all available samples in a collection now that rapid, cost-effective methods are available.

Y-STRs and The History Of Western Slavs

Marcin Woźniak¹, Boris Malyarchuk², Miroslava Derenko² and Tomasz Grzybowski¹ (Presented by Marcin Woźniak)

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Contemporary Western Slavs are defined by linguistic criterion as people using one of four Western Slavic languages: Czech, Kashubian, Polish or Slovakian. Despite their common cultural heritage and relatively close geographical distances between their lands, Western Slavs exhibit complex genetic relationships as far as Y-Chromosome polymorphism is regarded. Significant differences between Y-Chromosome haplotypes of Czechs and Slovaks on one side and Poles (including Kashubians) on the other side were reported recently by us as well as other teams. In the same time all Western Slavic populations share some haplotypes that might be regarded as originating from the area where Western branch of Slavdom was formed. We have collected and analyzed 752 Y-STR haplotypes of Western Slavic origin comprising 12 loci amplified by PowerPlex Y. Using haplotype network analysis combined with haplotype age calculations, haplogroup analysis and extensive YHRD searches we were able to analyse the phylogeny of at least two large Y-STR haplotype families that seem to be significantly frequent in the territories dwelled by Western Slavs. While the first of those haplotype families seems to be common in all Western Slavs and correlated with M458 polymorphism recently described, the second family seems to originate from the territory of Northern Poland and reaches its highest frequencies in Kashubian population. We conclude that our observations regarding Y-STR haplotypes of Western Slavs could facilitate resolving complex genetic history of Central Europe. Additionally, practical application of our data is possible as many of the observed haplotypes seem to represent useful ancestry markers (AIMs).

Analysis Of Y-SNPs In The HapMap3 Project

Yali Xue, Qasim Ayub, Sarah Hunt, Chris Tyler Smith (Presented by Yali Xue)

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The International HapMap3 project (<http://hapmap.ncbi.nlm.nih.gov/>) has generated genomewide SNP genotype data on 1,115 individuals from 10 worldwide populations (Extended HapMap Panel) using two platforms: the Illumina Human1M and the Affymetrix SNP 6.0 chips. Data from the two platforms have been merged for the public release set. In this dataset, genotypes of 1,385 Y-SNPs were included from the 567 males in the panel. Of these, 119 Y-SNPs overlap with the updated YCC set (Karafet ref) and are distributed across all the clades, although the SNPs are biased towards the European and African lineages. Using these SNPs, we can assign most of the samples to a YCC haplogroup, and see the expected worldwide patterns of haplogroup distribution among the populations. Haplogroups A3 and B2 were found in one of the African populations while E lineages are dominant in most of other African populations, such as YRI, LWK and MKK. Haplogroup R1 lineages are more common in European populations such as CEU and TSI while the O lineages form the most frequent haplogroups in Asian populations, such as CHB, JPD and CHD. Although over 1,000 of the other Y-SNPs were either un-callable or XYSNPs, we also found 106 new variable and useful Y-SNPs in this dataset. These are distributed across most of the lineages, and several of them subdivide the existing lineages. This analysis illustrates the usefulness of freely-available public datasets and provides a taste of what may be expected from ongoing projects, like the 1000 Genomes Project (<http://www.1000genomes.org/page.php>).

Comparative Analysis On 17 Y-STRs Between 5 Regional Mongolian Populations and A Japanese Population

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Seventeen Y-STR loci were haplotyped from 95, 100, 97, 84, and 117 unrelated males collected from Ulaangom (western), Dalanzadgad (southern), Ulaanbaatar (middle), Undurkhaan (eastern), and Choibalsan (northeastern) in Mongolia, respectively, using the AmpFISTR Yfiler kit. A total of 350 haplotypes were observed in the five regional Mongolian populations, and only two of which were shared among all the populations. The numbers of haplotypes were 76, 74, 87, 68, 96, and the haplotype diversities were 0.9915, 0.9828, 0.9966, 0.9891, and 0.9919 in Ulaangom, Dalanzadgad, Ulaanbaatar, Undurkhaan, and Choibalsan, respectively. The numbers of null alleles were 35 at DYS 448, 2 at DYS439, and each one at DYS19, DYS385 and DYS456 in those five populations. However, when the samples with the null alleles were amplified with each the primer set for each loci, only at DYS19, DYS385 and DYS439, each allele were genotyped. The duplicated alleles were observed at DYS19, DYS458 and DYS448, especially alleles 16-17 at DYS19 were observed in 53 individuals. The pairwise comparisons among the five regional populations using the pairwise genetic distances (RST) revealed that the Ulaangom population is genetically slightly different from the four other regional Mongolian populations. Additionally, we haplotyped 138 Japanese males for the 17 Y-STRs, and 132 haplotypes were observed with 0.9989 of haplotype diversity. Thus, Mongolian males seem to be less diverse than Japanese ones. A phylogenetic tree with the all haplotypes observed in the Mongolian and Japanese populations was constructed using PAUP software. Accordingly, two clusters with almost only Japanese haplotypes were formed, and the identical haplotypes between 5 regional Mongolian populations and a Japanese populations.

