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Online reference database of European Y-chromosomal short tandem repeat (STR) haplotypes

L. Roewer[a,*], M. Krawczak[b], S. Willuweit[a], M. Nagy[a], C. Alves[c], A. Amorim[c], K. Anslinger[d], C. Augustin[e], A. Betz[f], E. Bosch[g], A. Cagliá[h], A. Carracedo[i], D. Corach[j], A.-F. Dekairelle[k], T. Dobosz[l], B.M. Dupuy[m], S. Füredi[n], C. Gehrig[o], L. Gusmaõ[c], J. Henke[p], L. Henke[p], M. Hidding[q], C. Hohoff[r], B. Hoste[k], M.A. Jobling[g], H.J. Kärgel[s], P. de Knijff[t], R. Lessig[u], E. Liebeherr[v], M. Lorente[w], B. Martínez-Jarreta[x], P. Nievas[x], M. Nowak[y], W. Parson[z], V.L. Pascali[h], G. Penacino[j], R. Ploski[y], B. Rolf[d], A. Sala[j], U. Schmidt, C. Schmitt[q], P.M. Schneider, R. Szibor, J. Teifel-Greding, M. Kayser

[a]*Institut für Rechtsmedizin, Humboldt Universität, Hannoversche Straße 6, D-10115 Berlin, Germany*
[b]*Institute of Medical Genetics, University of Wales College of Medicine, Cardiff C14 4XN, UK*
[c]*IPATIMUP, Rua Dr. Roberto Frias, 4200 Porto, Portugal*
[d]*Institut für Rechtsmedizin München, Frauenlobstraße 7a, D-80337 München, Germany*
[e]*Institut für Rechtsmedizin, Butenfeld 34, D-22529 Hamburg, Germany*
[f]*Landeskriminalamt Baden-Württemberg, Taubenheimstraße 85, D-70372 Stuttgart, Germany*
[g]*Department of Genetics, University of Leicester, Adrian Building, University Road, Leicester LE1 7RH, UK*
[h]*Instituto di Medicina Legale, Universitá Cattolica del S. Cuore, Largo Franceso Vito 1, I-00168 Rome, Italy*
[i]*Institute of Legal Medicine, Faculty of Medicine, 15705 Santiago de Compostela, Spain*
[j]*Servicio de Huellas Digitales Geneticas, Facultad de Farmacia y Bioquimica UBA, Junin 956, 1113 Buenos Aires, Argentina*
[k]*Institut National de Criminalistique et de Criminologie, Ch. De Vilvorde 98-100, B-1120 Brussels, Belgium*
[l]*Institute of Forensic Medicine, Medical University Wroclaw, Mikulicza-Radeckiego 4, 50-368 Wroclaw, Poland*
[m]*Institute of Forensic Medicine, University of Oslo, Rikshospitalet, 0027 Oslo, Norway*
[n]*Institute for Forensic Sciences, P.O. Box 314/4, H-1903 Budapest, Hungary*
[o]*Institut de Médecine Légale Genève et Lausanne, 9 av. de Champel, 1211 Genève 4, Switzerland*
[p]*Institut für Blutgruppenforschung, Hohenzollernring 57, D-50672 Köln, Germany*
[q]*Institut für Rechtsmedizin der Universität zu Köln, Melatengürtel 60-62, D-50823 Köln, Germany*
[r]*Institut für Rechtsmedizin der Universität Münster, von-Esmarch-Straße 62, D-48149 Münster, Germany*
[s]*Landeskriminalamt Sachsen-Anhalt, Lübecker Str. 53-63, D-39124 Magdeburg, Germany*
[t]*MGC Department of Human and Clinical Genetics, Forensic Laboratory for DNA Research, Leiden University Medical Center, P.O. Box 9503, 2300 RA Leiden, The Netherlands*
[u]*Institut für Rechtsmedizin, Universität Leipzig, D-4103 Leipzig, Johannisallee 28, Germany*
[v]*Landeskriminalamt Berlin, Tempelhofer Damm 12, D-12101 Berlin, Germany*
[w]*Department of Legal Medicine, Facultad de Medicina, Universidad de Granada, Avda. de Madrid 12, 18071 Granada, Spain*
[x]*Department of Forensic Medicine, University of Zaragoza, C/Domingo Miral s/n, 50.009 Zaragoza, Spain*
[y]*Department of Forensic Medicine, Medical Academy Warsaw, ul. Oczki 1, 02-007 Warszawa, Poland*
[z]*Institut für Gerichtliche Medizin, Universität Innsbruck, Müllerstraße 44, A-6020 Innsbruck, Austria*
[A]*Institut für Rechtsmedizin, Klinikum der Albert-Ludwigs-Universität Freiburg, Albertstr. 9, D-79104 Freiburg, Germany*
[B]*Institut für Rechtsmedizin, Universität Mainz, Am Pulverturm 3, D-55131 Mainz, Germany*
[C]*Institut für Rechtsmedizin, Universität Magdeburg, Leipziger Str. 44, D-39112 Magdeburg, Germany*

