University of Szeged Department of Forensic Medicine
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Ph.D. THESIS

Molecular Biological tests and their application in parentage cases

written by

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ABBREVIATIONS

Bp	Base pair
Kb	Kilobase pair
U	Unit
ng	nanogramme
Tris	Tris [Hydroxymethyl]aminomethane
EDTA	Ethylenediaminetetraacetic acid disodium salt
SDS	sodium dodecyl sulphate
Hp	Haptoglobin
Gc	Group specific component
Pi	α_1- Antitrypsin
C3	Complement 3
Tf	Transferrin
PGM_1sub	Phosphoglucomutase subtype
AP	Acid phosphatase
GLOI	Glyoxalase I
ADA	Adenosindeaminase
AK	Adenylatkinase
EsD	Esterase D
PGP	Phosphoglycolat phosphatase
HLA	Histocompatibility antigen

Table of contents

1. Introduction

Due to the great development of molecular biology, DNA examinations have spread in all the fields of biology and medical sciences, including forensic sciences as well. They tend to gain ground all over the world in civil and criminal court cases together with blood-group determination tests.

Identification of criminals is based on the testing of biological traces (e.g. blood, saliva, sperm and hair) left at the scenes of crimes. In most cases their quantity is so little that traditional serological methods can produce no results. The DNA typing of the remains becomes necessary.

In the parentage cases the paternity of the alleged male cannot be either proven or excluded in many instances with the classical serological methods and it makes the extension of the testing methods necessary. It is provided by the DNA tests. The DNA analysis in the so-called status court cases is first used in Hungary in our laboratory.

1.1. Objectives

In the practice of the determination of paternity only those methods can be applied which are based on known allele frequencies and genotype occurrence with a well established and proven information on the inheritance of the traits. For their applicability in forensic sciences the validation of the DNA systems is required that can be carried out with population genetic tests.

Objectives of my PhD thesis are as follows:

- The examination of distribution of genotypes in 5 somatic chromosomal STR systems (HumVWA, HumTH01, HumF13B, HumFES/FPS, HumFGA) and 2 Y chromosomal STR systems (DYS19, DSY390) in the population of Szeged and its environs,
- the calculation of the allele frequency values of the individual STR systems,
- the probability of the exclusion of paternity,
- as well as the calculation of the combined probability of exclusion of the 7 DNA STR. systems
- By using the probabilities of exclusion I plan to compile a combined protocol of optimal blood group and DNA systems which yield a combined exclusion probability near 100 %.
- To challenge the practical applicability of the DNA examination in the parentage cases, I compare the results of the blood group systems that were combined with DNA analysis with the results of the blood group systems alone in cases from the year 1997.

1.2. Organization of the human genome

The human genome is made up by the DNA in the cell nucleus and in the mitochondria. Approximately 10% of the nuclear DNA consist of a great number of repetitive sequences. They are organised in the form of the interspersed repeats and the tandem repeats (Figure 1). The interspersed repeats are scattered in the genome, in small groups intermingled with the non repetitive DNA (Strachan and, Read 1996). They appear in two forms in the human genome.

SINE (short interspersed nuclear elements) consist about form 5% of the Human genome, the so-called Alu-family also belongs here. The repeats are ~ 280 bp long and occur at about every 4 kb within the human genome. The number of repeats is 750 000, therefore, this is the most known human genome sequence (Strachan and Read, 1996).

LINE (long interspersed nuclear elements): They are 500 bp -7 kb long and their function is not completely known. They may play a role in the DNA repair mechanisms (Strachan and Read, 1996).

The tandem repeats appear in blocks that are placed in succession or in blocks or in a row along the DNA strands.There can be so-called uncoded (intron) sequences inside the genes or sequences outside the genes. On the basis of the number of repeats and the length of the units three types can be distinguished by density gradient centrifugation, i.e. the satellite, the minisatellite and the microsatellite DNA (Strachan and Read, 1996).

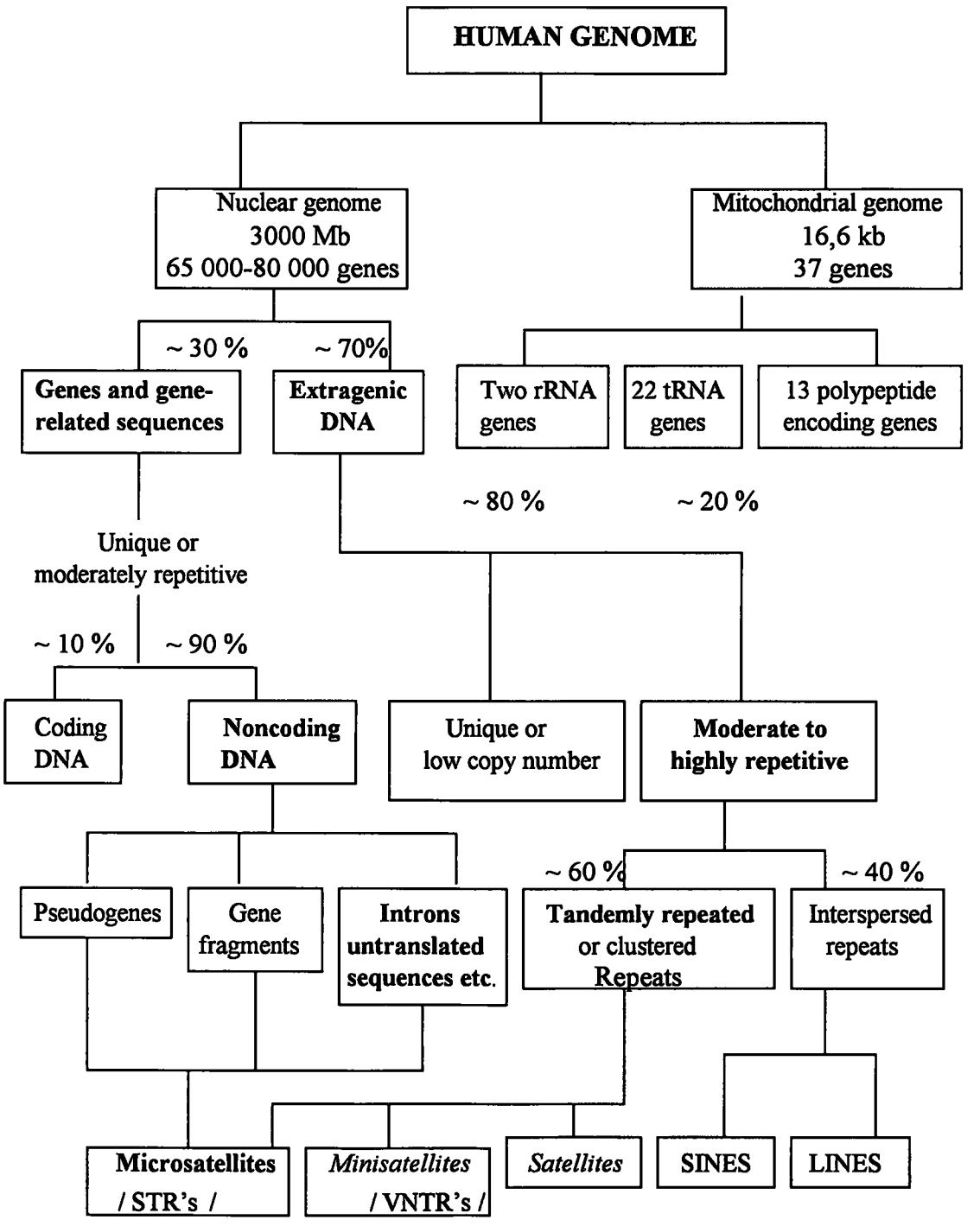
Satellite-DNA: The repeats form a long chain and are either localised in a heterochromatic region or at around the centromere.

Minisatellite-DNA: The medium-sized "tandem repeats" sequences belong here. They are 9-100 bp long sequences repeated several thousand times. Minisatellite DNAs are also known as the variable number of tandem repeat (VNTR) systems (Nakamura at al.,1987).

Microsatellite-DNA: The repeats are 1-6 bp long streches of DNA, their number is 100 000 within the genome. The repeats are l-6 bp long. The amplifiable fragment length polymorphism (AMPFLP) systems, where the sequences are 150-900 bp long with varying numbers of repeated bases,and the short tandem repeats (STR) systems, where the stretches are 150-350 bp long belong here (Edwards at al.,1992; Strachan and Read, 1996).

Fig.1: Organization of human genome

(modified according to Fowler et al, 1988 ; Strachan and Read, 1996)



1.3. Microsatellite systems

Regarding the basic biological function of the microsatellite systems there have been various ideas on whether they play a role in gene regulation or gene conservation or in the determination of sexes or they are regarded as recombination hot spots. According to current opinion, these sequences were created by slippage mutation and have no functional significance (Strachan and Read, 1996).

Similarly to the blood, enzyme and serum groups, the DNA sequences belonging to the microsatellite regions of the human genome also show genetic variability, they are polymorphic. Polymorphism is the form of a genetic variation where the human chromosomes include one or the other alleles. They are the result of a mutation in the DNA which can be passed further to the next generation.

1.3.1. Mutation models

We can draw conclusion for the mutational mechanisms from the two mutation models that lead to the development of various alleles in the microsatellite systems. Infinite allele model (IAM): It is a presumed that every new mutation results in a new allele (Wright, 1949; Kimura and Crow, 1964). In the microsatellite loci it means that the new alleles are the results of a complex mutational process that affects many repeat or the entire tandem region.

Stepwise mutation model (SMM): This model has been introduced to explain development protein polymorphisms (Kimura and Ohta, 1973). In the case of microsatellite loci it means the insertion or deletion of one repeat in the tandem region (Chakraborty, 1977; Chakraborty and Daiger 1991; Shriver at al 1993).

1.3.2. Mutational mechanisms in the microsatellite systems

Slipped strand mispairing: This occurs when the normal pairing between the two complementary strands of the double helix is altered by staggering of the repeats on the two strands, leading to incorrect pairring of repeats. Although slipped strand mispairing can be envisaged to occur in nonreplicating DNA. Replicating DNA may offer more opportunity for slippage, therefore the mechanism is often also called replication slippage or polymerase slippage. In addition to mispairing between tandem repeats, slippage replication has been

envisaged to generate large deletions and duplications by mispairing between noncontigous repeats (Strachan and Read, 1996).

Gene conversion: This describes a non reciprocal transfer of sequence information between a pair of nonallelic DNA sequences (interlocus gene conversion) or allelic sequences (intraallelic gene conversion).

One of the pair of interacting sequences , the donor, remains unchanged, but the other DNA sequence, the acceptor, is changed so that it gains some sequence copied from the donor sequence. Gene conversion events may be relatively frequent in tandemly repetitive DNA (Strachan and Read, 1996).

Unequal crossover: This is a form of recombination in wich the crossover takes place between nonallelic sequences on nonsister chromatids of a pair of homologs.

The crossover occurs between mispaired nonsister chromatids, the exchange results in a deletion on one of the participating chromatids and an insertion on the other.

Unequal crossover is also expected to occcur comparatively frequently in complex satellite DNA repeats and at tandemly repeated gene loci (Strachan and Read, 1996).

1.3.3. Types of mutation

The DNA of human genom is subject to a variety of different types of heritable change.

Large-scale chromosome abnormalities involve loss or gain of chromosomes (numerical abnormalities) or breakage and rejoining of chromatids (structural abnormalities).

Smaller-scale mutations involve changes a single DNA sequence (simple mutation) or they involve exchanges between two allelic or nonallelic sequences.

Three classes of small-scale mutation can be distinguished. (Table1)

Table 1

Small scale mutation classes in the human genome

Mutation class	Type of mutation	Incidence
Base subtitutions	All types	Common type in coding and in noncoding DNA
	Transitions-transversion	unexpectedly;transitions are commoner than transversions; in mitochondrial DNA
	Synonymous - nonsynonimous substitutions	synonymous substitutions commoner than nonsynonymous in coding DNA
	Gene conversion	Rare except at certain tandemly repeated loci or clustered repeats
Insertions	One or more nucleotides are inserted into a sequence	Very common in noncoding DNA
Deletions	one or more nucleotides eliminated from a sequence	Very common in noncoding DNA

1.3.4. Brief description of the examined DNA STR systems

HumVWA: The 40th intron of the von Willebrand Factor gene is located on the 12th chromosome in the 12p12-pter region. The fragment size is about 150 bp long and the TCTA/TCTG bases are repeated (Mercier et al., 1991, Kimpton et al., 1992).

HumTHO1: The 1st intron of the Tyrosine hydroxylase gene is located on the 11p15-15.5 chromosome, the fragment is about 160 bp long in which 4 pairs of bases (AATG) are repeated (Edwards et al., 1992).

HumF13B: It is the 3'-ended region of the XIII b coagulation factor gene that is located on the 1q31-q32 chromosome. It is about 200 bp long fragment inside which there are pairs of bases (ATTT) repeated. Its polymorphisms were first described by Nishimura and Murray (1992).

HumFES/FPS: It is the 5th intron of c-fes/fps protooncogene gene located on the 15q25-qter chromosome. Its is about 250 bp long fragment in which 4 pairs of bases (ATTT) are repeated. It was first described by Polymeropoulos et al. (1991a).

HumFGA: It is the 3rd intron of the alpha fibrinogen gene. Its chromosomal localisation is 4q28. It is about a 280 bp long fragment with the repeat of TCTT inside (Barber et al, 1996).

DYS19 is about 200 bp long section located on the short arm of the Y chromosome inside which 10-19 repeats of the CTAT/C sequences can be seen.(Roewer at al, 1992; Kayser at al, 1997).

DYS390 is about 220 bp long fragment located on the long arm of the Y chromosome inside which 18-27 repeats of the CTG/AT sequences can be seen. (Kayser et al. 1997).

2. Materials and methods

2.1. Materials

The DNA was extracted from blood samples applied on a piece of linen and EDTA blood samples that originated from the Albert Szent-Györgyi Medical University of Blood Transfusion Station and the Department of Forensic Medicine's routine parentage tests.There were 489 unrelated adult individuals tested in the case of the HumVWA and HumTHO1 systems, 465 in the HumF13B system, 360 in the HumFES/FPS and HumFGA systems, 308 in the DYS19 and 268 in the DYS390 systems.

2.2. Methods

2.2.1. DNA extraction from blood

The DNA extraction from EDTA blood samples was carried out with the NaCl extraction method of Miller et al. (1988).

2.2.1.1. Lysis of red blood cells

The EDTA blood is washed three times with 0.05 M KCl solution. The ratio between the blood and the KCl solution is 1:4. After the washing we gain a residue containing leukocytes and red blood cell membranes free from haemoglobins.

2.2.1.2. Lysis of leukocytes

The DNA isolation was carried out in a buffer containing Proteinase K (Pro K) enzyme to dissolves proteins, e.g. nucleases and SDS to emulsify the lipid bilayers of the cell membranes, making thus possible the action of the enzyme.

The process of digestion takes place in the presence of a so-called lysis buffer composed of:

10mM Tris/HCl pH:8,0
10mM NaCl
10mM EDTA-Na

The lysis of the leucocytes was done in incubation in a 2 ml lysis buffer containing 250 µl Pro K (2mg/ml) and 100ml 20% SDS in 37°C at overnight.

2.2.1.3. Protein extraction

Following the incubation the proteins in the solution were precipitated by addition of 1 ml 6M NaCl solution.After centrifugation (20 minutes at 3600 g) the DNA remained in the supernatant.

2.2.1.4. DNA extraction

The supernatant was transferred into a centrifuge tube. A 96% cold ethanol solution is added and the tube was tilted to precipitate DNA. The DNA is appears in the ethanol-phase, transferred into another centrifuge tube, washed in 70% ethanol and dried at room temperature. Finally, it is dissolved in TE buffer (10mM Tris, 1mM EDTA).

2.2.2 DNA extraction from blood stain

The DNA extraction from bloodstains was done with the Chelex method described by Walsh et al. (1991).