Now, those data were analyzed population-genetically with the data from the other ethnic and/or regional populations near/in Mongolia and Japan. Moreover, the haplogroup data in the Ulaanbaatar population will be reported.

Comparative Y-STR and Y-SNP Analysis In Two Vlachian Romani Population Groups From Eastern Hungary

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The single nucleotide polymorphisms (SNP) and short tandem repeat (STR) markers from NRY (Non Recombination Y) on Y-Chromosome are proper tools for studying male specific lineage evolution.

The aim of this study is to contribute to the knowledge of Gypsy genetic history by examining 12 Y-STR and 51 Y-SNP loci in two different Roma groups, as well as Hungarians.

Two lineages of the Romani paternal gene pool were identified: Indian ancestral and recent European. The reduced diversity and expansion signals of H1a-M82 paternal lineages imply descent from closely related paternal ancestors, who could have settled in the Indian subcontinent.

The recent Romani paternal gene pool is dominated by a specific subset of E1b1b1a-M78, J2*-M172, G2a-P15 and J2a2-M67 lineages from the Middle East and Anatolia. Additional admixture, evident in the low and moderate frequencies of typical European haplogroups I1-M253, I2a-P37.2, I2b-M223, R1b1-P25 and R1a1-M198, took place primarily during early settlement in the Balkans and subsequent influx of them in the Carpathian Basin.

Phylogeny Specific Filters For Quasi Median Network Analysis: Sharpening The Blade For mtDNA Error Detection

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The process of mitochondrial DNA data generation is challenging and, according to review summaries, still prone to error. The application of quasi-median networks provides an effective tool to check the quality of the produced haplotype data. Filtering of highly recurrent mutations prior to network analysis is required to detect data idiosyncrasies and potential artefacts as well as occasionally occurring homoplasy. While fine-grained filters pinpoint serious problems, problem-specific filters are needed to effectively highlight potential errors in data that are inconspicuous at first. The phylogenetic background of a dataset obviously determines the mutations to be filtered. For example, the transition at position 73G with respect to rCRS is a stable "mutation" in superhaplogroups L and M, whereas it shows considerable homoplasy within hg R0. Therefore, we suggest application of phylogeny-specific filters to increase the efficiency of quasi-median networks.

In this study we demonstrate the performance of a "west Eurasian filter" (hg N) for the examination of a variety of datasets including small and large sets of high quality and flawed data as well as the effect of distantly related datasets. The analyses are based on a west Eurasian etalon dataset that was carefully compiled for network purposes.

Rapid Screening For Mitochondrial and Y-Chromosome Haplogroups In Routine DNA Analysis.

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The population of Argentina, as that of other South American countries, is the result of an intercontinental admixture. Recent results confirmed this observation. In order to refine the admixture levels a rapid screening approach has been developed for discerning the major haplogroup to which a sample might belong for either the maternal or paternal lineages. The analysis is part of the DNA typing required for paternity testing or criminal casework and is only conducted proving the participant sign an informed written consent statement in which they accept to participate in this research. After extraction, DNA is quantitated by means of Real Time PCR (Rt-PCR) using commercial kits (Plexor HY-Promega or Quantifiler-ABI, United States). Quantitated samples are then submitted to two multiplex Rt-PCRs in order to determine the major Native American mtDNA and Y-chromosome haplogroups by means of High Resolution Melting (HRM) analysis. In the first, a cocktail of primers flanking nucleotide substitutions that define C mtDNA haplogroup and A2, B2, and D1 sub-haplogroups are used. The second include primers flanking Y-SNPs M242, M3, M269 and U179 that allow discriminating Q and non-Q Hgs. In all cases amplicons are less than 120 nucleotides long in order to increase the signal detection. This new working-flow strategy facilitates and speed-up the screening process that may lead to detailed sequencing analysis of particular samples, or for further molecular epidemiological investigations in which continental origin influences might be of relevance.