PII: S0379-0738(00)00478-3

[D]Bayerisches Landeskriminalamt, Maillingerstraße 15, D-80636 München, Germany
[E]Max-Planck-Institut für Evolutionäre Anthropologie, Abt. Evolutionäre Genetik, Inselstraße 22, D-4103 Leipzig, Germany

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Abstract

The reference database of highly informative Y-chromosomal short tandem repeat (STR) haplotypes (YHRD), available online at http://ystr.charite.de, represents the largest collection of male-specific genetic profiles currently available for European populations. By September 2000, YHRD contained 4688 9-locus (so-called ''minimal'') haplotypes, 40% of which have been extended further to include two additional loci. Establishment of YHRD has been facilitated by the joint efforts of 31 forensic and anthropological institutions. All contributing laboratories have agreed to standardize their Y-STR haplotyping protocols and to participate in a quality assurance exercise prior to the inclusion of any data. In view of its collaborative character, and in order to put YHRD to its intended use, viz. the support of forensic caseworkers in their routine decision-making process, the database has been made publicly available via the Internet in February 2000. Online searches for complete or partial Y-STR haplotypes from evidentiary or non-probative material can be performed on a non-commercial basis, and yield observed haplotype counts as well as extrapolated population frequency estimates. In addition, the YHRD website provides information about the quality control test, genotyping protocols, haplotype formats and informativity, population genetic analysis, literature references, and a list of contact addresses of the contributing laboratories.

Keywords: Database; Y chromosome; STR; Haplotype; Forensics

1. Introduction

Although uniparentally inherited genetic markers from Y-chromosomal and mitochondrial DNA are particularly useful in advanced forensic testing and kinship analysis, the problem arises as to how to evaluate matches of haplotype profiles (i.e. combinations of completely linked markers) rather than of genotype profiles comprising independently transmitted markers. Whilst the population frequency of the latter can be extrapolated from small locus-wise databases by multiplication, provided that the inter-marker distances involved are sufficiently large, frequencies of non-recombining haplotypes can only be estimated on the basis of complete genomes. The number and relative abundance of different haplotypes in a reference population is thus not only a function of the locus-specific level of polymorphism, but also depends critically upon the inter-individual patrilinear (Y chromosome) or matrilinear (mt-DNA) relationships.

In view of the huge diversity of Y-chromosomal short tandem repeat (STR) loci, it is immediately apparent that considerable efforts have been necessary to obtain a reliable database for the practical use of these markers. For forensic purposes, it would be particularly essential (i) to identify polymorphisms capable of discriminating between the majority of unrelated lineages in a given population, (ii) to establish a database representative of the geographical and ethnical structure of the populations of interest, and (iii) to aim at a database size that would allow accurate frequency estimation for rare haplotypes. A long-term international project, initiated in 1994 to facilitate the forensic exploitation of human Y-chromosomal STR markers, has resulted in a database of European haplotype data (YHRD) that should be equally useful in forensic analysis and anthropological or archaeological research. The database is available to all interested users who are also invited to become collaborating contributors, thereby ensuring that the ongoing expansion of YHRD will benefit the forensic and scientific community.