The Chelex 100 (Biorad) is a styrene resin having reactive iminodiacetate groups which chelate with multivalent metal ions. The ions, especielly Fe ions in the case of blood, that catalyse the fragmentation of the DNA at high temperature (Singer-Sam et al., 1989).

In the first step a 3x3 mm piece of the bloodstain was put in an Eppendorf tube and incubated in 1 ml sterile distilled water at room temperature for 30 minutes while vortex - mixing it, therefore, accelerating the solution of the haemoglobin from the conveying material. After the incubation period it was centrifuged for 5 minutes at 14 000g. The supernatant, except 50 µl, was removed and 150 µl 5% Chelex solution was added. After incubation in a 56°C water bath for 30 minutes the cells were boiled for 8 minutes in order to accelerate the lysis of the cells. After the centrifugation the supernatant can be used in the PCR.

2.2.3. Quantitative and qualitative DNA testing

The estimation and quality testing of the DNA isolated from blood is done with agarose gel electrophoresis followed by ethidium-bromide staining.

For the estimation of the DNA concentration a 50, 100, 200, 400 and 600 ng/ml solution of the DNA originating from the K562 cell line (Promega , Madison) is added into adjacent lanes of the same gel. For the electrophoresis we prepare an 0,8 % (w/v) gel concentration.

Components: 1,6 g Agarose (Serva, Heidelberg)

20 ml 10% Loening buffer (0,4 M Tris,
10 mM EDTA)
200 mM Na-acetate (pH 7,4)
200 ml Deionised water

Prior to application the samples have been prepared as follows:

1µl DNA sample + 11µl TE buffer (10 mM Tris, 1 mM EDTA) + 3µl Stopp-mix

Stopp-mix components:5 ml Glycerol

1 ml 10X TBE buffer (1.34 m Tris,749 mM Boric acid, 25,5 mM Na_2-EDTA)
1 ml Bromophenolblue (7 %)
1 % Xylenecyanole (10 %)

The electrophoresis lasts for 2 hours in the 1X Loening electrode buffer at 250 mA.
The extracted DNA is stained by incubating with a 0,2 % (w/v) ethidium-bromide solution for 30 minutes.The DNA bands become visible in UV light (320 nm) with the help of a transilluminator.

2.2.4. PCR (Polymerase Chain Reaction)

The in vitro synthesis (amplification) of the DNA region of interest is performed by PCR (Mullis and Faloona, 1987; Saiki et al., 1988) in the presence of thermo-stable Taq-DNA polymerase.
The reaction takes place in three phases. The first phase includes the separation of the double-stranded DNA into single strands at 90-95°C (denaturation). The second phase includes the annealing of primers (synthetic oligonucleotide) to the opposite DNA strand (50-64°C). In the third phase (extension) a new DNA strand is formed from the nucleotides included in the system from the point where the primers are annealed (70-72°C). The process is repeated in 30 cycles.

PCR mixture

2 µl 10x Reaction buffer (Promega)
1-2 µl Deoxynucleotide (2.5-2.5 mM dGTP, dATP, dCTP, dTTP)
1,5µl 25mM $MgCl_2$
0.2 µl (1U) Thermus aquaticus (Taq)-DNA.polymerase (Promega)
x µl (1µM) Primer 1 and 2
x µl (2ng) "Template" DNA (sample to be typed)
ad 25µl Deionised water

To prevent evaporation 25 µl Mineral oil (SIGMA) is layered on the top of the reaction mixture.

The amplification reaction is done with a PTC 100MJ Research thermocycler.

The primers used in the examinations are listed in Table 2. The optimal parameters of the amplification reaction for each individual primer are described in Table 3.

2.2.4.1 The verification of the PCR product

A 3.5 µl of amplification product is applied into a polyacrylamide gel with 123 bp molecular weight marker," the ladder" (SIGMA), as control and it is visualized with silver nitrate staining (See detailed description in Chapter 2.2.5).

2.2.5. Detection of the genotypes

The alleles are visualized in native discontinuous buffer (Allen et al.,1989) by polyacrylamide gel electrophoresis (PAGE) followed by silver nitrate staining (Budowle et al.,1991). During the electrophoresis it is as the number of the tandem repeat sequences is directly proportional to the lenght of the examined DNA strand, e.g.: the longer the DNA section it is nearer to the inoculation and the smaller its wandering speed is, therefore, it is further to the inoculation. With lower number of tandem repeats we can observe a longer distance of migration from the site of loading.

The DNA samples can be typed by comparison with an allelic ladder constructed by the known alleles and standardised in our laboratory.

Table 2

The used primers in each STR systems

	Primer 1	Primer 2
HUMVWA Kimpton et al. /41/	5' CCC TAG TGG ATG ATA AGA ATA ATC 3'	5' GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG 3'
HUMTH01 Edwards et al. /22/	5' GTG GGC TGA AAA GCT CCC GAT TAT 3'	5' GTG ATT CCC ATT GGC CTG TTC CTC 3'
HUMF13B Nishimura , Murray /63/	5' TGA GGT GGT GTA CTA CCA TA 3'	5' GAT CAT GCC ATT GCA CTC TA 3'
HUMFES/FPS Polymeropoulos et al. /66/	5' GGG ATT TCC CTA TGG ATT GG 3'	5' GCG AAA GAA TGA GAC TAC AT 3'
HUMFGA /Mills et al. /56/	5' GCC CCA TAG GTT TTG AAC TCA 3'	5' TGA TTT GTC TGT AAT TGC CAG 3'
DYS19 Roewer et al /67/	5' CTA CTG AGT TTC TGT TAT AGT 3'	5' ATG GCA TGT AGT GAG GAC A 3'
DYS390 Kayser et al. /38/	5' TAT ATT TTA CAC ATT TTT GGG CC 3'	5' GAC AGT AAA ATG AAC ACA TTG C 3'

Table 3

The parameters of the amplifikation (PCR) in each STR systems

HUMVWA /2/	**HUMTH01 /2/**	**HUMF13B /3/**	**HUMFES/FPS /2/**	**HUMFGA /4/**	**DYS19 /38/**	**DYS390 /38/**
					94 °C 180s	94 °C 180s
94 °C 60s	94 °C 60s	96 °C 60s	95 °C 60s	95 °C 60s	94 °C 30s	94 °C 15s
50 °C 60s	64 °C 60s	60 °C 60s	54 °C 60s	60 °C 60s	51°C 30s	58 °C 20s
72 °C 90s	70 °C 120s	70 °C 90s	70 °C 90s	72 °C 60s	72 °C 90s	72 °C 20s
30 cycles	10 cycles	10 cycles	30 cycles	30 cycles	30 cycles	5 cycles
	90 °C 60s	90 °C 60s	72 °C 10min	72 °C 10min		94 °C 15s
	64 °C 60s	60 °C 60s				54 °C 20s
	70 °C 120s	70 °C 90s				72 °C 20s
	17cycles	20 cycles				30 cycles

2.2.5.1. Polyacrylamide gel electrophoresis

Solutions necessary for an electrophoresis:

1./ 29,1% Acrylamide (SERVA)
0,9 % Piperazine diacrylamid (PDA, SIGMA)
2./ 0,28 M 2-Cyclohexylamino ethane-sulfonicacid (CHES)
3./ 0,5 M Tris/Formiate pH:9,0
4./ 0,35 M Tris/Sulphate pH:9,0

The gel polymerisation takes place on the effect of the ammonium peroxo-disulphate (APS) and N,N,N',N'-tetramethylediamine (TEMED). To check the amplification product we use an 8% gel of 10 cm running distance, the thickness of which is 0.45 mm. The electrode buffer is 0.28 M Tris/Boric acid, which contains 0.05% (w/v) bromophenolblue. The contact between the electrode and the gel is provided by a 230 mm x 10 mm x 5 mm sized, 2% agarose gel saturated with electrode buffer.

The electrophoresis is carried out in a multiphore-2 gel electrophoresis chamber with upper electrodes (Pharmacia) at 100 V and 20 mA, until the bromophenolblue band reaches the anode. The sample is loaded at 2 cm from the cathode by a strip of Schleiher & Shuell 2013 type filter paper. In order to indicate the genotypes we used a 0.75 mm thick 6% gel with 20 cm running distance. The electrophoresis is carried out for 90 minutes at 5W and for 90 minutes at 10 W and finally at 15 W, until the bromophenolblue reaches the anode.

2.2.5.2. Silver - staining

The indication of the DNA phenotypes was carried out with the method described by Budowle et al. (1991). The DNA fragments are fixed onto the gel with in 1 % (v/v) HNO_3 for 5 minutes and the gel was washed 3 times with deionised water. The staining is carried out for 30 minutes in 0.2% (w/v) $AgNO_3$ with continuous shaking, then it is rinsed with deionised water then washed with a 0.28M Na_2CO_3 solution also containing 0.05% (w/v) formaldehyde. It is repeated as many times as it is necessary in order to have reddish or greyish-brownish DNA bands on the light coloured gel basis. Then the gel is fixed in 10% (v/v) acetic acid solution for 3 minutes, washed in deionised water and befor air - drying it is transferred into 5% (v/v) glycerol 5 minutes (Figures 2-8).

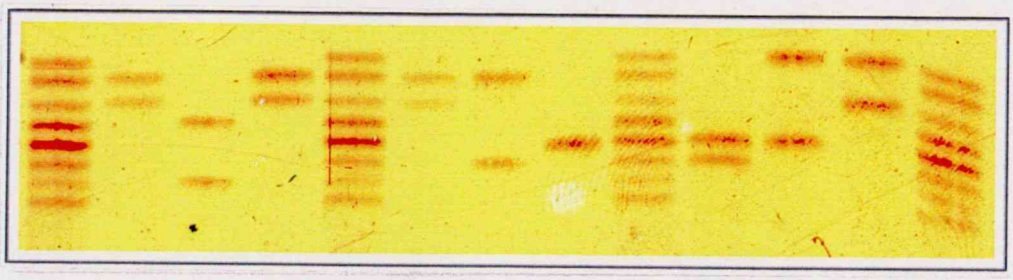


Fig.2. ***HumVWA genotypes***: allelic ladder /from left to right/:allelic ladder (13-20); 14/15;16/19;14/15; allelic ladder(13-20); 14/15;14/18;17/17; allelic ladder (13-20);17/18;13/17; 13/15;allelic ladder (13-20

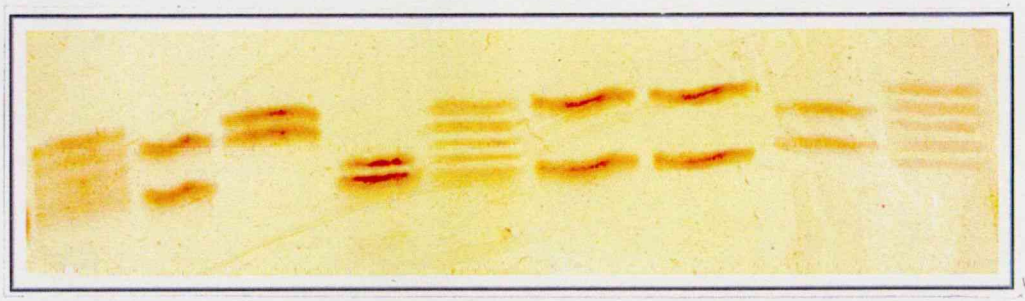


Fig.3. ***HumTH01 genotypes***: / from left to right /: allelic ladder(6-9.3);7/9.3;6/7;9/9.3;allelic ladder (6-9.3);6/9.3;6/9,3;7/9; allelic ladder(6-9.3);

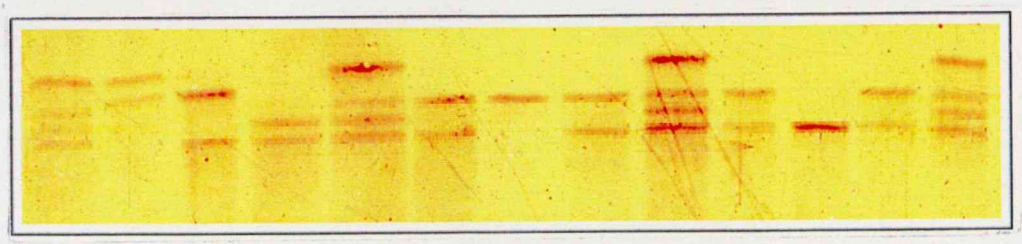


Fig.4. ***HumF13B genotypes*** **:** /from left to right /: allelic ladder(6,8-10); 6/7;7/10;9/10; allelic ladder(6,8-10); 8/10;8/8;8/10; allelic ladder(6,8-10); 8/10;10/10; 8/10; allelic ladder(6,8-10)

Fig.5. *HumFES/FPS genotypes*: / from left to right /: allelic ladder(9,10A-14);10A/12; allelic ladder (9,10A-14)10/12;10/11;11/11; allelic ladder(9,10A14); 11/11;10A/11;10A/10A; allelic ladder(9,10A-14);10A/11;11/12; 11/11;
allelic ladder (9,10A-14)

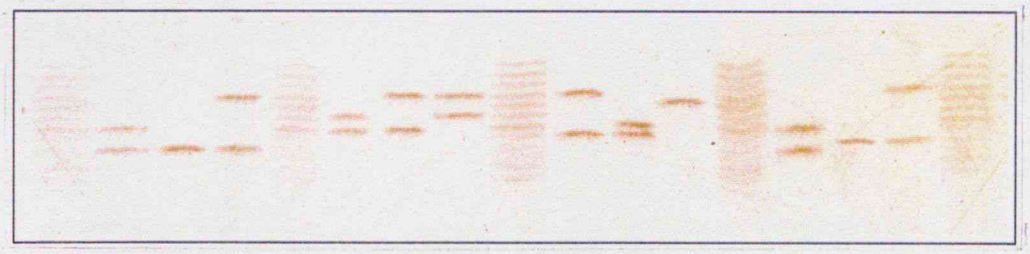


Fig.6. *HumFGA genotypes*: / from left to right /: allelic ladder(17-28);23/25; 25/25;20/25;allelic ladder (17-28);22/23;20/23;20/22; allelic ladder (17-28);20/24;23/24; 21/21; allelic ladder(9,10A-14)24/26;25/25;20/25; allelic ladder(17-28)

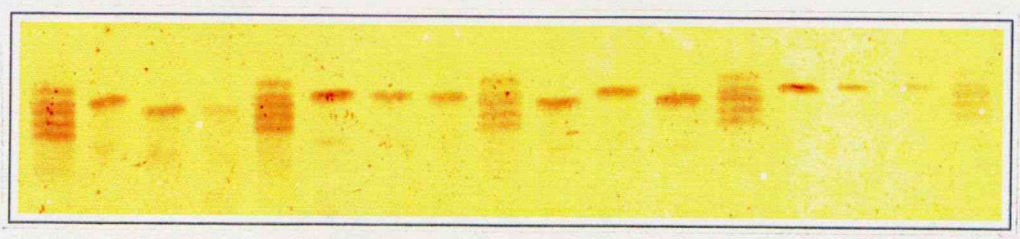


Fig.7. *DYS19 genotypes*: / from left to right /: allelic ladder(13-17);14;15;15; allelic ladder(13-17); 14;14;14; allelic ladder(13-17);15;14; 15; allelic ladder(13-17); 14;14;14; allelic ladder(13-17)

Fig.8. ***DYS390 genotypes*****:** / from left to right /: allelic ladder(21-25);22;23;23; allelic ladder (21-25); 22;22;21; allelic ladder (21-25);21;24;24; allelic ladder(21-25);

3. Results

The blood samples of unrelated adults from the Blood Transfusion Station of Albert Szent-Györgyi Medical University and from parentage cases of the Forensic Medicine Institute of Albert Szent-Györgyi Medical University have been tested 7 DNA-STR systems and the allele frequency values were calculated.