Y-Chromosomal Variation In Sub Saharan Africa and The Bantu Expansion: Insights From Biallelic and Microsatellite Markers

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One of the most significant and well-known human migrations in Sub-Saharan Africa is the Bantu expansion, which has been associated – although not unanimously – with agricultural innovations and at a later stage with iron technologies. Bantu languages belong to the Niger-Congo linguistic phylum, and are thought to have expanded from the area around Cameroon/Nigeria beginning about 5,000 years ago; Bantu-speaking groups now encompass most of sub-Saharan Africa. Previous studies of African Y-Chromosomal variation associated haplogroup E-M2 (or E1b1a) with the Bantu expansion. However, the frequency of EM-2 and associated diversity levels do not differ appreciably between Bantu and other Niger-Congo populations. Here, we further investigate the Y-Chromosomal footprint of the Bantu expansion by analysing 35 biallelic markers of which three delineate sub-lineages of E-M2, and associated diversity for 12 microsatellites, in 1234 males from 15 ethno-linguistic groups in Sub-Saharan Africa.

The sub-lineage typing identifies significant haplogroup frequency differences among Niger-Congo Bantu and non-Bantu groups, where the former showed high frequencies of E-U174 and E-U175. However, there are no clear geographic patterns associated with the Y-STR haplotype diversity or variance for either E-U174 or E-U175. This suggests that there were no strong bottlenecks associated with the expansion of these haplogroups throughout Sub-Saharan Africa. Correspondence Analysis and Analysis of Molecular Variance indicated that groups clustered mainly according to linguistic criteria, and to a lesser extent with geographic location. Nevertheless, we found that an isolation by distance model could explain the levels of haplotype sharing among groups ($r=0.30$, p -value: 0.002).

These results highlight the importance of linguistic boundaries in shaping the paternal demographic history of Sub-Saharan Africa.

Contrasting Male Population Substructure Among African Agriculturalists and Hunter Gatherers

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Socio-economic and cultural factors might play an important role in explaining differences in human population genetic structure. To explain patterns in population substructure, studies so far have analyzed genetic differences among widely dispersed populations, and did not consider differences within the same tribe and/or village. We conducted two detailed tribal specific micro-geographic studies to investigate the influence of socio-economic and anthropological factors on population genetic structure. We analyzed the DNA of two groups: (i) males ($n=500$) from the Bimoba tribe living in the Upper East Region of Ghana, and (ii) males ($n=500$) from the Mbenzele or Biaka, pygmies that live in tropical rainforests in the western Congo basin. Bimoba samples were collected as part of ongoing research of colleagues from the LUMC. Biaka samples were collected by Luca Cavalli-Sforza in the 1960-ies. Isolated white cells of these samples have been kept ever since in Leiden.

We will discuss the sharp contrast in patterns of Y-chromosome diversity between these two different groups of isolated African populations. For this we use the genetic information obtained using 15 Y-Chromosomal Short Tandem Repeats (STRs) and an extensive series of biallelic Single Nucleotide Polymorphisms defining all relevant African Y-chromosome haplogroups (A, B, E, and their sublineages).