2. Database organization

2.1. Haplotype format

A previously defined core set of Y-STR markers (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385), compatible with the ISFG guidelines for forensic STR analysis [1,2] has proven to represent a means of safe, sensitive and efficient genotyping of the human Y chromosome and provides a high discrimination potential [3–5]. Whilst seven of these markers are unilocal, marker DYS385 detects variation at two loci simultaneously, thereby bringing the total number of loci tested to nine. Profiling with the core marker set has since been established by many laboratories performing forensic diagnosis and kinship testing as well as anthropological and archaeological research. The core set was therefore chosen as a backbone for the Y-STR database (Fig. 1a, Table 1), and the respective profiles were termed ''minimal haplotypes'' in order to emphasize the authors' view that they represent the minimum requirement for sufficiently informative haplotyping in forensic casework (current haplotype diversity for the core set $h = 0.9972$). Nevertheless, the database structure allows

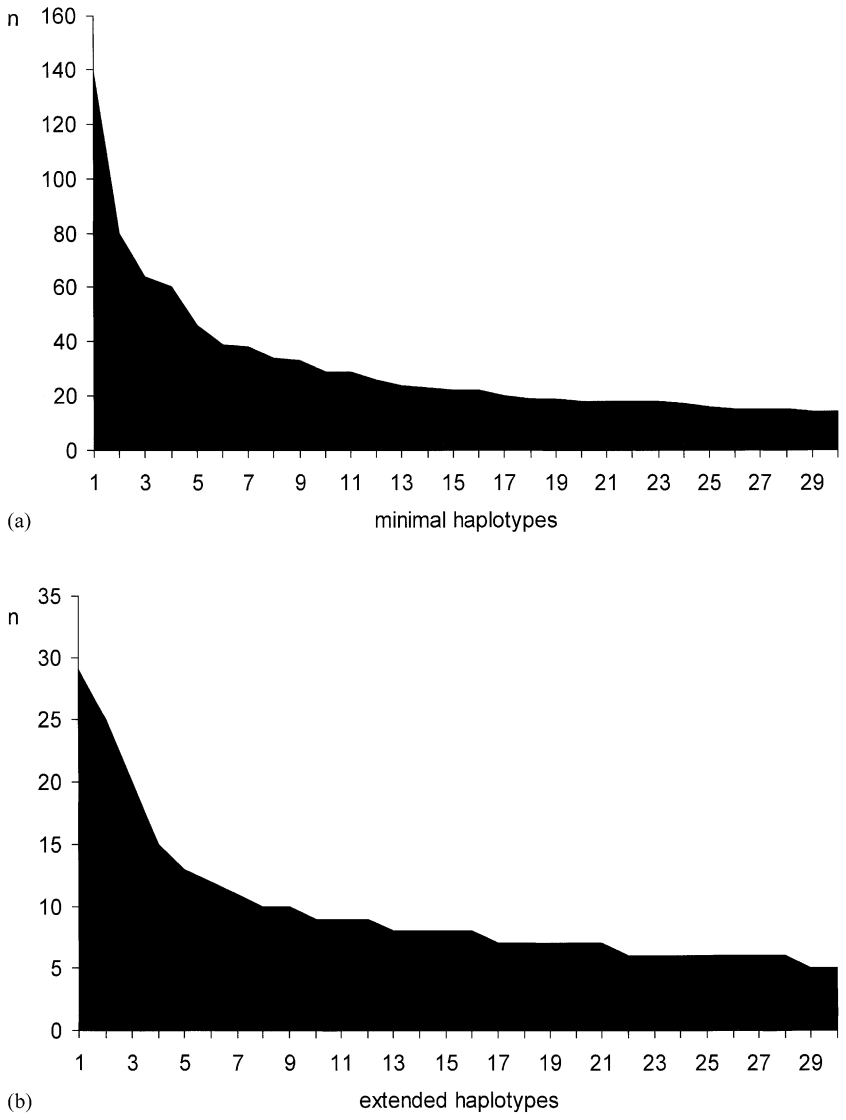

Fig. 1. (a) Observed frequency distribution for the 30 most frequent minimal haplotypes (by September 2000; $n = 4688$). (b) Observed frequency distribution for the 30 most frequent extended haplotypes (by September 2000; $n = 1957$). For a list of minimal and extended haplotypes, see Tables 1, 2).