3.1 Population genetic analysis

We have typed 489 individuals for the HumVWA and HumTH01 systems, 465 for the HumF13B, 360 for the HumFES/FPS and the HumFGA systems. The distribution of the genotypes is given in Tables 4/A, B, C, D, and E respectively.

3.2. Statistical analysis

3.2.1. Calculation of significance

With the knowledge of the allele frequencies I calculated the theoretical genotype occurrence. With χ^2 probe I tested if there is a significant difference between the found and calculated genotypes, i.e. a difference from the Hardy-Weinberg equilibrium (Table 7).

3.2.2. Expected heterozygosity (Table 8)

$H = 2n\,(1-\sum x_{ij}^2)/2n-1)$ (Nei, 1978) x_{ij} = allele frequency
n = total number of alleles

3.2.3. Power of discrimination (Table 8)

$DP = 1 - \sum y_{ij}^2$ (Jones, 1972) y = genotype frequencies

3.2.4. Gene/haplotype diversity (Table 8)

$h = 1- \sum q_{ij}^{\ 2}$ (Nei, 1987) q = gene /haplotype frequences

3.2.5 Polymorphism information content (Table 8)

$PIC = 1-\sum p_i^2 - \sum 2p_i^2\, p_j^2$ (Botstein et al., 1980) p = allele frequency

3.2.6.Comparative study of the allele frequency values of the various populations

On the basis of G. Carmody's (Ottawa, Canada) RxC contingency table (Tables 10/A, B).

3.2.7. Genetic distance

By the calculation of genetic distances in the knowledge of the allele frequency values we can draw conclusions about the genetic relationships among various ethnic groups. (Table 11).

$D_{(i,j)} = \Sigma[p_i - p_j] \times 1n\ [p_i/p_j]$

pi = allele frequency one population
pj = allele frequency other population

(Hummel and Kazarinowa-Fukshansky, 1991)

4. Evaluation of the results

Among the results gained on the basis of the genotype occurrence in the 5 tested somatic DNA systems there were 26 genotypes typed in the HumVWA system (Table 4/A), where the occurrence frequency of the type 17/20 is quite low (0.082%), whereas that of genotype 17/18 is high (11.042 %).

Table 4/A

Genotype frequencies in the STR systems HumVWA

Genotype	Found %	Calculated %	Genotype	Found %	Calculated %
13/16	0.204	0.036	16/17	10.225	9.610
14/14	1.227	1.416	16/18	7.566	7.498
14/15	3.067	2.547	16/19	2.658	3.703
14/16	4.703	4.332	17/17	7.975	6.969
14/17	4.498	6.283	17/18	11.042	10.877
14/18	5.521	4.902	17/19	4.908	4.752
14/19	2.454	2.142	17/20	0.082	0.005
14/20	1.431	1.618	18/18	4.294	4.243
15/15	1.431	1.145	18/19	4.089	4.656
15/16	4.089	3.894	19/19	1.840	0.81
15/17	5.930	5.649	19/20	0.613	0.342
15/18	4.908	4.408			
15/19	0.814	1.926			
15/20	0.204	0.410			
16/16	3.681	3.312			

Among the 23 genotypes typed in the HumTH01 system (Table 4/B) the occurrence frequency of 5/8, 5/9.3, 9/10 is the lowest (0.204%), whereas at two genotypes (6/8.2, 9/9.3) there were high values if compared to others (11.040 % and 13.088%).

Table 4/B

Genotype frequencies in the STR systems HumTH01

Genotype	Found %	Calculated %	Genotype	Found %	Calculated %
5/8	0.204	0.113	7/9.3	5.725	7.506
5/9	0.410	0.166	7/10	0.613	0.251
5/9.3	0.204	0.221	8/8	1.840	1.932
6/6	6.540	5.570	8/9	5.521	5.616
6/7	7.360	6.561	8/9.3	7.566	7.506
6/8	6.750	6.561	8/10	0.408	0.251
6/9	8.380	9.534	9/9	3.272	4.080
6/9.3	11.040	12.744	9/9.3	13.088	10.908
6/10	0.610	0.425	9/10	0.204	0.364
7/7	2.040	1.932	9.3/9.3	7.770	7.290
7/8	3.476	3.864	9.3/10	0.613	0.486
7/9	6.339	5.616			

Among the 15 HumF13B genotypes (Table 4/C) found in the samples of the Szeged region,the allele frequency of 9/11 was the lowest (0.22%) whereas that of the 8/10 was 24.95%.

Table 4/C

Genotype frequencies in the STR systems HumF13B

Genotype	Found %	Calculated %
6/6	1.080	0.876
6/8	7.090	4.790
6/9	3.440	4.790
6/10	6.020	7.810
7/9	1.075	0.420
7/10	0.860	0.810
8/8	4.730	6.540
8/9	9.250	11.060
8/10	24.950	21.340
8/11	0.430	0.390
9/9	5.160	4.670
9/10	18.270	18.030
9/11	0.220	0.330
10/10	16.560	17.410
10/11	0.860	0.630

Among the 22 genotypes defined in the HumFES/FPS STR system (Table 4/D) homozygote 11 had the highest frequency value (21.94%), whereas the lowest values belonged to the 7/11 and 8/10 (0.278 %).

Table 4/D

Genotype frequencies in the STR systems HumFES/FPS

Genotype	Found %	Calculated %	Genotype	Found %	Calculated %
7/11	0.278	0.140	10A/12	6.944	6.464
8/10	0.278	0.236	10/12	2.222	2.336
8/11	1.389	1.027	10/13	6.389	6.278
8/12	0.278	0.352	11/11	21.944	21.809
9/9	1.120	1.160	11A/11	0.570	0.682
9/10	0.556	0.496	11/12	14.167	14.944
9/11	3.333	3.176	11/13	4.722	4.016
9/12	1.111	1.090	12/12	2.220	2.560
9/13	0.290	0.292	12/13	1.120	1.376
10A/10A	4.444	4.080			
10A/10	2.778	2.949			
10/10	5.277	5.329			
10A/11	18.055	18.867			

The HumFGA (table 4/E) is the most polyphormic system in which we found 46 different genotypes, the frequency values vaied between 0.310 % and 7.98 %.

On the basis of the distribution of the genotypes occurrence we calculated the allele frequency values for each STR system.

Table 4/E

Genotype frequencies in the STR systems HumFGA

Genotype	Found %	Calculated %	Genotype	Found %	Calculated %
16/22	0.310	0,060	21/23	7,980	6.110
18/19	0.310	0,210	21/24	5,520	5.100
18/20	1.230	0,460	21/25	2,450	2.900
18/21	0.920	0,600	21.2/24	0,610	0.110
18/22	0.310	0,600	22/22	3,370	3.920
18/23	0.310	0,410	22/22.2	0,310	0.160
19/19	0.610	0,480	22/23	4,910	5.300
19/20	0.920	2,110	22/24	4,910	5.200
19/21	2.450	2,650	22/25	2,150	2.900
19/22	2.760	2,740	22/26	0,920	0.800
19/23	1.840	1,900	22.2/24	0,310	0.110
19/24	2.150	1,900	23/23	2,150	1.800
19/25	1.530	1,000	23/24	2.450	3.500
19/26	0.310	0,250	23/25	2.450	2.000
19/27	0.310	0.100	23.2/22	0.310	0.240
20/20	2.150	2.350	23.2/24	0.310	0.160
20/21	4.910	5.900	23.2/25	0.610	0,100
20/22	7.970	6.100	24/24	1.840	1.720
20/23	3.370	4.100	24/25	0.920	1.900
20/24	4.290	4.010	24/26	0.610	0.500
20/25	2.760	2.210	24/27	0.310	0.110
20/26	0.310	0.560	25/26	1.500	0.260
21/21	3.070	3.690			
21/22	7.980	8.000			

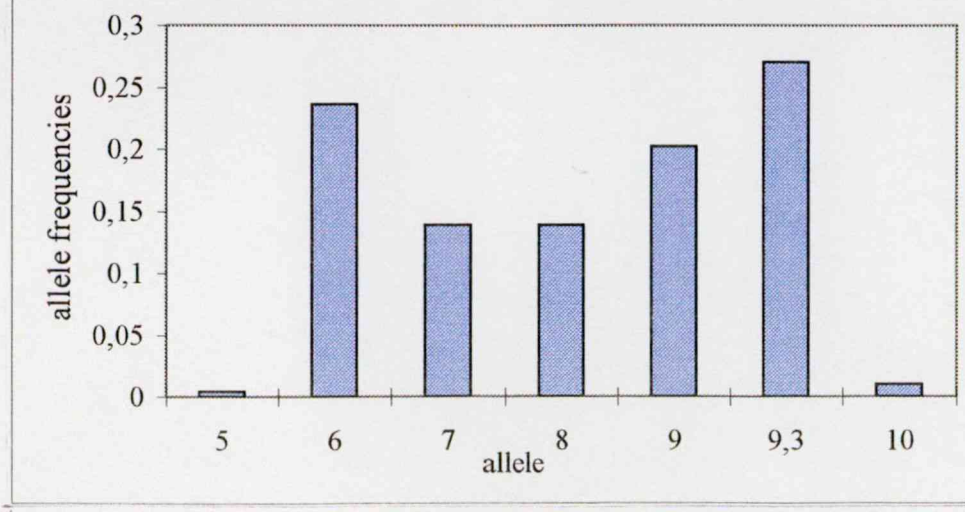
In the HumTH01 (Table 5/A; Fig.9) system we typed 7 alleles among which allele 9.3 occurred the most frequently (27%) and allele 5 the least frequently (0.4%).

Table 5/A

Distribution of HumTH01,HumF13B and Hum FES/FPS allele frequencies in the in the population of Szeged and its environs

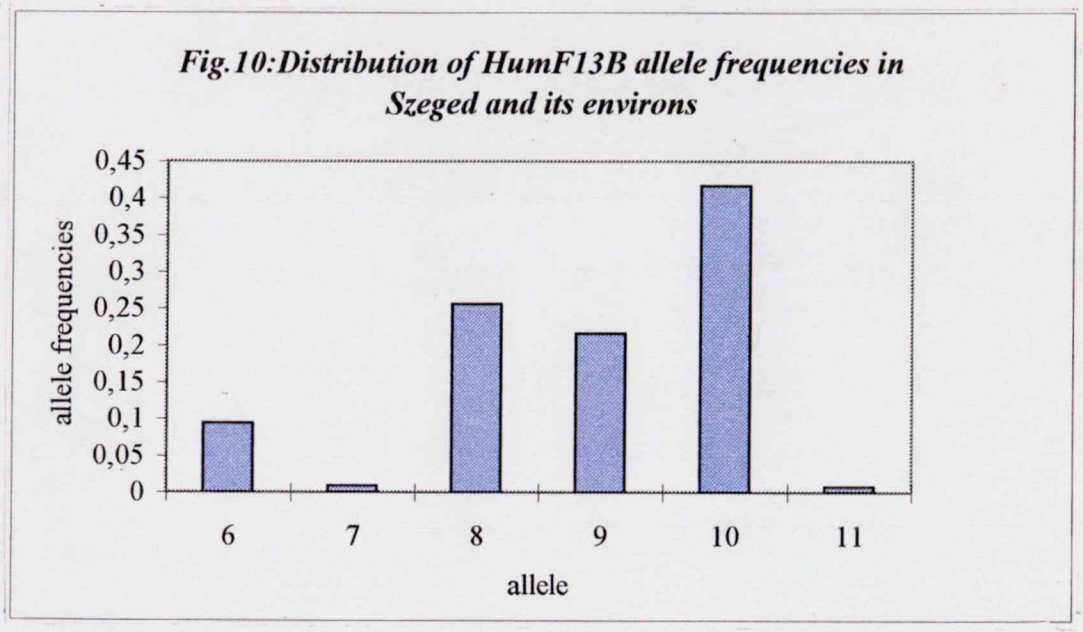
Allele	HumTH01 n=489	HumF13B n=465	HumFES/FPS n=360
5	0.004		
6	0.236	0.094	
7	0.139	0.009	0.002
8	0.139	0.256	0.011
9	0.202	0.216	0.005
9.3	0.270		
10	0.01	0.417	0.073
10A			0.261
11		0.008	0.431
11A			0.012
12			0.160
13			0.043
14			0.002

Fig.9:Distribution of HumTH01 allele frequencies in Szeged and its environs



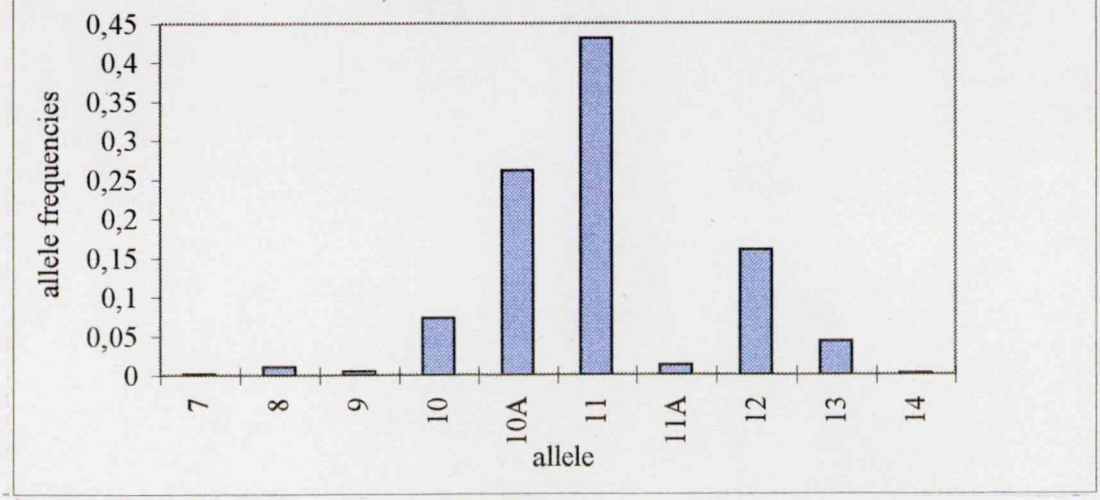
In the HumF13B system 6 alleles were typed among which most 10 that occurred frequently (41.7%) in the tested samples. At the same time, however, the frequency of alleles 7 and 11 were very low, between 0.9% and 0.8% (Table 5/A; Fig. 10).

Fig.10:Distribution of HumF13B allele frequencies in Szeged and its environs



In the HumFES/FPS STR system 10 alleles were typed among which allele 11 occurred at a frequency of 43.1% and it is the highest value. At the same time, however, the frequency value of allele 7 and 14 is 0.2%. (Table 5/A ; Fig. 11).

Fig.11:Distribution of HumFES/FPS allele frequencies in Szeged and its environs

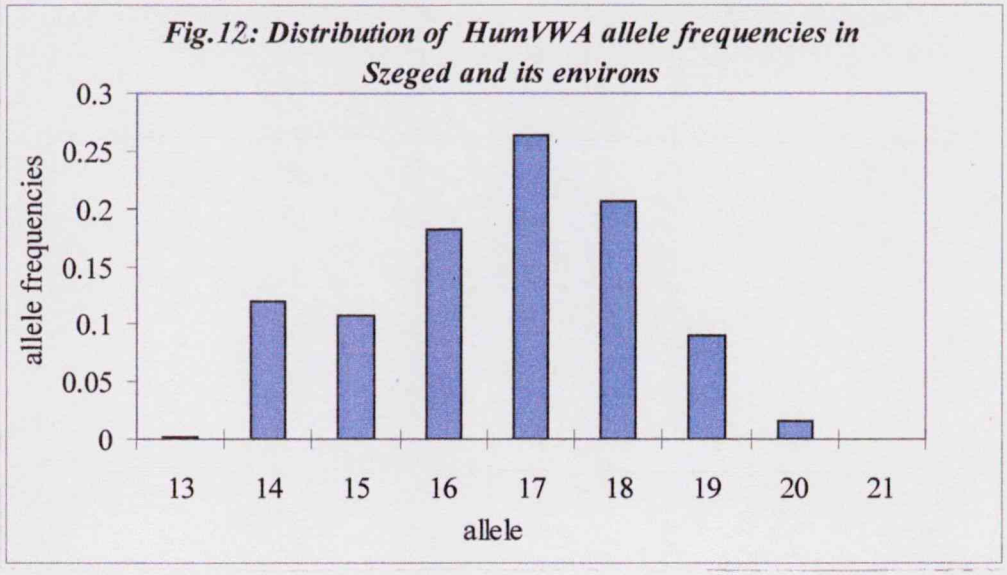


In the HumVWA system (Table 5/B ; Fig 12) 9 alleles were typified among which the most frequent one was allele 17 (26.4%), whereas the occurrence frequency values of allele 13 and 21 were low (0.1%).

