Forensic Science International: Genetics 4 (2009) 11–20

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

A comparative analysis of two different sets of Y-chromosome short tandem repeats (Y-STRs) on a common population panel

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ARTICLE INFO

Article history:
Received 20 June 2008
Received in revised form 15 March 2009
Accepted 29 March 2009

Keywords:
Forensic science
Short tandem repeats
Microsatellites
Y-chromosome
Y-STR

ABSTRACT

A comparative analysis of two Y-STR loci sets was conducted on a population sample of 224 individuals, 114 Caucasians and 110 African Americans. One set of loci, designated the OSU 10-locus set, comprises variable, single copy, male-specific loci that are dispersed across the Y-chromosome. Parallel evaluations were performed using the 10 Y-chromosome loci most frequently used for forensic analysis, the loci chosen as the SWGDAM Y-STR loci. The OSU 10-locus set had a greater average number of alleles per locus and higher average gene diversity than the SWGDAM loci. The OSU 10-locus set found 220 unique haplotypes in 224 individuals. In ~6000 pairwise haplotype comparisons for each population with each set of loci, the OSU 10-locus set also yielded a greater average number of allelic differences per pair than the SWGDAM loci. Finally, the overall linkage disequilibrium levels were lower for the OSU 10-locus set in the Caucasian population. In general, the OSU 10-locus set revealed a higher power of discrimination than the SWGDAM set.

1. Introduction

Gender specific markers, such as Y-chromosome short tandem repeats (Y-STRs), are valuable tools in DNA forensic analyses because men commit the majority of violent crimes [1]. Y-STR loci are utilized for many forensic applications which include the preferential amplification of the male DNA contribution in mixed samples and the determination of the number of male individuals in multiple male contributor cases. Y-STRs are also useful in kinship cases to identify patrilineage, particularly in situations involving a deceased putative father. In evolutionary studies, Y-STRs have aided in the identification of paternal migration patterns, contrasting with maternal migration patterns identified by mitochondrial DNA [2,3].

Until recently, approximately 50 Y-STR loci had been identified and/or characterized in the literature [4–14]. In 2004, 166 new loci were identified and examined in eight individuals, each from a different SNP-based haplogroup [15]. In terms of physical location within the Y-chromosome, most loci are limited to two small regions fairly close to the Y-chromosome centromere. Even though there is a lack of normal meiotic recombination on the Y-chromosome there is the potential for non-random association. In this paper we will refer to non-random association between loci as "linkage disequilibrium." Since recombination is expected to be absent for loci on the male-specific region of the Y-chromosome, decay of linkage disequilibrium will occur only by the accumulation of new mutations on pre-existing haplotypes. Higher mutation rates would result in lower linkage disequilibrium. Loci located within the same small region of the Y-chromosome may have similar mutation rates. If loci in the same region were to have low mutation rates there is the potential for high linkage disequilibrium. Choice of loci randomly dispersed would reduce the possibility of correlated mutation rates. Additionally, potential intrachromosomal recombination and conversion within the Y-chromosome as described by several researchers compounds the problem of potential non-random association between closely located loci [16,17]. In addition to the distribution within the Y-chromosome, some Y-STR loci have additional limitations; these include limited discrimination power, and multi-allelic profiles. Information from the human genome project and from the literature indicates that a number of the current loci are duplicated

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doi:10.1016/j.fsigen.2009.03.004

elsewhere on the X- or Y-chromosome. For some loci duplicate copies and extra alleles routinely appear [8,18–27]. While these loci may be variable, for forensics they present more complicated interpretations, particularly for resolving mixture profiles from two or more male contributors. For other loci, detection of a multiple allele profile is less problematic particularly when detected only occasionally [8,17–19,24–26,28]. Thus, selection of additional forensically useful Y STR loci is based on those loci that tend to yield one allele per individual in the population.