for a possible extension of all logged haplotypes by additional Y-STR or Y-SNP loci. So far, genotypes for the highly informative Y-STR marker YCAII, which also detects variation at two loci at a time, have been included in 40% of cases (''extended haplotype'', $h = 0.9983$, Fig. 1b, Table 2). Some 52% of minimal haplotypes have been found to be different among the European samples logged, a proportion that increases to 71% upon the inclusion of YCAII (Table 3).

2.2. Quality assurance and data submission

In order to ensure accurate Y-STR genotyping and the use of an approved standard nomenclature, a quality assurance test has been introduced that represents an obligate requirement for all participating laboratories prior to any data submission. This quality test involves blind haplotyping of five high molecular weight DNA samples for the 9-locus core set (plus YCAII for laboratories submitting extended 11-locus haplotypes). The test is evaluated and certified by the Institute of Legal Medicine, Humboldt-University, Berlin.

Once a contributing laboratory has passed the quality control test, haplotype profiles can be submitted electronically to the Institute of Legal Medicine, Humboldt-University, Berlin, for immediate storage in the online database. Data files are transmitted in standardized format so as to

Table 1

	Minimal haplotypes (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385)	*n*
1	14, 13, 29, 24, 11, 13, 13, 11–14	139
2	14, 13, 29, 23, 11, 13, 13, 11–14	80
3	14, 13, 29, 24, 10, 13, 13, 11–14	64
4	14, 12, 28, 22, 10, 11, 13, 13–14	60
5	16, 13, 29, 25, 10, 11, 13, 11–14	46
6	16, 13, 30, 25, 11, 11, 13, 11–14	39
7	17, 13, 30, 25, 10, 11, 13, 10–14	38
8	14, 13, 29, 23, 10, 13, 13, 11–14	34
9	14, 12, 28, 23, 10, 11, 13, 13–14	33
10	14, 13, 29, 24, 11, 13, 13, 11–15	29
11	16, 13, 30, 25, 10, 11, 13, 11–14	29
12	14, 12, 28, 23, 10, 11, 13, 14–14	26
13	14, 14, 30, 24, 11, 13, 13, 11–14	24
14	15, 13, 29, 25, 10, 11, 13, 11–14	23
15	14, 13, 30, 24, 11, 13, 13, 11–14	22
16	14, 12, 28, 22, 10, 11, 13, 14–14	22
17	14, 13, 29, 25, 11, 13, 13, 11–14	20
18	14, 13, 29, 24, 10, 13, 13, 11–15	19
19	15, 13, 30, 25, 11, 11, 13, 11–14	19
20	16, 13, 31, 24, 11, 11, 13, 14–15	18
21	14, 13, 29, 24, 11, 13, 13, 12–14	18
22	14, 13, 28, 23, 11, 13, 13, 11–14	18
23	13, 13, 30, 24, 10, 11, 13, 16–18	18
24	15, 13, 29, 24, 11, 13, 13, 11–14	17
25	14, 13, 29, 24, 11, 13, 13, 11–13	16
26	16, 13, 30, 25, 10, 11, 13, 10–14	15
27	16, 13, 29, 24, 10, 11, 13, 11–14	15
28	14, 13, 29, 23, 11, 13, 13, 11–13	15
29	15, 13, 30, 25, 10, 11, 13, 11–14	14
30	14, 12, 28, 22, 10, 11, 13, 13–15	14