Table 5/B

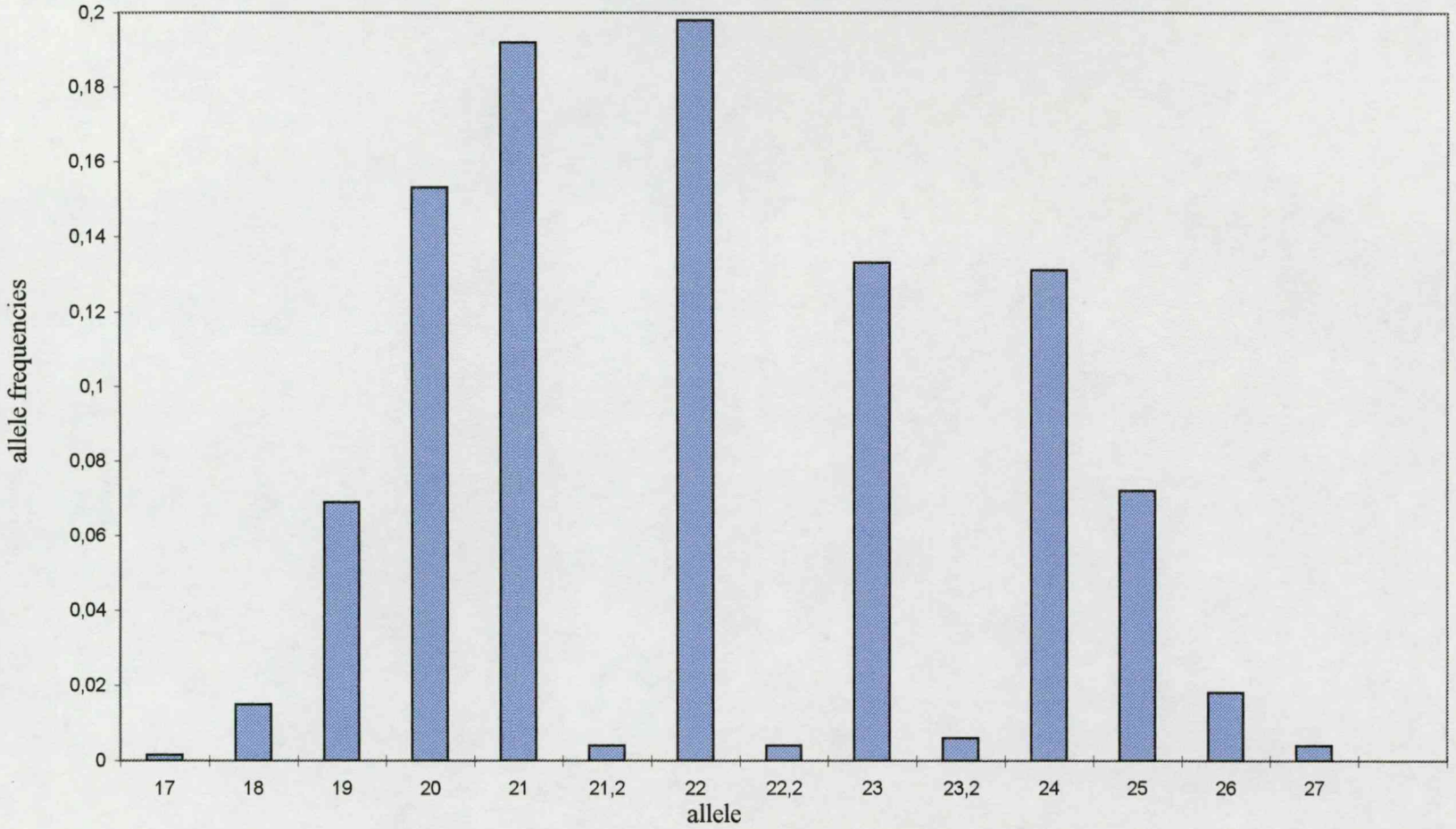
Distribution of HumVWA and HumFGA allele frequencies in the population of Szeged and its environs

Allele	HumVWA n=489	HumFGA n=326
13	0.001	-
14	0.119	-
15	0.107	-
16	0.182	0.0015
17	0.264	-
18	0.206	0.015
19	0.090	0.069
20	0.015	0.153
21	0.001	0.192
21.2		0.004
22		0.198
22.2		0.004
23		0.133
23.2		0.006
24		0.131
25		0.072
26		0.018
27		0.004



The occurrence frequencies of the 14 alleles typed in the HumFGA system vary between 0.15% and 19.8% (Table 5/B; Fig.13).

Fig.13: Distribution of HumFGA allele frequencies in Szeged and its environs



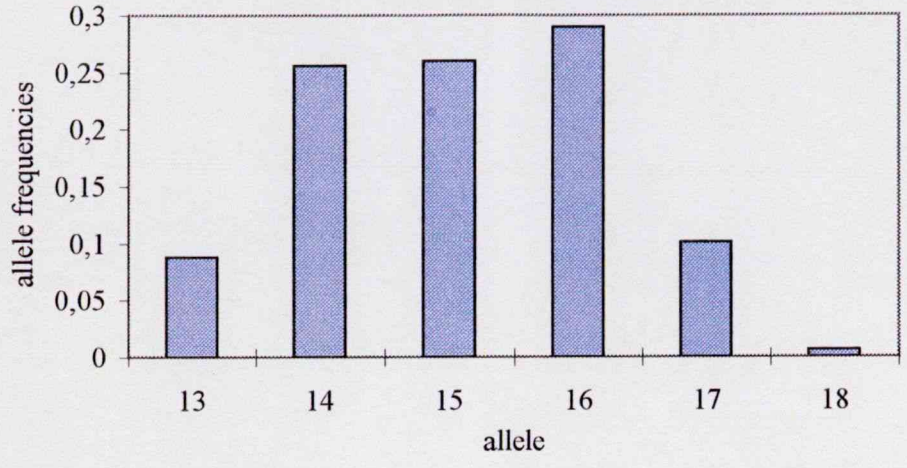
Among the STR traits localised on the Y chromosome 6 alleles were typed in DYS19 system (Table 5/C; Fig.14) in which alleles 14, 15 and 16 occured nearly with the same frequency values (15.6% -29%) while allele 18 occured at a low frequency, 0.6%.

Table 5/C

Distribution of DYS19 and DYS 390 allele frequencies in the population of Szeged and its environs

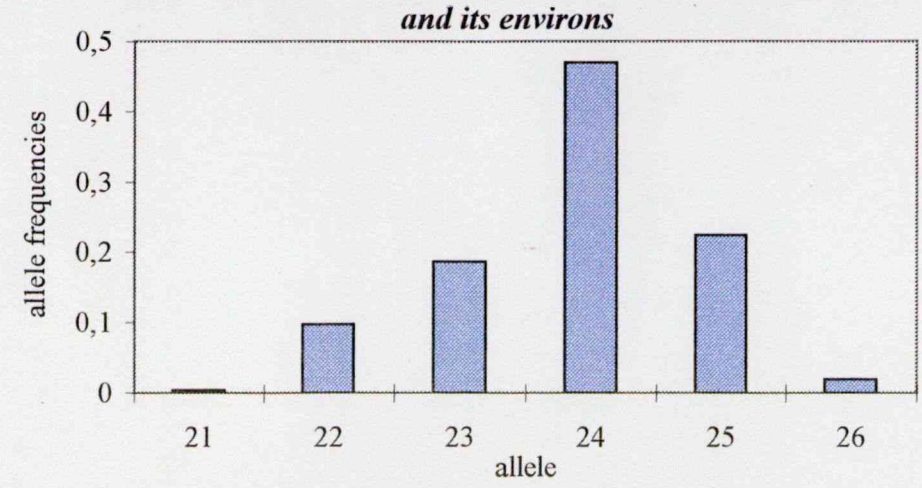
Allele	DYS19 n=308	DYS390 *n*=268
13	0.088	
14	0.256	
15	0.260	
16	0.290	
17	0.101	
18	0.006	
19		
20		
21		0.004
22		0.097
23		0.187
24		0.470
25		0.224
26		0.019

Fig.14: Distribution of DYS19 allele frequencies in Szeged and its environs



At the same time, however, of the 6 alleles typed in the DYS390 system (Table 5/C; Fig.15) the frequency of allele 24 is very high (47%) as compared to those of the others that have frequency values between 0.4% and 22.4%.

Fig.15: Distribution of DYS390 allele frequencies in Szeged and its environs



The frequency occurrence of the haplotypes defined on the basis of the combination of the typified alleles in the two systems of the tested Y chromosome is indicated in Table 6. On the basis of this it is haplotype 16/24 that has the highest frequency of occurence (12.69%), whereas haplotype 18/24 is the least frequent one (0.75%).

Table 6

DYS19/DYS390 haplotype frequencies in the population of Szeged and its environs

Haplotype	obs. (n= 268)	frequencies
13/23	5	0.0187
13/24	18	0.0671
13/25	5	0,0187
14/22	10	0.0373
14/23	23	0.0858
14/24	26	0.0970
14/25	9	0.0336
15/21	4	0.0149
15/22	11	0.0410
15/23	19	0.0709
15/24	22	0.0821
15/25	10	0.0373
15/26	3	0.0112
16/22	4	0.0149
16/23	8	0.0298
16/24	34	0.1269
16/25	22	0.0821
17/23	5	0.0187
17/24	14	0.0522
17/25	9	0.0336
17/26	5	0.0187
18/24	2	0.0075

On the basis of the allele frequencies I performed an χ^2 test to see the level of significance difference between the calculated genotype and the found genotype with.
With the χ^2 tests performed in all 5 somatic systems, there were no significant difference among the found and the calculated genotypes. There was no divergence from the Hardy-Weinberg equilibrium. (Table 7)

Table 7

Value of the χ^2 test in each STR systems

	Σχ2	df	P
HUMVWA	6.4586	16	0.98<P<0.99
HUMTH01	6.5000	14	0.95<P<0.98
HUMF13B	3.3406	8	0.90<P<0.95
HUMFES/FPS	6.7558	12	0.80<P<0,90
HUMFGA	6.6869	20	P>0.99

The expected heterozygosity calculated from the allele frequency value (Table 8) in the 5 somatic STR systems vary between 70% and 86%. The observed heterozygosity value is high in the HumVWA (80.42%) and the HumFGA (85.68%) systems, whereas they are the lowest in the HumFES/FPS (70.83%).In the case of more hypervariable systems like in HumVWA and in HumFGA the value of the heterozygote is higher, whereas in the STR systems of lower allele numbers the number of heterozygotes is lower.
In the HumVWA the expected heterozygosity varies between 80.41% and 83.9%, between 77.43% and 81.09% in the HumTHO1, between 68.46 % and 72.68 % in the HumF13B, between 68.39% and 73.2% in the HumFES/FPS and between 85.55% and 85.77% in the HumFGA systems. The expected values correlate with the observed heterozygosity values.
On the basis of the results of the discrimination index (Table 8) it is the HumVWA, HumTH01 and HumFGA systems that result in a greater possibility of distinction among the individuals (0.9247-0.9571), whereas the values are lower in the HumF13B and HumFES/FPS systems

(0.8531; 0.8564). In both systems there is an allele, therefore, they are allele 10 in HumF13B and allele 11 in HumFES/FPS that has a frequency higher than 40% in our samples, therefore, there is a lower discrimination possibility.

Table 8

Statistical parameters for 7 STR systems in Szeged and its environs
(H_{obs} observed heterozygosity, H_{exp} expected heterozygosity, DP discrimination indices, h gene/haplotype diversity, PIC polymorphic information content)

STR	H-obs	H-exp (± SE)	DP	h	PIC
HumVWA	0.8042	0.8214 ± 0.0173	0.9402	0.8210	0.8329
HumTH01	0.7853	0.7926 ± 0.0183	0.9247	0.7918	0.8023
HumF13B	0.7247	0.7057± 0.0211	0.8531	0.7049	0.7273
HumFES/FPS	0.7083	0.7079 ± 0.024	0.8564	0.7130	0.7392
HumFGA	0.8568	0.8566±0.0011	0.9571	0.8551	0.8572
DYS19	-	-	-	0.7649	-
DYS390	-	-	-	0.7116	-
DYS 19/ DYS390	-	-	0.9403	0.9757	0.9405

It can be stated on the basis of the values of the genes/haplotypes diversity and of the polymorphism information content (Table 8) that the HumFES/FPS and the HumF13B are less polymorphic and the variability of the alleles is smaller than in other three somatic STR systems.

A high discrimination index (0.9403), a high haplotype variability (0.9757) and polymorphism (0.9405) are characteristics of the haplotypes that can be determined in the two STR systems located on the Y chromosome. (Table 8)

The allele frequency value in the Szeged population and in the Szeged region have been compared to the data of other European and Asian populations, other regions in Hungary

(Budapest, Pécs) and a Romany population in the neighbourhood of Pécs in the HumVWA and HumTH01 systems (Tables 9/A-G; Fig.16-22).

The comparison of the data of Budapest and Germany in the HumVWA (Table 10) system to their own allele frequency values did not show a significant difference, whereas the frequency values of the Romany population in Pécs differed from our data considerably (χ^2 = 109.669; P = 0.000). In this population the frequency of allele 16 is higher (33.3%) than in Hungarian, other European and Asian populations (18.2-22.5%).

When our frequency values in the HumTH01 were compared (table 10) to those of Budapest (χ^2 = 5.6317;P = 0.4668) and the German data (χ^2 = 16.5319; P = 0.0110), no significant differences could be seen. But it is not the case if we compare our data to the frequency values of Romany in Pécs, the Turkish, and the Spanish populations.

Our allele frequency values in the HumF13B system (table 10) significantly differ from the Thailander (χ^2 = 183.7589; P = 0) and Turkish frequency values (χ2 = 32.4912; P = 0) where the frequency value of allele 8 is higher (38.8%) than in Europe. In the Thailander sample allele 10 has a frequency value of 64%, whereas in the German, the Spanish, the Budapest and in our own sample it is approximately 40%.

On the basis of the comparative tests on the population in the HumFES/FPS system (Table 10) it can be seen that there is no significant difference between the frequency values in the German population and our frequency value data (χ2 = 15.1032; P = 0.0830). At the same time, however, our frequency value data differ significantly from those of the Budapest and the Pécs samples. Alleles 10 and 11A were not found in the samples from either Budapest or Pécs, this can be the reason for the significant difference in the samples within one country.

In the HumFGA system (Table 10) it is only the frequency values of the Austrian and the Dutch populations that there is no significant difference from our own data. At the same time, however, the allele frequency values found during the typification of 127 individuals in Pécs has a minor but still significant difference (χ2= 49.8552;P = 0).