We identified a series of new Y-STR loci that address the aforementioned concerns associated with previous loci (Maybruck et al., submitted for publication). Utilizing resources of the Human Genome Project, we constructed a library of 465 potential Y-STR loci (tri-to-hexanucleotide repeats) located outside of the two regions of concentrated loci. A set of 229 potential loci was evaluated. The remaining 236 loci were not assessed because of close proximity to the 229 loci tested. BLAST searches of the genome revealed that 73% of the 229 loci are duplicated elsewhere in the human genome, mostly on the X- and/or Y-chromosome. Allelic variation and potential multi-allele profiles per locus were assessed at the remaining 62 loci in a test sample of 30 male individuals, 26 of which were Caucasian and African American (16 Caucasians, and 10 African Americans) to further narrow the candidate loci. The final subset of 10 loci, The Ohio State University (OSU) 10-locus set, is comprised of the following loci: DYS471, DYS448, DYS487, DYS488, DYS504, DYS576, DYS685, DYS688, DYS703, and DYS707. During the course of our study several researchers identified some of the same loci [14,15,29]. In order to prevent multiple designations for the same locus, albeit with different primer sequences, the earliest locus designation is utilized as suggested by the DNA Commission of the International Society of Forensic Genetics (ISFG) [30]. The loci were further screened in several females to test for potential amplification from non-Y sources. Examination of the OSU 10-locus set revealed 26 unique haplotypes in 26 individuals, suggesting a potential high haplotype diversity. One of the loci, DYS448, has been included in the AmpFℓSTR® Yfiler® PCR amplification kit (Applied Biosystems, Foster City, California) [31].

Since the OSU 10-locus set was highly informative in our test sample, two larger populations were examined and a comparative study was conducted to evaluate the power of these combined loci relative to the 10 Scientific Working Group on DNA Analysis Methods (SWGDAM) selected loci: DYS19, DYS385, DYS389I and II, DYS390, DYS391, DYS392, DYS393, DYS438 and DYS439 [32].

The intent of this study is to evaluate the discrimination power of the newly identified loci, and whether they have the potential to enhance the discrimination power of Y-STR analysis for forensic applications. The SWGDAM loci are firmly established. In previous studies several common haplotypes were observed [18,19,33,34]. In this study, individuals with identical haplotypes for the SWGDAM loci were differentiated by the OSU set.

2. Materials and methods

2.1. Sample collection

A population of 224 unrelated individuals was screened for the study: 114 Caucasian, and 110 African American. The Biomedical Sciences Institutional Review Board at The Ohio State University approved the use of human subjects and the protocol. Buccal samples were collected from 26 male individuals from Ohio-16 from non-criminal material were made available by the State of Ohio Bureau of Criminal Investigation and Identification (BCI), all of which were stripped of their identifiers; the remaining 10 samples were amassed from unrelated residents of Columbus, Ohio. No personal identifiers were associated with these samples, thus providing anonymity for the donors. Each individual was provided with instructions for buccal cell collection, using sterile swabs. Participants from Columbus, Ohio, collected their own sample under our supervision. All buccal samples were stored at 2–8 °C until extraction.

One hundred and ninety-eight additional samples were typed at the National Center for Forensic Science, Orlando, FL. The human use procedures used were approved by the University of Central Florida's Institutional Review Board. The samples were obtained from the Virginia Division of Forensic Science (bloodstains). All samples were stored at −47 °C until needed.

2.2. DNA extraction

Three different types of DNA extraction procedures were conducted. At The Ohio State University, DNA was obtained from buccal swabs through either the QIamp® DNA Mini Kit Buccal Swab Spin Protocol (QIAGEN Inc., Valencia, California) or the BuccalAmp™ DNA Extraction Kit (Epicentre, Madison, Wisconsin) in accordance with the manufacturers' instructions. QIamp® extracted samples were stored at 2–8 °C, and BuccalAmp™ extracted samples were stored at −20 °C.