Table 2

	Extended haplotypes (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385, YCAII)	*n*
1	14, 13, 29, 24, 11, 13, 13, 11–14, 3–7	29
2	16, 13, 29, 25, 10, 11, 13, 11–14, 3–7	25
3	14, 13, 29, 23, 11, 13, 13, 11–14, 3–7	20
4	14, 12, 28, 22, 10, 11, 13, 13–14, 3–5	15
5	16, 13, 30, 25, 10, 11, 13, 11–14, 3–7	13
6	16, 13, 30, 25, 11, 11, 13, 11–14, 3–7	12
7	14, 13, 29, 24, 10, 13, 13, 11–14, 3–7	11
8	15, 13, 29, 25, 10, 11, 13, 11–14, 3–7	10
9	14, 13, 29, 24, 11, 13, 13, 11–15, 3–7	10
10	14, 13, 29, 23, 10, 13, 13, 11–14, 3–7	9
11	14, 14, 30, 24, 11, 13, 13, 11–14, 3–7	9
12	17, 13, 30, 25, 10, 11, 13, 10–14, 3–7	9
13	14, 12, 28, 23, 10, 11, 13, 13–14, 3–5	8
14	16, 13, 29, 24, 10, 11, 13, 11–14, 3–7	8
15	14, 13, 29, 25, 11, 13, 13, 11–14, 3–7	8
16	13, 13, 30, 24, 10, 11, 13, 16–18, 3–5	8
17	16, 12, 30, 24, 11, 11, 13, 14–15, 4–4	7
18	14, 13, 29, 24, 11, 13, 13, 11–14, 3–6	7
19	14, 12, 28, 22, 10, 11, 13, 14–14, 3–5	7
20	14, 13, 29, 24, 11, 13, 12, 12–14, 3–7	7
21	16, 13, 29, 25, 11, 11, 13, 11–14, 3–7	7
22	15, 14, 30, 23, 10, 14, 14, 11–13, 2–4	6
23	15, 13, 29, 24, 11, 13, 13, 11–14, 3–7	6
24	15, 13, 30, 25, 11, 11, 13, 11–15, 3–7	6
25	14, 13, 29, 25, 10, 11, 12, 16–17, 3–6	6
26	14, 13, 29, 24, 10, 13, 13, 11–15, 3–7	6
27	15, 13, 29, 23, 11, 14, 14, 11–14, 2–4	6
28	16, 13, 30, 25, 11, 11, 13, 11–15, 3–7	6
29	14, 12, 28, 23, 10, 11, 13, 14–14, 3–5	5
30	14, 13, 29, 23, 11, 13, 13, 11–15, 3–7	5

avoid extra work (and the introduction of errors) prior to storage. Observed haplotypes are logged in YHRD as single-line entries. Each record comprises the respective allele designations, the population affiliation, and a unique proband identifier that is not available via Internet but is used internally to allow the identification of haplotypes for expansion.

2.3. Website features

In addition to the haplotype search facilities, the YRHD website includes several documentation and help menus plus a section containing PCR multiplex protocols and primer sequences for Y-STR analysis. An introductory "**About**" page provides information on the current state of the database (i.e. the number of minimal and extended haplotypes logged) and an introduction to forensic Y-STR haplotype analysis. The main menu contains links to all important parts of the program, featuring a list of contributing laboratories (**forensic Y-User Group**), a description of polymorphic Y-STR sequences, a list of recommended primers and PCR multiplex protocols (**Primers and Protocols**), the "**Haplotype formats**" used for databasing, and results from analysis of molecular variance (AMOVA) of the population samples (**Population Analysis**). Laboratories willing to participate in the database project can receive further information under program items "**Quality control analysis**" and "**Haplotype contribution**". A "**References and**

Table 3
Forensic efficiency values for European Y-STR haplotypes

Haplotype	*n*	*h*	Most frequent haplotype	Discrimination capacity (%)
Minimal (9-locus)	4688	0.9972	139 (2.96%)	52
Extended (11-locus)	1957	0.9983	29 (1.48%)	71