Table 9/A

Comparison of HumVWA allele frequencies in several populations

Allele	Hungarian-Szeged n=489	Hungarian-Budapest n=446 /26/	Hungarian Romany n = 135 /24/	German n=321 /57/	Spanish n= 100 /2/	Turkish n= 228 /33/
11	-	-	-	-	-	
13	0,001	0,002	-	0,001	-	-
14	0,119	0,108	0,085	0,095	0,100	0,103
15	0,107	0,114	0,070	0,099	0,135	0,114
16	0,182	0,206	0,333	0,204	0,225	0,162
17	0,264	0,307	0,252	0,280	0,275	0,333
18	0,206	0,173	0,233	0,220	0,200	0,173
19	0,090	0,072	0,019	0,085	0,065	0,072
20	0,015	0,016	0,007	0,013	-	0,037
21	0,001	0,002	-	0,002	-	0,004

Table 9/B

Comparison of HumTH01 allele frequencies in several populations

Allele	Hungarian-Szeged n=489	Hungarian-Budapest n=446 /26/	Hungarian Romany n=135 /24/	German n=110 /75/	Spanish n=100 /2/	Turkish n=174 /33/
5	0,004	0,002	-		-	-
6	0,236	0,220	0,237	0,207	0,190	0,333
7	0,139	0,159	0,085	0,180	0,160	0,118
8	0,139	0,114	0,233	0,126	0,095	0,126
9	0,202	0,209	0,181	0,171	0,165	0,172
9.3	0,270	0,283	0,259	0,302	0,365	0,221
10	0,011	0,013	0,004	0,014	0,025	0,023
11	-	-	-	-	-	0,006

Table 9/C

Comparison of HumF13B allele frequencies in several populations

Allele	Hungarian-Szeged n=465	Hungarian -Budapest n=223 /27/	German n=555 /3/	Spanish n=392 /53/	Turkish n=188 /33/	Thailander n=127 /30/
6	0,094	0,092	0,103	0,115	0,096	0,012
7	0,009	0,004	0,012	0,010	0,005	0,004
8	0,256	0,278	0,224	0,250	0,388	0,104
9	0,216	0,217	0,225	0,189	0,181	0,228
9C	-	-	0,001	-	-	0,004
10	0,417	0,401	0,432	0,431	0,330	0,644
10C	-	-	0,001	-	-	-
11	0,008	0,007	0,001	0,005	-	0,004

Table 9/D

Comparison of HumFES/FPS allele frequencies in several populations

Allele	Hungarian -Szeged n=360	Hungarian-Budapest n=446 /25/	Hungarian-Pécs n = 164 /46/	German n=414 /3/	Spanish n=100 /2/	Turkish n=:211 /33/
7	0,002	-	0,006	-	-	0,002
8	0,011	0,018	-	0,012	0,015	0,005
9	0,005	-	0,006	0,006	-	-
10A	0,261	0,247	0,274	0,248	0,295	0,166
10	0,073	-	-	0,056	0,060	0,026
10.3	-	-	-	-	0,005	-
11A	0,012	-	-	0,030	-	0,009
11	0,431	0,448	0,482	0,413	0,360	0,445
12A	-	-	-	-	-	0,002
12	0,160	0,224	0,189	0,188	0,220	0,291
13	0,043	0,061	0,043	0,045	0,045	0,050
14	0,002	0,002	-	0,002	-	0,002

Table 9/E

Comparison of HumFGA allele frequencies in several populations

Allele	Hungarian-Szeged n=326	Hungarian-Pécs n=127 /48/	Austrian n=525 /42/	Dutch n=205 /65/	Egyptian n=100 /42/
16	0,0015	-	-	-	-
17	-	-	0,001	-	-
18	0,015	0,004	0,010	0,015	0,015
19	0,069	0,13	0,063	0,059	0,075
20	0,153	0,098	0,147	0,137	0,095
21	0,192	0,169	0,170	0,180	0,140
21.2	0,004	-	0,001	-	0,010
22	0,198	0,205	0,207	0,166	0,160
22.2	0,004	0,008	0,008	0,017	0,005
23	0,133	0,138	0,131	0,144	0,165
23.2	0,006	0,004	0,001	0,007	-
24	0,131	0,146	0,145	0,154	0,185

Table 9/E (continued)

Allele	Hungarian-Szeged n=326	Hungarian-Pécs n=127 /48/	Austrian n=525 /42/	Dutch n=205 /65/	Egyptian n=100 /42/
24.2	-	-	-	-	-
25	0,072	0,075	0,083	0,083	0,075
25.2	-	-	-	-	0,005
26	0,018	0,016	0,025	0,029	0,040
27	0,004	0,008	0,007	0,010	0,010
28	-	-	0,001	-	0,010
29	-	-	-	-	0,005
>29	-	-	-	-	0,005

Table 9/F

Comparison of DYS19 allele frequencies in several populations

Allele	Hungarian-Szeged n=308	German-Münster n=272 /43/	Italian-Roma n=100 /43/	Slovakian-Bratislava n=57 /43/	Japanese n=221 /43/	Mongolians n=40 /43/	Chinese n=36 /43/
10	-	-	0,010	-	-	-	-
11	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-
13	0,088	0,040	0,130	0,070	0,080	-	-
14	0,256	0,570	0,520	0,190	0,030	0,270	0,250
15	0,260	0,230	0,260	0,210	0,510	0,320	0,280
16	0,290	0,120	0,050	0,310	0,230	0,300	0,390
17	0,101	0,040	0,030	0,210	0,150	0,050	0,050
18	0,006	-	-	-	0,010	0,050	0,030

Table 9/G

Comparison of DYS390 allele frequencies in several populations

Allele	Hungarian-Szeged n=268	German-Münster n=114 /43/	Italian-Roma n=100 /43/	Japanese-Osaka n=150 /43/	Mongolian n:40 /43/	Chinese n:36 /43/
19	-	-	-	-	-	-
20	-	-	-	-	-	-
21	0,004	0,030	0,010	-	-	0,030
22	0,097	0,160	0,150	0,160	0,070	0,050
23	0,1876	0,260	0,390	0,190	0,250	0,530
24	0,470	0,370	0,390	0,220	0,370	0,250
25	0,224	0,170	0,050	0,350	0,270	0,130
26	0,019	0,010	0,010	0,070	-	-
27	-	-	-	-	0,020	-