At the University of Central Florida, the dried bloodstains were incubated overnight at 56 °C in 400 μl of DNA extraction buffer (100 mM NaCl, 10 mM Tris–HCl, 25 mM EDTA, 0.5% SDS) and 0.1 mg/ml proteinase K. The samples were placed into a spin ease basket and subjected to centrifugation at 14,000 × *g* for 5 min. An equal volume of phenol/chloroform/isoamyl alcohol was added to the crude extract. The aqueous phase of the extracts containing the DNA were purified using Centricon 100™ concentrators (Millipore, Bedford, MA), according to the manufacturer's instructions.

2.3. DNA quantification

At The Ohio State University the quantity of DNA was determined, using the QuantiBlot® DNA Quantification Kit (Applied Biosystems, Foster City, California) in accordance with the manufacturer's protocol. At the University of Central Florida, the quantity of DNA was determined by comparison of ethidium bromide induced fluorescence on a 1% agarose yield gel with a reference set of DNA standards of known concentration.

2.4. Polymerase chain reactions (PCRs)

The 10 OSU loci were amplified in two multiplex reactions. The 25 μl reaction mix of Multiplex Maybruck 1(MPM1) contained: 3 ng of template DNA, 0.38–0.88 μM primers (DYS576, 0.38 μM; DYS504, 0.80 μM; DYS688, 0.44 μM; DYS487, 0.50 μM; DYS707, 0.88 μM (Invitrogen, Grand Island, NY and Applied Biosystems, Foster City, CA)), 1 mM dNTPs, 1× PCR buffer II (10 mM Tris–HCl, pH 8.3, 50 mM KCl), 2.25 mM $MgCl_2$, 10 μg of non-acetylated bovine serum albumin (Sigma–Aldrich, St. Louis, MO), and 1.25 units of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA). The 25 μl reaction mix of Multiplex Maybruck 2 (MPM2) contained: 3 ng of template DNA, 0.25–0.44 μM primers (DYS448, 0.25 μM; DYS488, 0.25 μM; DYS471, 0.25 μM; DYS685, 0.25 μM; DYS703, 0.44 μM (Invitrogen and Applied Biosystems)), 1 mM dNTPs, 1× PCR buffer II, 2.25 mM $MgCl_2$, 10 μg non-acetylated bovine serum albumin, and 1.75 units of AmpliTaq Gold DNA polymerase. The PCR cycling conditions for both multiplex reactions were: (1) 11 min heat-soak at 95 °C, (2) 28 cycles of 1 min at 94 °C, 1 min at 60 °C and 30 s at 72 °C, and a final extension at 60 °C for 60 min. Primer sequences for the OSU 10-locus set are listed in Table 1. The SWGDAM loci were amplified according to the methods in Daniels et al. [35].

Table 1
Primer sequences for OSU 10-locus set. The loci contained in the two multiplex sets, MPM1 and MPM2, are listed in ascending order according to the DYS number. The size in base pairs is based upon the reference sequence found in GenBank.

Locus	Primer Sequence (5′-3′)	Repeat	Reference allele size	Approximate allele range
MPM1				
DYS487	F-TCAGGAGAAAATTCCAAAAGC R-CAGTGAGCCAAGATGGTGAC	Tri	163	161–173
DYS504	F-CACCACTGTGCCAAGCTATT R-CAGAGCAACCCTCTGTCAAG	Tetra	283	256–286
DYS576	F-GAATATCCTAGCTGTGAATCTCCTC R-CATGGGAAAAACCCAACAC	Tetra	432	405–441
DYS688	F-GAAATTGTGACATACCGCTGAC R-CGAGCAACAGTGCAAGACTC	Tri	422	389–455
DYS707	F-ATTATATCCCGTCCGATTCC R-TTGGTGTGAACTGGAGTGG	Penta	339	324–367
MPM2				
DYS448	F-GGTGGGTTTTAGTTGGCTATG R-TTCTTGATTCCCTGTGTTGG	Hexa	388	358–406
DYS471	F-GGCATTATGTGTTTGTGAGTGC R-ACAGACTGGCAACCAAAAGG	Tri	219	208–254
DYS488	F-TTGTGCTCATGTACCCTGGA R-CCTCCTGTCTGCCATTTTGT	Tri	246	244–259
DYS685	F-CTGGGTGTGCATTCGAGAC R-CCTGGGTGACAGACTCCATC	Tetra	382	339–416
DYS703	F-TGCTTGAACCTTGGAGACAG R-TTGACTTGTTGACCCTGTGG	Tri	252	254–266