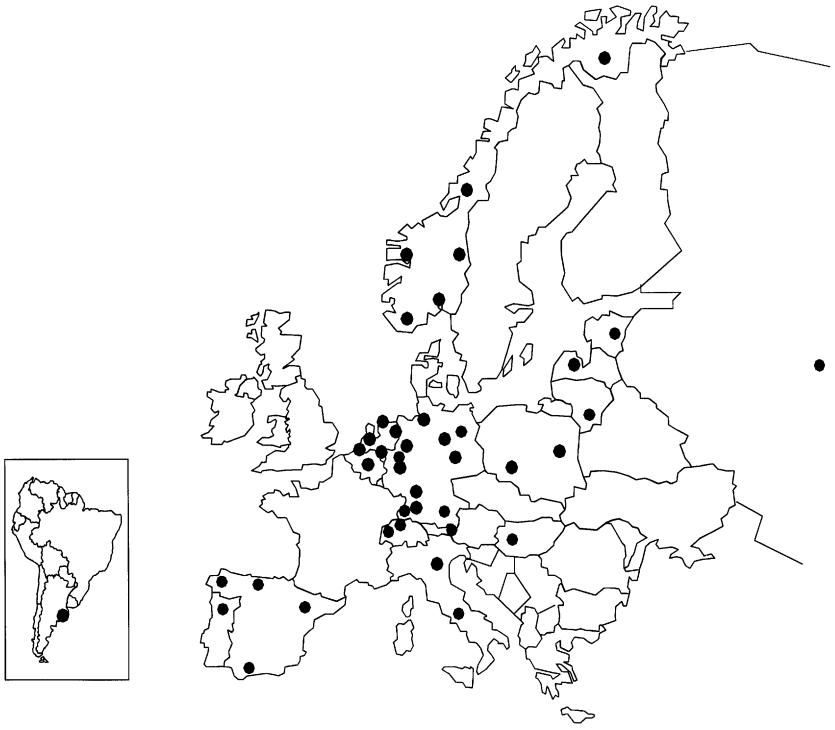

Fig. 2. Geographical origin of population samples logged in YHRD (by September 2000).

Links'' section lists relevant citations and weblinks; extra ''**Contact**'' and ''**Help**'' menus are also provided.

The inclusion of new haplotypes into the database automatically changes all database-dependent values, tables and graphs (e.g. haplotype diversity, number of different haplotypes, allele numbers, frequency distributions, etc.) since these features have been dynamically programmed.

2.4. Sampling scheme and phylogenetic analysis

All populations referred to in the database are of European extraction, and each contributing laboratory is requested to provide data from at least 75 males originating from a defined geographical region (Fig. 2). In most instances, the definition of a regional haplotype pool is based upon surnames and/or birth places. As is revealed by AMOVA, the different regional affiliations cause a subtle stratification of the database even although the overall inter-population variability is low (Fig. 3). Most pair-wise Φ_{st} values were substantially smaller than 0.1, and turned out to be statistically non-significant, especially for the closely related central and southern European populations (Φ_{st} values were calculated on basis of 7-locus haplotypes excluding the bilocal systems DYS385 and YCAII and are available from the two first authors upon request). Haplotype sharing within relatively young European patrilineages (Jobling et al., this issue), urban admixture and recurrent single step mutations (average $\mu = 2.8 \times 10^{-3}$, Kayser and Sajantila, this issue; [6,7]) all contributed to the observed homogenization of the European Y-STR haplotype pool. Common haplotypes, probably the modal haplotypes of particular lineages, appear throughout Europe. A typical example is provided by the most frequent minimal haplotype 14, 13, 29, 24, 11, 13, 13, 11–14 (139 database counts out of a total 4688 chromosomes, Fig. 1a, Table 1) which is present in 34 of the 41 populations covered. The Baltic, Norwegian, and Hungarian populations are slightly more distantly related to the rest of the European populations (Fig. 3). This is in agreement with the finding of Zerjal et al. [8] and Jobling et al. (this issue) who observed an over-representation of SNP-defined Y-chromosomal haplogroup 16, which is probably of Asian descent, in the Baltic region and in Scandinavia. For comparison, two populations of non-European descent have also been included in the AMOVA (Fig. 3), namely, German Turks and Hungarian Roma (Baranya district). In view of the significantly larger genetic distances pertaining to these two populations, they have not, however, been included in YHRD which is aimed at haplotypes of European descent.