Fig.16.
HumVWA allele frequencies in some other populations

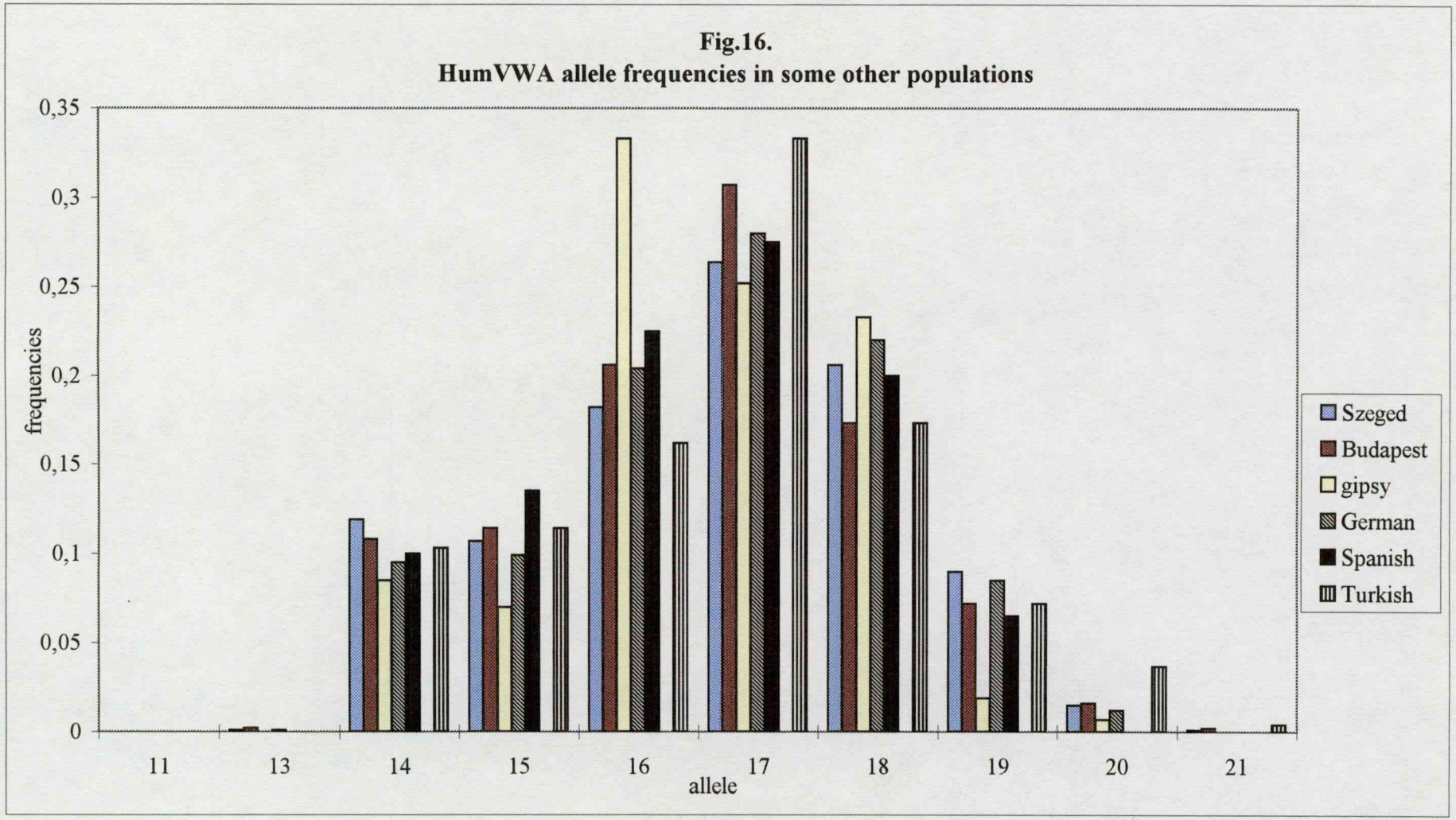


Fig.17.
HumTH01 allele frequencies in some other populations

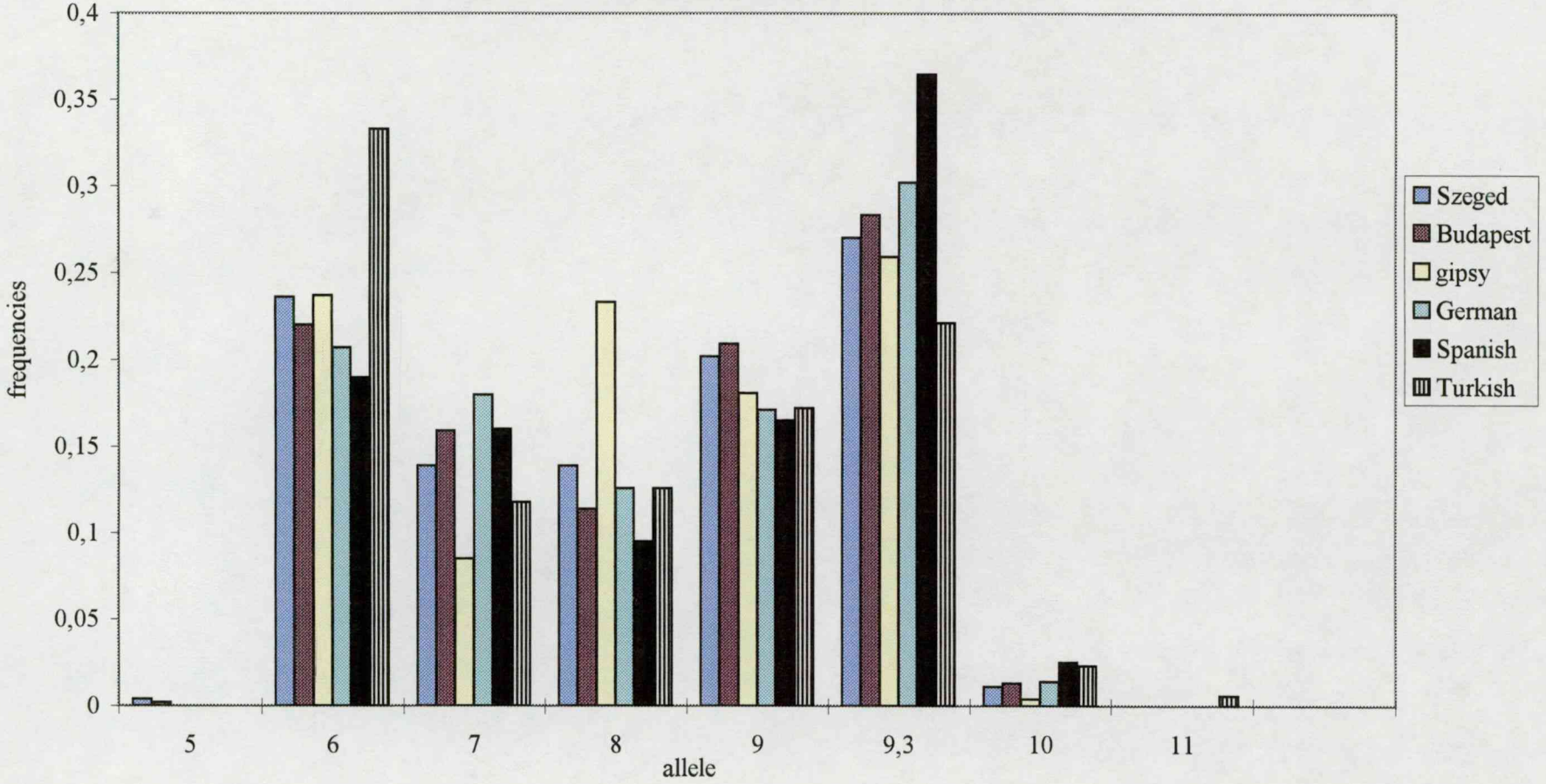


Fig.18.
HumF13B allele frequencies in some other populations

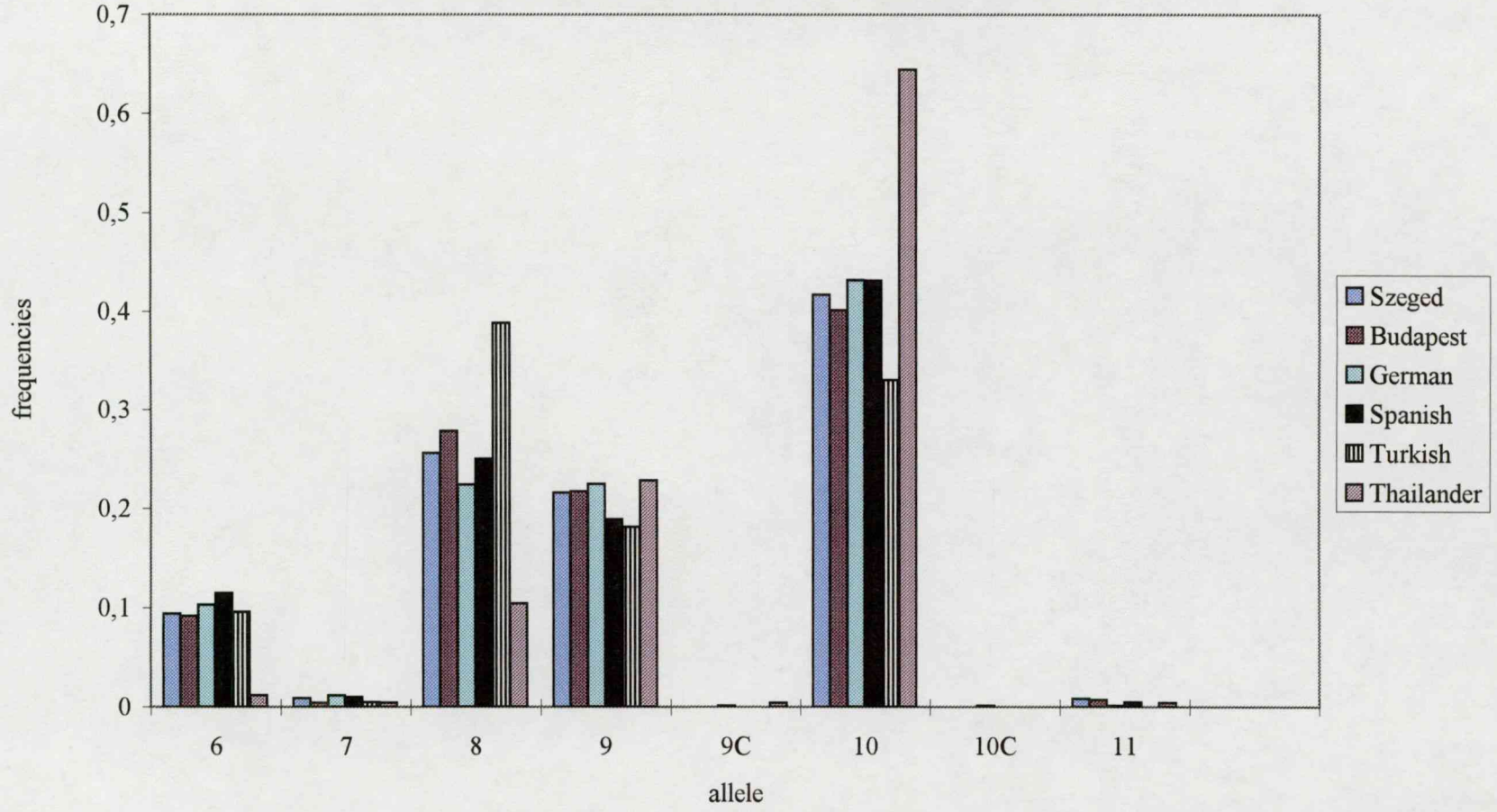


Fig.19.
HumFES/FPS allele frequencies in some other populations

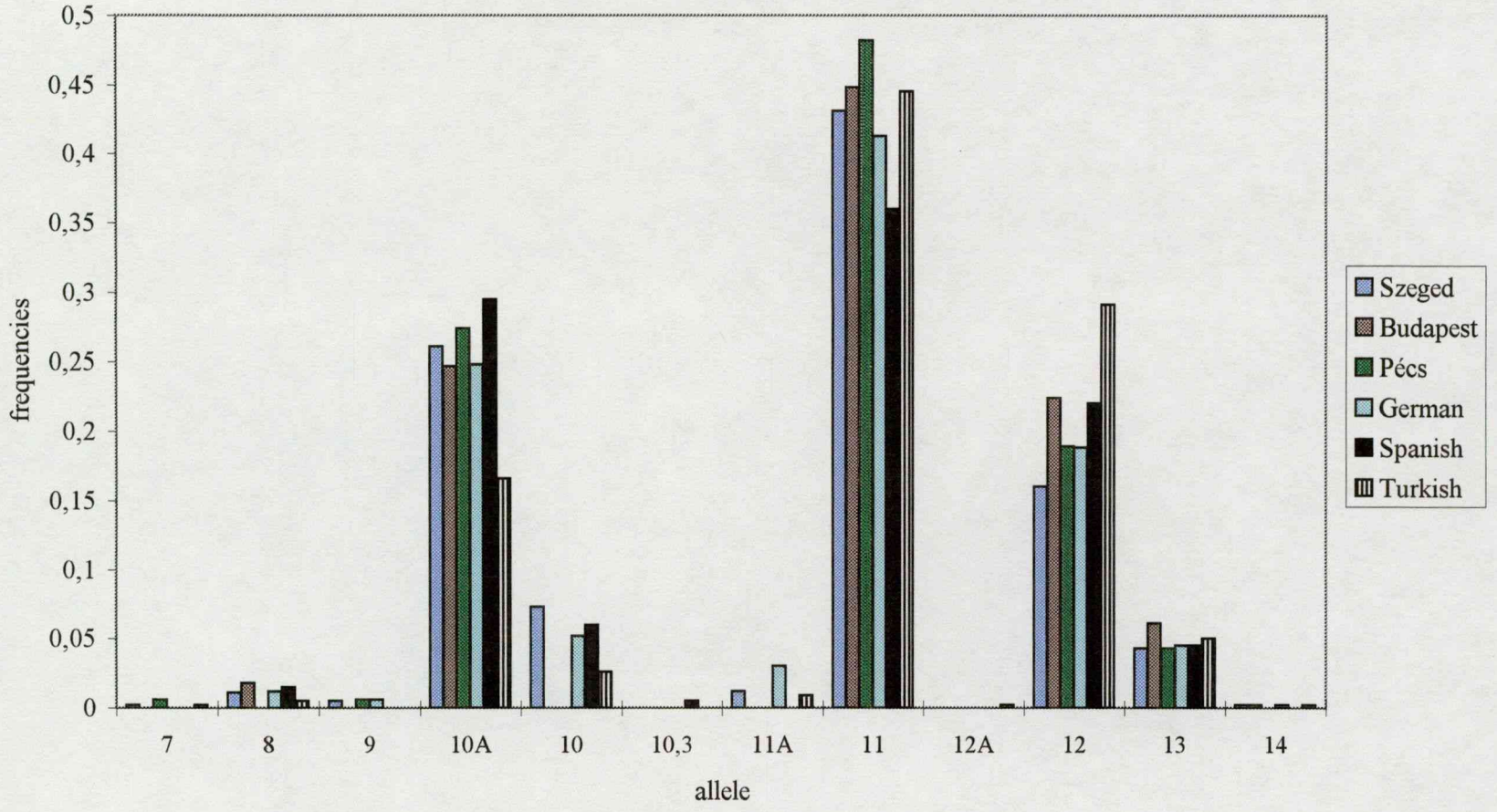


Fig. 20.
HumFGA allele frequencies some other populations

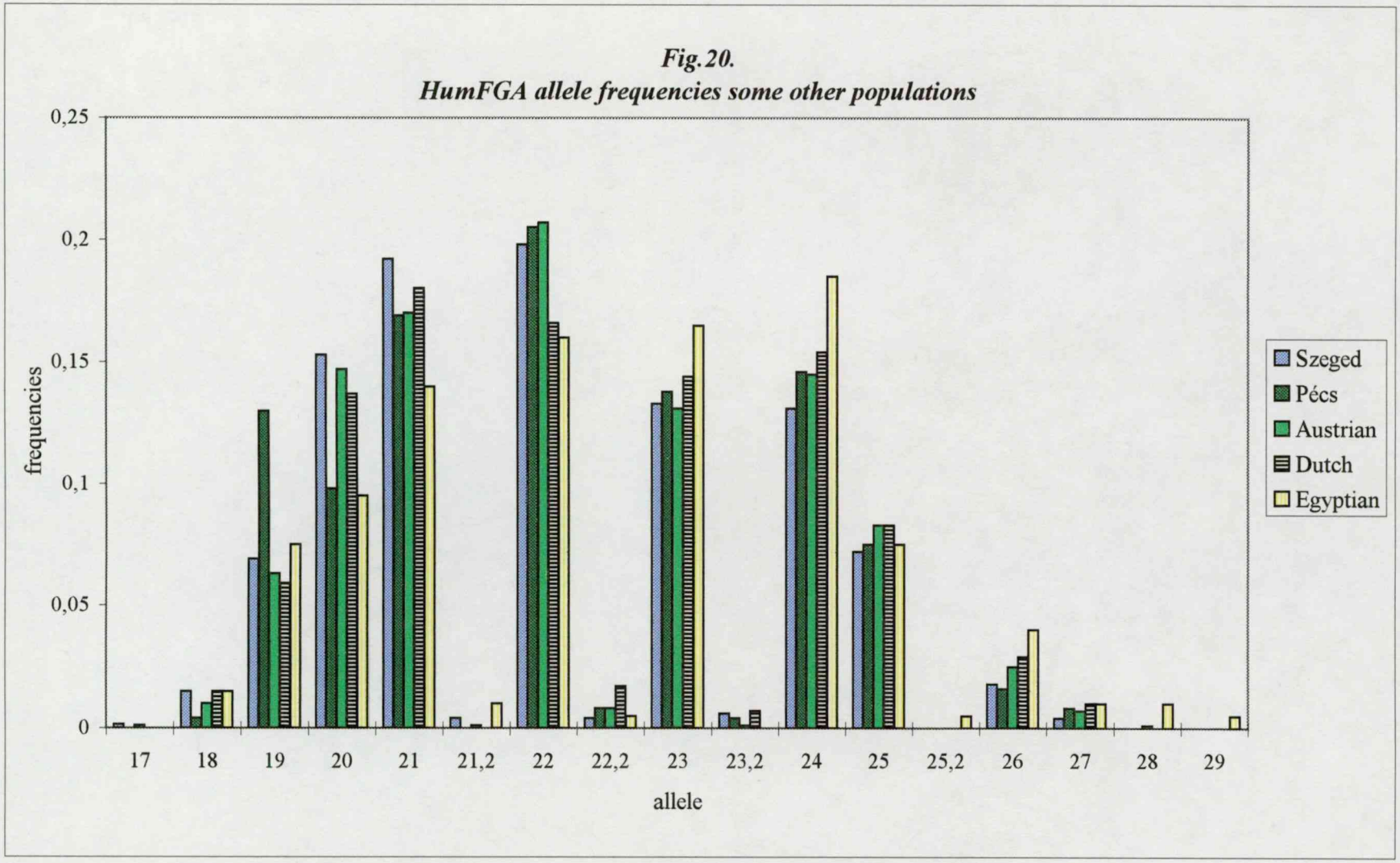


Fig.21.

DYS19 allele frequencies in some other populations

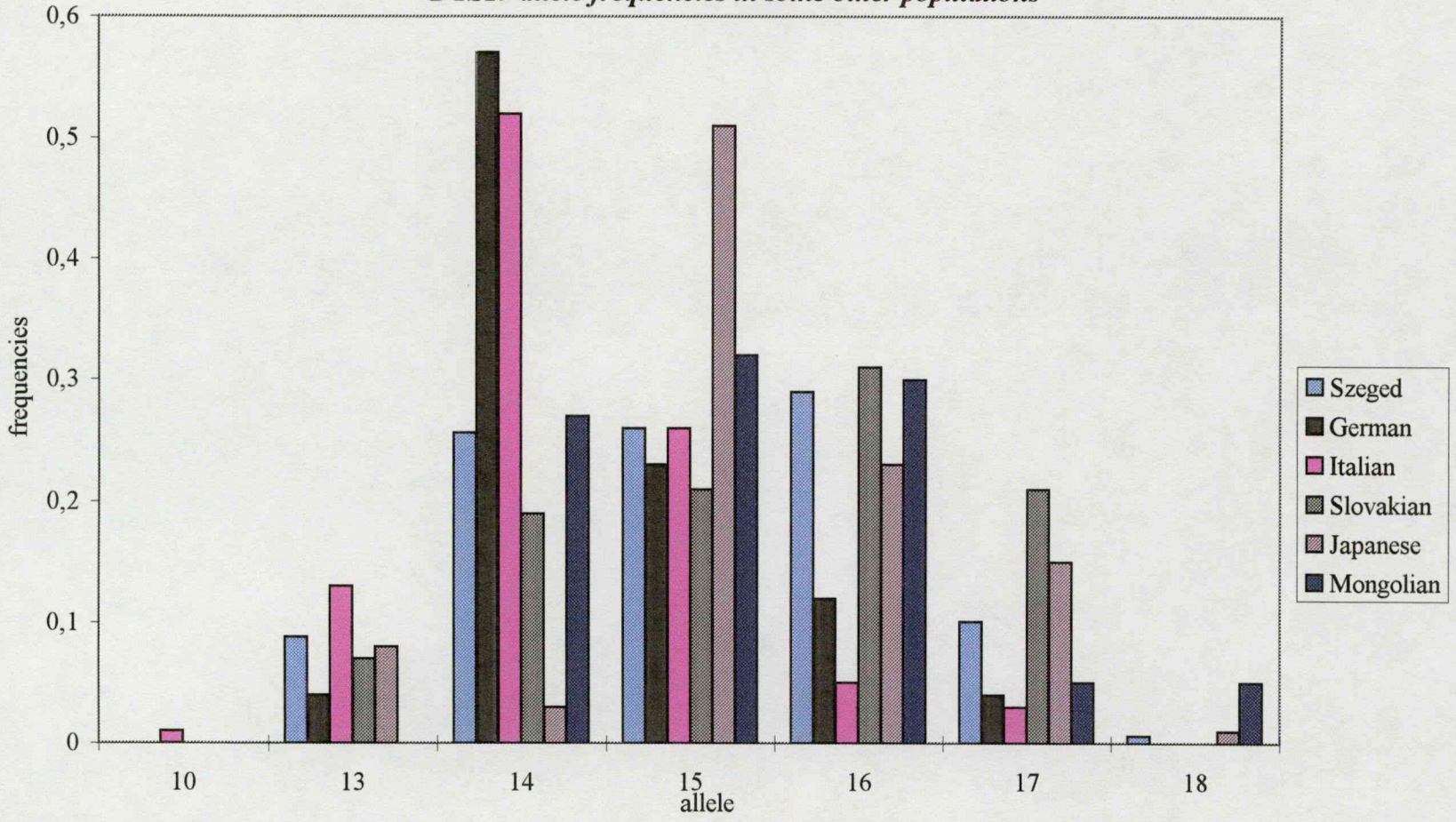


Fig.22.
DYS390 allele frequencies in some other populations

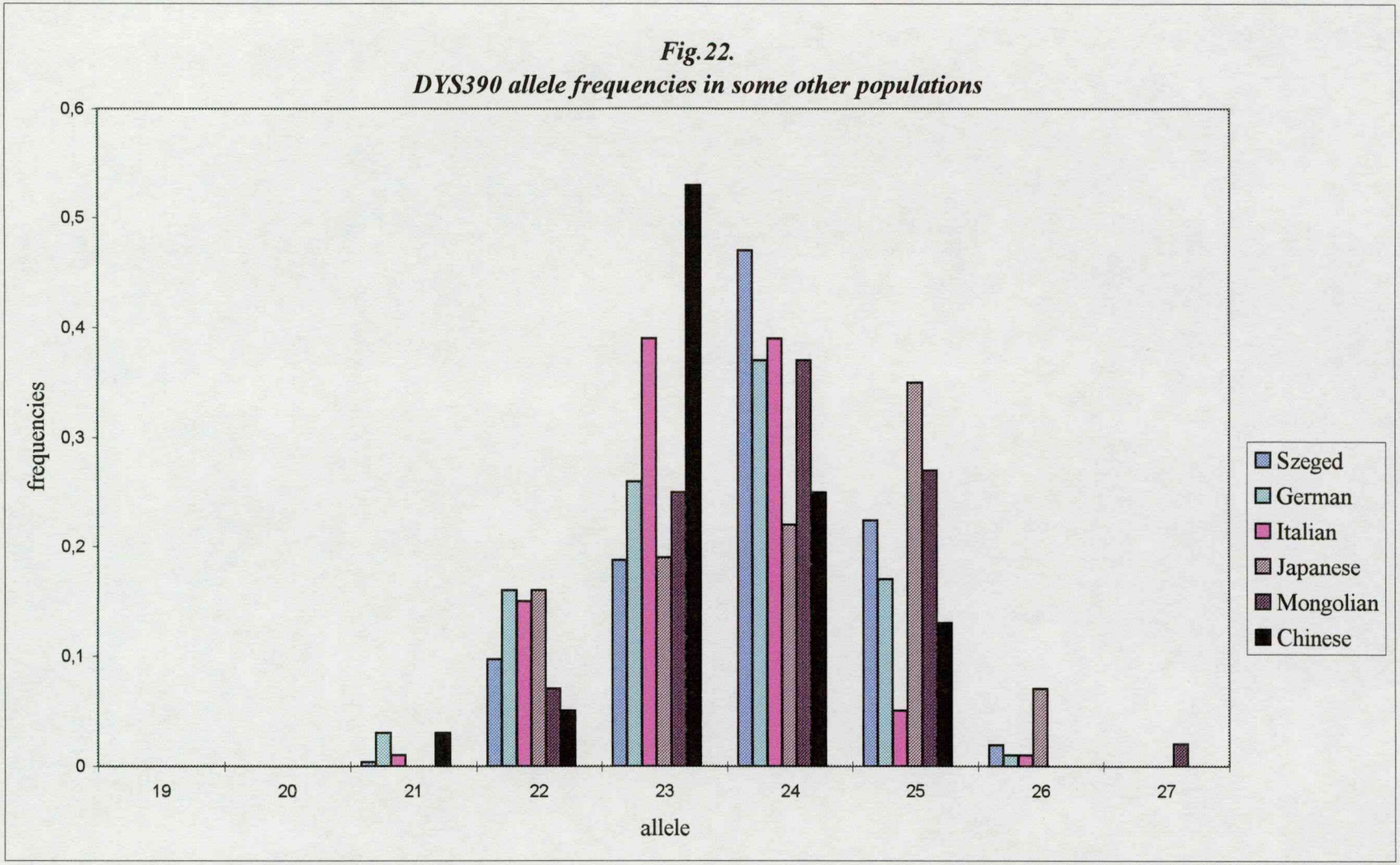


Table 10.