PhyloTree An Updated Human mtDNA Tree

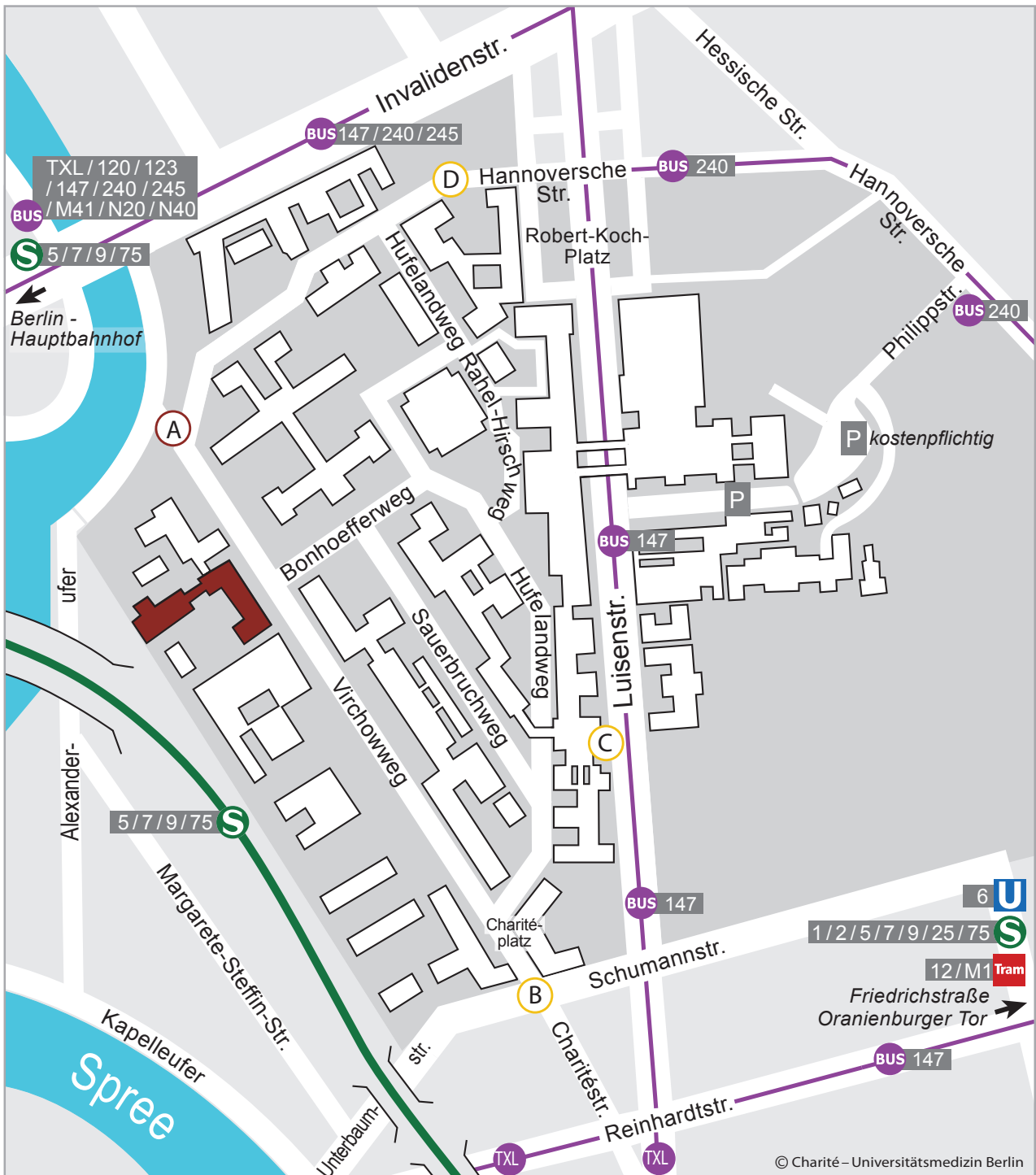
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Mitochondrial DNA is widely used in human population studies as well as in forensics, due to its convenient properties such as matrilineal inheritance, lack of recombination, small size, and high copy number. The availability of a large and growing number of entire mtDNA sequences (soon reaching 7000) allows the construction of a detailed phylogeny providing insights into the evolutionary history of the human species (Torroni et al. 2006). The PhyloTree project (van Oven and Kayser 2009) was started in October 2008 and aims at combining all available mtDNA phylogenetic knowledge into one public resource (<http://www.phylotree.org>). The tree contains both coding and control region mutations and includes haplogroup nomenclature. Since its launch we have updated the tree several times to incorporate newly released data. In this process we proposed solutions for several nomenclature conflicts (e.g. van Oven 2010) and added a considerable amount of novel (sub)haplogroups. From a forensic perspective the tree can be used to infer candidate haplogroups based on (partial) control region sequence data. This then facilitates the selection of coding-region SNPs, which can additionally be typed to increase discrimination power (Coble et al. 2004). Another application is quality control as phylogenetic analysis can help to identify sequencing errors (Yao et al. 2004). We encourage any effort to produce high-quality complete mtDNA sequences as this will enable further improvement of the mtDNA tree. Finally, we would like to thank the many users of PhyloTree who have provided valuable feedback.

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A Entrance Central Station (Airport Shuttle, Train)

B Entrance Charitéplatz

C Entrance Poliklinik

D Entrance Hannoversche Straße 11

(delivery entrance, cars & trucks, open Mo - Fr from 6am to 6pm)



Venue