2.5. Allelic detection and genotyping

The PCR product was detected in the same manner for both multiplex reactions. A 1.0 μl aliquot of the amplified product was added to 8.7 μl of deionized formamide (Applied Biosystems) and 0.3 μl GeneScan 500 LIZ internal lane standard (Applied Biosystems). Ninety-six well plates containing the prepared samples were heated at 95 °C for 3 min and snap-cooled on ice for 3 min. Samples were injected using Module G5 (5 s injection, 15 kV, 60 °C). Fluorescently labeled products were separated and detected using a 3130 Genetic Analyzer (data collection v3.0) (Applied Biosystems), and data were analyzed with GeneMapper Analysis Software v3.7. A peak detection threshold of 50 RFUs was used for allele designation.

The PCR product of DYS389II includes the DYS389I repeat; therefore, DYS389II alleles were scored by subtracting the DYS389I allele. As a result, DYS389I was not included twice in the analysis.

2.6. Genetic analysis

The number of alleles observed in the population samples for all 19 single copy loci was calculated by the direct count method. The alleles for the duplicated locus, DYS385, were described as genotypes. Allele frequencies, and gene diversities were calculated using Genepop on the Web software v.3.4 Option 5 (http://genepop.curtain.edu.au) [36] for both sets of loci. Independent segregation analyses (linkage disequilibrium) were calculated using software developed by Chakraborty and Lee (http://cgi.uc.edu/downloads/YSTR) as described by Sinha et al. [34]. Analysis of linkage disequilibrium among pairs of loci was conducted for all locus pairwise comparisons in each population group. When pairs of loci were compared, there were a total of 171 pairwise tests within and between both sets of loci for each population group. Since DYS385 is composed of multiple loci and the alleles could not be unequivocally assigned to a locus, the allele frequency, gene diversity, and linkage disequilibrium analyses were not calculated for DYS385 to avoid incorrect calculations.

The discrimination power of both sets of 10 loci was evaluated by comparing the number of haplotypes for both sets of loci. Then a pairwise comparison of every individual profile with every other individual profile was carried out and the number of allelic differences between each pair for each set of loci was noted.

3. Results

3.1. Allelic comparisons

Based upon an initial screen of a 30-individual test sample, the OSU 10-locus set appears to be highly informative (Maybruck et al., submitted for publication). To further examine the discriminative power of the OSU 10-locus set, a comparative study with the 10 SWGDAM loci was conducted using two sample populations (Caucasian, $N = 114$; African American, $N = 110$). The number of alleles for all 19 single copy loci examined in the same individuals was compared in Fig. 1. In the whole population, SWGDAM single copy loci contained an average of 6.0 ± 0.87 alleles (Caucasian 4.8 ± 1.5 and African American 5.9 ± 0.87). For the 9 single copy loci, 5–7 alleles were observed and for the multicopy locus, DYS385, 14 alleles were observed. Since the alleles for DYS385 could not be assigned unequivocally [37,38] the duplicated locus was scored as a genotype (Supplemental Table 1) as recommended by the DNA Commission of the International Society of Forensic Genetics [30,39]. The OSU loci showed an average of 9.6 ± 4.7 alleles in 224 individuals (Caucasian 7.4 ± 3.4 and African American 8.4 ± 4.3). All 10 OSU loci are single copy, and from 5 to 19 alleles were observed. Therefore, in the same 224 individuals, we observed an average of 3.6 more alleles per locus, using the OSU 10-locus set (Caucasian 2.6 and African American 2.5). A two-sample t significance test showed that the average difference between the two sets is significant with $p < 0.02$ (Caucasian $p < 0.02$ and African American $p < 0.05$).