Comparison between different populations
(RxC contingency table)

Population	χ^2	P	G stat.	P
HumVWA				
Szeged/Budapest /26/	10.9370	0.1290	10.9651	0.2258
Szeged/Romany /24/	109.669	0.0000	114.853	0.0000
Szeged/(Pécs)German /57/	5.7074	0.6944	5.7202	0.0237
Szeged/Turkish /33/	27.4095	0.0000	28.2479	0.0000
Szeged/Spanish /2/	24.9543	0.0038	25.4254	0.0038
HumTH01				
Szeged/Budapest /26/	5.6317	0.4668	5.6500	0.4720
Szeged/Romany /24/	45.417	0.0000	47.481	0.0000
Szeged/(Pécs)German /75/	16.5319	0.0110	18.1012	0.0070
Szeged/Turkish /33/	40.4195	0.0000	44.4692	0.0000
Szeged/Spanish /2/	42.1023	0.0000	43.9157	0.0000
HumF13B				
Szeged/Budapest /27/	3.2324	0.6533	3.2816	0.6500
Szeged/German /3/	10.8657	0.0753	12.3949	0.0645
Szeged/Turkish /33/	32.4912	0.0000	35.5887	0.0000
Szeged/Spanish /53/	4.9573	0.4550	4.9692	0.4650
Szeged/Thailander /30/	183.7589	0.0000	196.4555	0.0000
HumFES/FPS				
Szeged/Budapest /25/	34.6147	0.0000	42.7623	0.0000
Szeged/Pécs /46/	25.066	0.0000	30.1919	0.0000
Szeged/German /3/	15.1032	0.0830	16.1411	0.0930
Szeged/Turkish /33/	91.9272	0.0000	96.3469	0.0000
Szeged/Spanish /2/	45.8573	0.0000	55.9511	0.0000
HumFGA				
Szeged/Pécs /48/	49.8552	0.0000	53.4326	0.0000
Szeged/Austrian /42/	17.0977	0.3120	19.2106	0.286
Szeged/Dutch /65/	27.2083	0.0111	30.2537	0.0062
Szeged/Egyptian /42/	85.3162	0.0000	98.6637	0.0000

The reason for this may be the small number of samples (127) as the population in Pécs, similarly to the population in Budapest and Szeged, is a so-called mixed population.
We have calculated the genetic distances between the various populations for each somatic system from the allele frequency values of the population of Szeged and its environs and the frequency values found in the various populations (Table 11/A).

Table 11/A

Genetic distances in the population of Szeged and its environs to some other populations

Population	HumVWA	HumTH01	HumF13B	HumFES/FPS	HumFGA
Szeged-Romany	0.2725	0.0879	-	-	-
Szeged-German	0.0115	0.0251	0.0472	0.0273	-
Szeged-Austrian					0.0250
Szeged-Spanish	0.0290	0.0761	0.0099	0.0399	-
Szeged-Turkish	0.0549	0.0616	0.0839	0.1769	-
Szeged-Thailander	-	-	0.4215	-	-
Szeged-Dutch	-	-	-	-	0.0456
Szeged-Egyptian	-	-	-	-	0.1071

We found a high value between our population and the Romany one. It was 0.2725 in the HumVWA system, whereas it was 0.0879 in the HumTH01 system. Then we analysed the differences between the various populations and our data in 2, 3, 4 DNA STR systems (Table 11/B). The joint evaluation of the HumVWA and HumTH01 systems show that the greatest genetic distance is with the Romany population in Pécs (0.36039) and the smallest is with the German population (0.03657). If we take into account the HumFES/FPS system as well, the German population is standing the nearest to that of the town of Szeged (0.06390) and the farthest is the Turkish (0.29340). The Austrian and the Spanish population data show average distance values (0.1769 and 0.1450 respectively).
When the analysed STR polymorphisms were supplemented with the HumF13B, it could be seen that there is a closer genetic relationship with the German population (0.1111) and a distant one with the Turkish population (0.3773). The Austrian and the Spanish population data show average distance values (0.1833 and 0.1549 respectively).

Table 11/B

Genetic distances of the population of Szeged and its environs to some other populations by reason 2,3 and 4 STR systems

Population	**2 systems** /HumVWA,HumTH01/	**3 systems** /HumVWA,HumTH01,HumFES/FPS/	**4 systems** /HumVWA,HumTH01,HumFES/FPS,HumF13B/
Szeged-Romany	0.36039	-	-
Szeged -German	0.03657	0.06390	0.11110
Szeged -Austrian	0.13220	0.17690	0.18330
Szeged -Spanish	0.10513	0.14500	0.15490
Szeged -Turkish	0.11650	0.29340	0.37730

We examined the difference from the European and Asian populations separately for the two systems located on the Y chromosome (Table 11/C).

Table 11/C

Genetic distances in the population of Szeged and its environs to some other populations

Population	DYS19	DYS390
Szeged-German	0.4983	0.150
Szeged-Italian	0.7116	0.4594
Szeged-Slovakian	0.1154	-
Szeged-Japanese	0.6896	0.343
Szeged-Mongolian	0.1420	0.059
Szeged-Chinese	0.1062	0.630

In contrast to the data calculated in the somatic STR systems, the population of Szeged and Szeged region is farther from the European populations in genetic terms, with the exception of the Slovakian (Bratislava) data, whereas it is nearer to the Mongolian population, especially on the basis of the DYS390 system (0.059).

If we want to make reliable statements about the genetic relatedness on the basis of the Y system, there is a need for the analysis of more Y systems and comparison of the frequencies of their calculated haplotypes is more informative than the separate evaluation of the two systems.Unfortunately, the haplotype frequencies values haven't been found in the data of the examined populations.

On the bases of the results of the genetic distance examinations in the somatic STR systems show a closer genetic connection with the European populations which is probably the result of the migrations in the cours of history.

The Y chromosome examinations tend to suggest a closer genetic relatedness with an Asian (Mongolian) population.This is supported by the Y chromosome test by Lahermo et al. (1999). Their data in the Finnish population were compared to the results of tests on 18 populations, including a mixed population sample from Hungary (Budapest) and a Chango sub-population sample. Our test indicate that genetically the Hungarian population is nearer to the Asian (Mongol) than to the European population.

5. Applicability of the DNA tests in parentage cases

The DNA tests have been applied effectively in criminal and civil law cases all over the world. In my dissertation I wish to report on the national applicability of the DNA examinations in status cases that have been introduced in Hungary first.

5.1. About parentage examination

Our Institution has been conducting blood group tests since 1979. In the beginning we analysed 14 and since 1984 17 blood group systems.

The blood group tests are the so-called negative proving in the judicial practice. If the paternity is excluded, the judge dismisses the case.

The exclusion may be based on:

Lack of characteristics (rule of Bernstein I)

In the disputed child has an allele that cannot be found either in the mother or the father.

Mother: MM
Child: MN
Putative father: MM

Opposite homozygosity (rule of Bernstein II)

The man and the child have opposite homozygosity for a given blood group characteristic.

Mother: MN
Child: NN
Putative father: MM

In the non-excluded cases we provide a mathematical probability. It is a matter of the judge to decide on the paternity.

The calculation of probability is based on the occurrence frequency of the blood group characteristics in a given population. As for the probability of paternity, there are the following categories:

1.01% - 5%	Paternity is improbable
5.01% - 94.99%	No probability opinion can be issued
95.0% - 98.99%	Paternity is probable
99.0% - 99.74%	Paternity is probable to a great extent
99.75% - 99.99%	Paternity is practically proven

(On the basis of the 17rd Methodology Letter of the National Institute of Forensic Medicine, 1998)

Since the blood group tests are rated as negative proving, it is useful to determine characteristics the theoretical exclusion probability of which is relatively high.
The theoretical exclusion probability means the number of males out of 100 who are not fathers and who can be excluded with the given method.
In order to determine the theoretical exclusion probability there is a need for the allele frequency values of the various systems. The formula of the theoretical exclusion probability according to Hummel and Gerchow (1981) is as follows:

$$PE = \sum P_i(1-P_j)^2 + \sum(P_iP_j)^2 (3P_i+P_j-4)$$

P_i = the first allele frequency
P_j = the second allele frequency

The so-called combined exclusion probability (Table 12) can be calculated from the theoretical exclusion probabilities of the various systems, the formation of which according to Jancik and Speiser (1952) is as follows:

$$Pcomb = 1-(1-PE_1)\ (1-PE_2)\ (1-PE_n)$$

The exclusion probability of the 17 systems that can be typified in a laboratory is 95.79%, which means that the paternity 4 of 100 tested males is not excluded, yet they are not the biological fathers.
For this reason we need to introduce systems and characteristics that can increase the combined paternity exclusion probability to nearly 100%.

Table 12

Probability of exclusion of blood groups systems and combined paternity exclusion

Serial no.	System	Exclusion probability/PE/	Comb.paternity exclusion /Pcomb/
Blood groups antigens			
1	ABO /28/	0.186	
2	MNSs /28/	0.311	0.439
3	Rh /28/	0.277	0.594
4	Kell/cellano /28/	0.034	0.608
5	Duffy /28/	0.187	0,681
Serum groups			
6	Hp /28/	0.184	0.740
7	Gc /28/	0.161	0.782
8	Pi /17/	0.226	0.831
9	C3 /50/	0.123	0.852
10	Tf /16/	0.195	0,880
Enzymes			
11	PGM_1sub. /15/	0.288	0.915
12	AP /28/	0.223	0.934
13	GLOI /45/	0.182	0.946
14	ADA /28/	0.039	0.948
15	AK /28/	0.019	0.949
16	EsD /44/	0.084	0.953
17	PGP /14/	0.105	***0,958***

The results of the blood group tests of the past 20 years (Table 13 and Fig.23;Fig 24) indicate that the paternity probability is not very great in 34% of the cases, i.e. 30-99%. It makes necessary to perform further tests that can either increase the probability value or result in the exclusion of the paternity.

Table 13

Results of the paternity examinations between 1979 and 1995

Probability of paternity	Paternity cases	
	N	%
< 95%	188	12.29
95.01 % - 99 %	329	21.50
99.01 - 99,75 %	297	19.41
99.75 % <	339	22.16
Exclusion		
1 system		
opp.homozyg.	58	3.79
lack of charact.	36	2.35
more systems	283	18.50
Sum total	1530	100.00

Fig. 23.
Results of paternity examination between 1979 and 1995

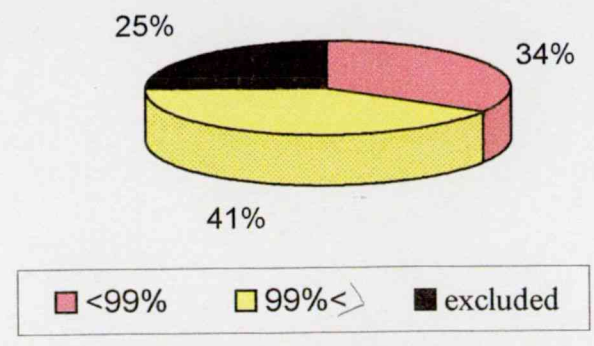
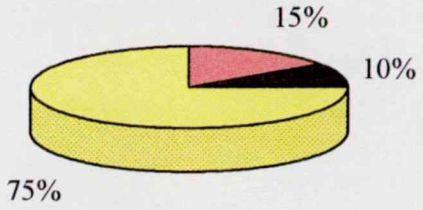


Fig.24.
Distribution of exclusions between 1979 and 1995



opp.homozyg. 1 syst. ■ lack of charact. 1 syst.
more systems

In 15% of the exclusions, however, paternity was excluded on the basis of the rule of homozygosity in one system. This could as well be a false exclusion as well because the phenotypically homozygotic characteristic can be heterozygotic where one allele is silent, i.e. cannot be typed by the applied blood group methods. Its presence can only be proven or excluded with the so-called extended family tests, i.e. in an indirect way.

The extended family tests mean that the male's ascending relatives are also tested. If incompatibility can be found there as well, especially between the male and his mother, we can speak about a silent allele that will also appear in the child. In our Institute there were 58 such cases between 1979 and 1995 but we could perform extended tests in 7 cases altogether and could only prove our hypothesis in 2 cases (Table 14).

Table 14

Result of the extended family test in the paternity cases

	Number of -cases	Rare allele	
		Proved	Not proved
Extended family test	**7**	2	5
Father of the defendant died	14		14
Mother of the defendant died	6		6
Parents died	9		9
Dwelling-place of the parents unknown	22		22
Sum. total	**58**	2	56

When it was not possible to perform a family test or it did not have any result, we did not consider it an evident exclusion but we suggested to the court that further tests (HLA) should be carried out.

In professional practice, however, incompatibility based on the lack of the characteristics can be regarded as exclusions even if they are of one system. It occurred in 1-2% of our cases and they were assessed as exclusions until the beginning of the early 1990s.

Bender et al.(1991) reported on a paternity case where an exclusion constellation was found between the male and the child that was based on the lack of a characteristic in the Pi system. With a DNA test the replacement of a nucleotide was proven as a result of crossing-over inside a gene of meiosis. It induced a change in the primary structure of the protein took place that resulted in the transformation of the phenotype.

This report warns us that we have to take into account the possibility of a mutation in the exclusions within one system. It needs exclusion within more systems so that the result can be interpreted as exclusion.

Bein et al.(1998) reported a paternity case where the male's paternity was excluded on the basis of both the blood group and the DNA systems located on the same chromosome. It was proven with a sequence analysis that at the birth of the mother's gamete the 6^{th} chromosome was inherited in diploid form with meiosis non disjunction. At the same time there were not three but two 6^{th} chromosomes in the zygote that developed from the mother's and the father's chromosome eliminated. This statement supports the hypothesis that exclusion can only be accepted if it is located in more than one system and on various chromosomes. It can rarely be done with blood group tests only.

The confirmation of the exclusions that are based on the Bernstein I and Bernstein II rules in one system as well as the low paternal blood group probability values in 30-40% of all cases make it necessary that the systems that can be tested and the methods should include DNA tests as well.

5.2. Results and evaluation of the DNA tests

In our institute we started to utilise the possibilities provided by the DNA STR systems in paternity cases in 1996. At first three somatic STR systems and since 1997 five somatic and two Y-STR systems were tested.

The exclusion probability and the combined exclusion probability of the various DNS STR systems have been summarised in Table 15.

Table 15

Probability of exclusion of DNA STR systems and combined paternity exclusion

Serial numb.	System	Exclusion probability /PE/	Comb.paternity exclusion /P comb/
1	HumVWA	0.878	-
2	HumTH01	0.659	0.957
3	HumF13B	0.134	0.964
4	HumFES/FPS	0.345	0.977
5	HumFGA	0.888	0.997
6	DYS19/DYS390	0.790	**0.9995**

Among the somatic STR systems the exclusion probability of the HumFGA is the highest (88.8%) and that of the HumTH01 is the lowest (65. 9%). It is alone greater than that of any blood group system. In most of the blood groups only 2-3 alleles are combined in the population and it does not make greater discrimination possible.