Although the most salient feature of the population genetic analysis was the close relationship that emerged between the majority of European haplotypes, some distinct geographical clusters (e.g. Scandinavia, eastern Europe) nevertheless became apparent. For these populations, haplotype frequency estimation should be performed on the basis of a partial (regional) database.

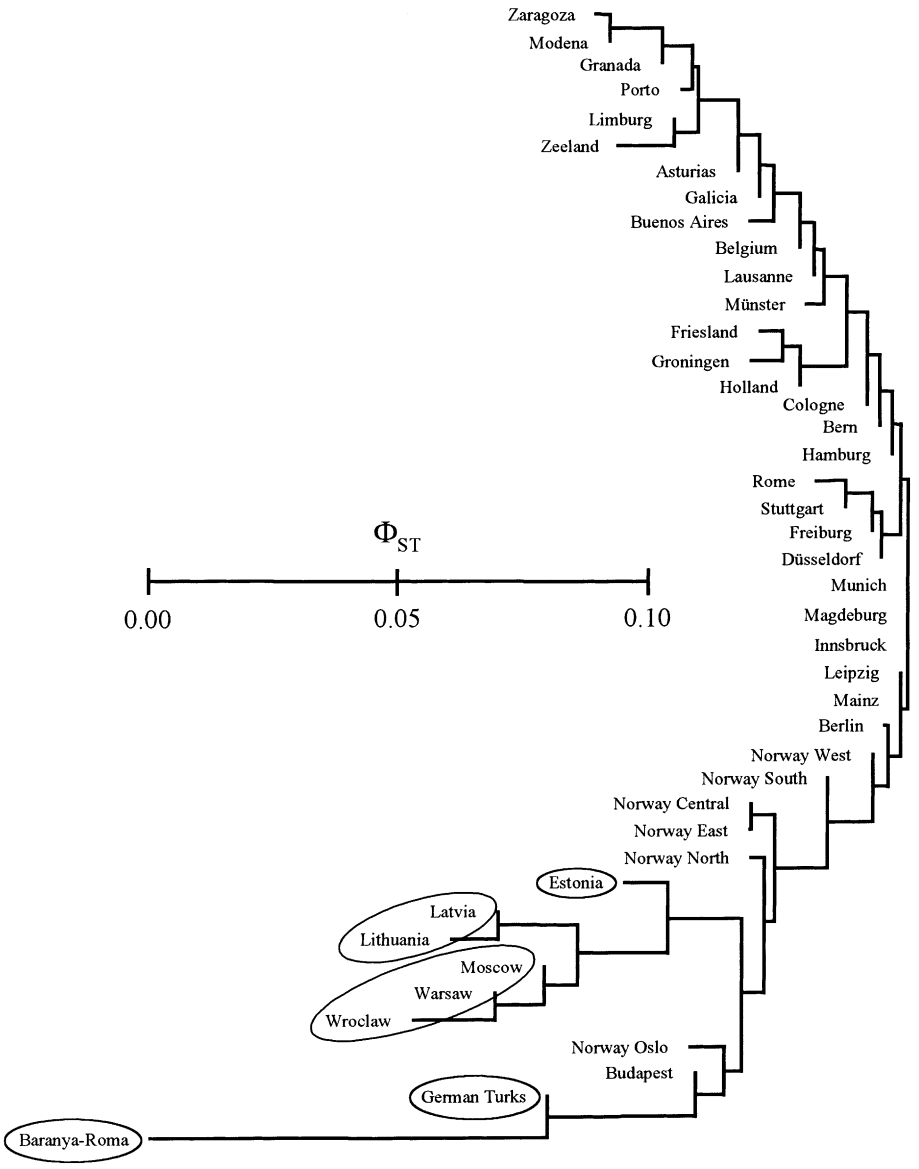

Fig. 3. Neighbor joining tree constructed from pair-wise Φ_{st} values between the 41 populations covered by YRHD (by September 2000). Ellipses mark population clusters characterized by a statistically significant Φ_{st} value ($P < 0.05$) separating all populations inside the cluster from all populations outside the cluster. Significance assessment was performed as described in [15].