With the application of 7 DNA STR systems alone we can achieve an exclusion probability of 99.9% that would make the test of all 17 blood group characteristics unnecessary. We should not advise to disregard the blood group tests because they can be used as filters on one hand and on the other hand with their joint application it is possible to test various – protein and DNA – systems located on several different chromosomes.

On the basis of the previously quoted reference data and our own data, it is not necessary to make any further tests where with a blood group test an exclusion constellation can be detected in a characteristic that can be found on at least two different chromosomes.

In the case of the exclusion of one system and where the probability of paternity is below 99%, it is necessary to include the DNA tests as well.

Among the blood group tests only those are worth being carried out the costs of which are not too high and the probability of exclusion is relatively high.

I recommend the application of nine blood group systems in the paternity cases. The combined exclusion probability of the nine systems is 85.4% (Table 16).

Table 16

Probability of exclusion and combined paternity exclusion of nine blood group systems

Serial numb.	System	Exclusion probability /PE/	Comb.paternity exclusion /Pcomb/
1	ABO	0.186	
2	Hp	0.184	0.336
3	Tf	0.195	0.466
4	Pi	0.226	0.587
5	C3	0.123	0.638
6	PGM_1sub.	0.288	0.742
7	AP	0.223	0.800
8	PGP	0.105	0.821
9	GLOI	0.182	**0.854**

The exclusion probability in the case of females (with the blood group and DNA tests – 5 DNA-STR systems) it is 99.97%, whereas in the case of males (with 7 DNA-STR systems) it is 99.99% (Table 17).

Table 17

Combined paternity exclusion with application of the blood groups and DNA STR systems

Combined paternity exclusion /Pcomb./	**Female**	**Male**
Pcomb blood group systems	0.854	0.854
Pcomb DNA	0.997	0.999
Pcomb blood group systems +Pcomb.DNA	**0.9997**	**0.9999**

It means that where the child is a girl the paternity of 3 out of 10 000 males can not be excluded, although, they are not the necessarily the biological fathers.

Where the child is a boy the paternity of 1 out of 10 000 man can not be excluded. In contrast however the analysis 17 blood group tests where the paternity of 400 men out of 10 000 is not excluded, apart from the paternity of the biological father.

In 1996-98 we performed DNA-tests together with blood group tests in nearly 150 family in paternity cases. Our test results in the year 1997 are indicated in Table 18 and Fig. 25.

Blood group tests indicated a probability rate below 95% in 27 cases out of 119. In 30 cases paternity was probable and in 43 cases probability was above 99%. Exclusion was found in 19 cases, 7 of which were from one system and 12 of them were from more systems. Blood group tests with paternity probability below 95% indicated probability in only 6 cases above 95%, whereas in 18 cases it was above 99% and in 3 cases it was excluded with DNA examinations. Blood tests with probability results between 95%-99%, except 2 cases,indicated probability above 99% with DNA tests. At a serological probability value of 99.01%-99.75% we found one exclusion on the basis of HumVWA system where:

Mother	HumVWA 15/18
Child	HumVWA **16**/18
putative father	HumVWA **17**/19

Probability of paternity in the case of blood group tests was 99.72%. The child had a 16 allele

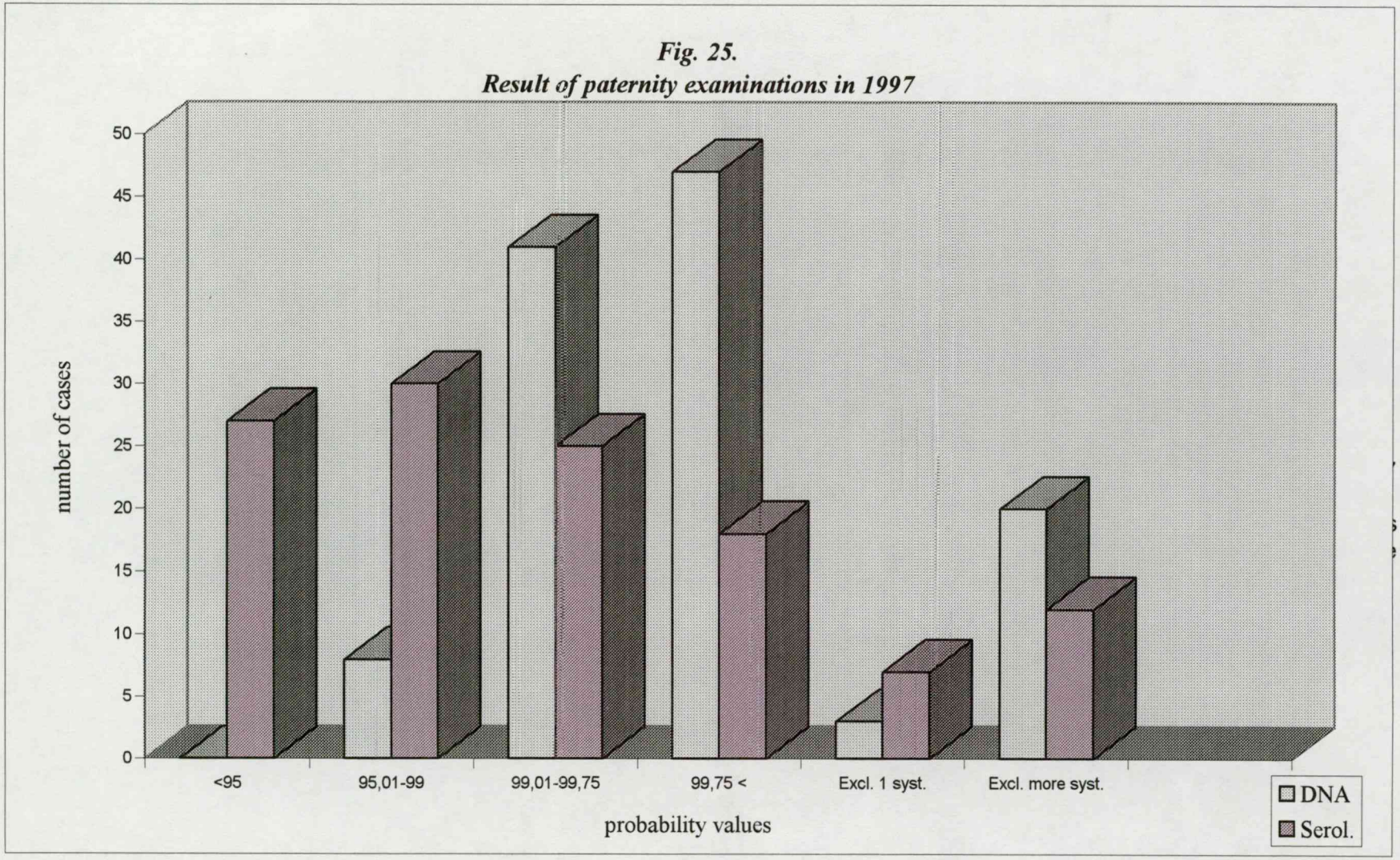
Table 18

Result of the paternity examinations in 1997

DNS / Serol	< 95 %	95.01% - 99 %	99.01% - 99.75 %	99.75 % <	Exclusion 1 system	Exclusion more systems	Sum. total
< 95 %	-	-	-	-			
95.01 %- 99 %	6	2	-	-			8
99.01 %-99,75 %	8	12	21	-			41
99.75 % <	10	16	3	18			47
1system			1		2		3
Exclusion		-		-		-	
more systems	3				5	12	20
Sum.total	27	30	25	18	7	12	**119**

Fig. 25.

Result of paternity examinations in 1997



and the putativ father had 17 allele,this incompatibility indicated the possibility of mutation. In the HumVWA system the mutation rate is 0.199% as stated by Brinkmann (1998), that made

the determination of the sequence of the HumVWA locus in the Institute of Forensic Medicine in Münster. The sequence analysis assumed the insertion at the child or deletion at the putative father of „TCTG" tandem repeat sequence in the HumVWA locus (Table 19).

Table 19

Sequence structure of 16 and 17 allele HumVWA system

Allele	bp	Sequences
16	146	5' -TCTA $(TCTG)_3$ $(TCTA)_{12}$ TCCA TCTA-
17	150	5' -TCTA $(TCTG)_4$ $(TCTA)_{12}$ TCCA TCTA-

In 2 cases among the blood group incompatibilities in one system we found exclusion on the basis of the DNA system. As well as they were found in a 2 different systems located 2 different chromosomes, we excluded the alleged male's paternity.

In another case of exclusion in one system we excluded paternity on the basis of more than one DNA systems as well.

Where paternity is not excluded with either a blood group test or a DNA test, we calculate a combined probability. The combined probability values calculated from the blood group tests and DNA tests done in 1997 are included in Table 20 and Fig.26.

TABLE 20.

Combined probability values of the blood group tests and DNA tests in 1997

Szerol.+ DNA / Szerol.	< 95 %	95.0% - 99 %	99.0% - 99.75 %	Sum. total
< 95 %	-	-	-	
95.0 %- 99 %	-	-	-	-
99.0 %-99.75 %	6	6	3	15
99.75 % <	18	24	21	63
Sum. total	24	30	24	**78**

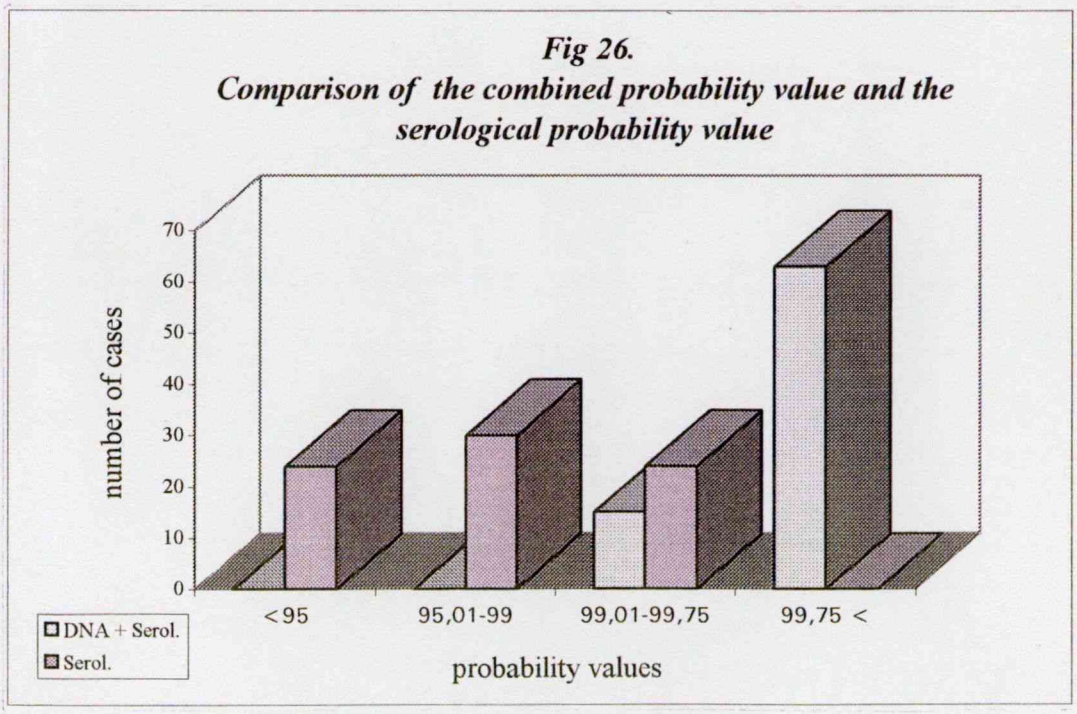


Fig 26.
Comparison of the combined probability value and the serological probability value

In 6 cases where probability of paternity was below 95% the DNA tests indicated probability between 99% and 99.75%, whereas in 18 cases it was above 99.75%. In 6 out of 30 cases where paternity was "probable" according to the blood group tests, the DNA tests indicated that paternity was "probable to a great extent" and in the remaining 24 cases it was "practically proven". In 3 cases where blood group tests showed paternal probability values between 99% and 99.75%, the probability value was not changed, whereas in 21 cases it rose above 99.75%.

Our results indicate that with the joint application of the blood group and the DNA tests in the not-excluded cases we can achieve so high probability values that are suitable for the proving of the so-called positive paternity, too.

Evident exclusions – by taking into account the possibilities of mutations (Bender, 1991; Bein,1998 and Henke, 1993) – only those exclusions can be accepted that are located on different chromosomes in more than one systems and can mostly be achieved only with the joint application of blood group and DNA tests.

If with the joint methods we cannot achieve an exclusion value on more chromosomes, mutation can be assumed the proving of which is possible with the sequence analysis of the DNA section.

Both our tests and reference data suggest the extension of the Bernstein rules with the exception that incompatibilities located on more than one chromosomes can only be regarded as exclusions based on either the lack of a characteristic or the rule of opposite homozygosity.

6. Summary

In 35% of the paternity cases serological tests do not provide great certainty which make the introduction of new methods and systems and the DNS tests necessary. In Hungary these tests have been used in civil court cases since 1996.

To introduce new systems in forensic medical practice it is necessary to perform so called population genetic tests in order to learn about the allele frequency that is the basis of the parental probability calculations.

In the first part of my paper I report on the frequency of the occurrence of the allele frequency values of HumVWA, HumTH01, HumFGA, HumF13B, HumFES/FPS, DYS19 and DYS390 STR in the population of the town of Szeged and its vicinity.

489 unrelated adults were tested for the characteristics in the HumVWA and HumTH01 systems, 465 in the HumF13B system, 360 in the HumFES/FPS and HumFGA systems, 308 in the DYS19 and 268 in the DYS390 systems.

The frequency values do not differ from the Hardy-Weinberg equilibrium.

In the knowledge of the allele frequency values of the expected hereozygosity (H), discrimination index gene and haplotype variance (h) and data characteristic of the polymorphism of the systems (PIC) can be determined in order to characterise the individual STR systems.

The value of the heterozygosity, the variance of genes and the degree of polymorphism in the systems of higher allele number, i.e. in the hypervariable systems (HumVWA, HumFGA), are higher. At the same time the statistic values are lower in systems of lower allele number or in systems where one allele can occur with greater probability than any other allele (HumTH01, HumF13B, HumFES/FPS).

It can be seen in the comparison of the allele frequency values of European, Hungarian, Gypsy and Asian populations that in the somatic STR groups there is no significant difference between our data and the European population data.

The tests on genetic distance show that the allele frequency values of the somatic STR systems in the European population groups do not differ significantly which is probably the result of the migrations in the course of history.

However, the characteristics of the Y chromosome that is inherited on the father's line show a close connection with the Asian (Mongolian) population will be tested in our laboratory.

In the second part of my paper I analyse the applicability of the DNA STR systems in parentage cases.In 30-40% of the analysed cases the probability value of the parentage with blood group tests was between 30-99% and in 2-6% of the cases in one system it was excluded which require more tests in order to prove paternity or its exclusion without doubt. By testing only the 7 DNA STR we can merely get a paternal probability over 99% or an exclusion. Despite this fact, it is not recommended to exclude blood group tests because with the joint application of the blood group and the DNA test it is possible to examine the different protein and DNA systems that are located on several different chromosomes. With their help any incorrect opinion can be eliminated that might be caused by probable mutations. At the same time the 16 blood group characteristics become unnecessary. Only those should be used that has a relatively high exclusion probability and the costs of which are not high. I recommend a series of tests of 9 blood group characteristics and 7 DNA systems with the help of which parental exclusion can be defined with near 100% probability. Data from other authors and our own data also indicate that the classical exclusion rules (Bernstein I and Bernstein II) can no longer be applied in their original form. I recommend the alteration of the exclusion rules in a way that they should only be acceptable if the characteristics are located on more chromosomes than one different chromosomes, because of probable mutations.

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