2.5. Database query and reports

The database can be queried for single alleles, partial or complete haplotypes using the "start search" feature. Haplotypes must be entered using ISFG compatible nomenclature [1–3]. Pull-down menus providing lists of locus-specific alleles already detected in the database serve to avoid typing errors. If YCAII is included in a haplotype profile, the subset of extended haplotypes is used as a reference database; in all other cases, the full reference database comprising all minimal haplotypes is used. As a result of a query, the observed number of haplotypes in either of the two databases is given. In addition, the geographical distribution of the haplotype of interest is presented in the form of both a table and a map. Using the "calculate frequency" option, the mean and modal (i.e. most probable) haplotype frequency can be obtained from a posterior distribution as described [5]. These values are calculated with and without making the assumption that the haplotype of interest has been observed once outside the database, as is the case for a trace or specimen haplotype. For a detailed discussion, see the related article by Michael Krawczak in this issue.

Since most of the haplotype profiles stored in YHRD represent original data that have not been published elsewhere, copyright issues preclude the database from being downloadable in its entirety. However, full access to the database for scientific purposes can be made available upon request.

2.6. Current state

By September 2000, laboratories from 19 different countries (Germany, Austria, Switzerland, The Netherlands, Belgium, Spain, Portugal, Italy, Hungary, Poland, Croatia, Slovenia, Sweden, Norway, Finland, Denmark, UK, USA, Argentina) have joined the database project. These 31 groups have contributed a total of 4688 Y-chromosomes, completely typed for the 9-locus minimal haplotype and covering 41 European populations (Fig. 2). For 1957 chromosomes (42%), extended 11-locus haplotyping has been completed. Some 165 different alleles/allelic classes have been observed so far. During the first six months on the Internet, YHRD has been accessed more than 5000 times by an average of 15 users per day.

3. Conclusions and outlook

Human Y-chromosomal DNA provides a valuable source of information for forensic analysis to resolve male–female DNA mixtures or to identify paternal lineages. The use of Y chromosome-specific STR polymorphisms in forensic casework has been reported several times before and this technique has been accepted by the courts in various countries (Betz et al., Honda et al., Corach et al., this issue, [9–14]). To support the presentation of Y-STR evidence in court cases, YHRD has been established as the central repository for European Y-STR haplotypes, allowing population frequency estimates to be obtained in a fast, non-commercial and comprehensively documented way.

Frequencies estimates extrapolated by means of the ''surveying'' method [5] fit the actually observed values exceptionally well for prevalent haplotypes. This suggests that the database might also yield reliable frequency estimates for haplotypes with frequencies as small as 1 in 10^5. However, further expansion of the database is necessary to increase the absolute number of rare haplotypes for which extrapolation does still yield large confidence intervals. Currently, 77% of all minimal haplotypes occur only once and, theoretically, 233 million different haplotypes could potentially exist. For extended haplotypes, 7.7 billion different copies are possible (based upon a total of 165 different alleles detected so far at the 11 Y-STR loci).

To expand the pool of European haplotype data, contributions from western, northern and southern Europe would be especially welcome. Moreover, the database may in the near future also serve as a reference for non-European haplotypes since a number of laboratories in North America, Asia and other regions have started collecting haplotype data according to the sampling scheme described herein.

Citation of articles in this special issue of Forensic Science International

M. Krawczak, Forensic evaluation of Y-STR haplotype matches: a comment (this issue).

M. Kayser, A. Sajantila, Mutations at Y-STR loci: implications for paternity testing and forensic analysis (this issue).

D. Corach, L. Filgueira Risso, M. Marino, G. Penacino, A. Sala, Routine Y-STR typing in forensic casework (this issue).

M.A. Jobling, Y-chromosomal SNP haplotype diversity in forensic analysis (this issue).

K. Honda, Z. Tun, D. Young, T. Terao, Examination of Y-STR mutations in sex chromosomal abnormality in forensic cases (this issue).

A. Betz, G. Bäßler, G. Dietl, X. Steil, G. Weyermann, W. Pflug, DYS-STR analysis with epithelial cells in a rape case (this issue).

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