The allele frequencies for the SWGDAM set and the OSU 10-locus set are presented in Supplementary Tables 1 and 2, respectively. Gene diversity was also calculated (Table 2). The gene diversity for the single copy SWGDAM loci ranged from 0.489

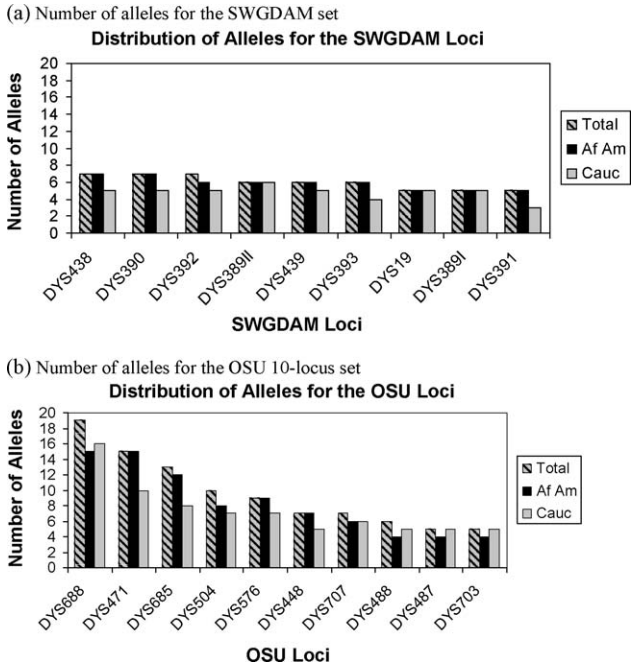

Fig. 1. Distribution of alleles for OSU 10-locus and SWGDAM sets. A comparison of the number of alleles present in the same 224 individuals, 114 Caucasians and 110 African Americans, using the (a) SWGDAM set and the (b) OSU 10-locus set.

Table 2
Gene diversity for SWGDAM and OSU 10-locus sets. The loci are listed according to their total gene diversity in descending order.

Locus	Gene diversity		
	Caucasian	African American	Total
SWGDAM			
DYS385[a]	NA	NA	NA
DYS390	0.704	0.659	0.781
DYS389II	0.590	0.701	0.685
DYS438	0.617	0.498	0.668
DYS439	0.666	0.652	0.667
DYS19	0.457	0.718	0.658
DYS392	0.602	0.416	0.585
DYS389I	0.541	0.553	0.546
DYS393	0.322	0.608	0.494
DYS391	0.522	0.417	0.489
OSU			
DYS688	0.890	0.899	0.910
DYS471	0.844	0.902	0.887
DYS685	0.786	0.859	0.843
DYS576	0.788	0.805	0.813
DYS504	0.752	0.718	0.810
DYS448	0.602	0.699	0.715
DYS707	0.553	0.644	0.677
DYS703	0.537	0.549	0.596
DYS487	0.444	0.301	0.381
DYS488	0.252	0.138	0.199

[a] Gene diversity was not calculated for DYS385 due to the compound nature of the locus.

to 0.781 (Caucasian 0.322–0.704 and African American 0.416–0.718). Since DYS385 is a duplicated locus, gene diversity could not be calculated.

The gene diversity for the OSU 10-locus set ranged from 0.199 to 0.910 (Caucasian 0.252–0.890 and African American 0.138–0.902). The average gene diversity was 6.4% higher in the OSU 10-locus set even with the low gene diversity value for DYS488. In the original screen of 30-individuals, DYS488 was the best candidate for the region of interest on the Y-chromosome and seemed to have

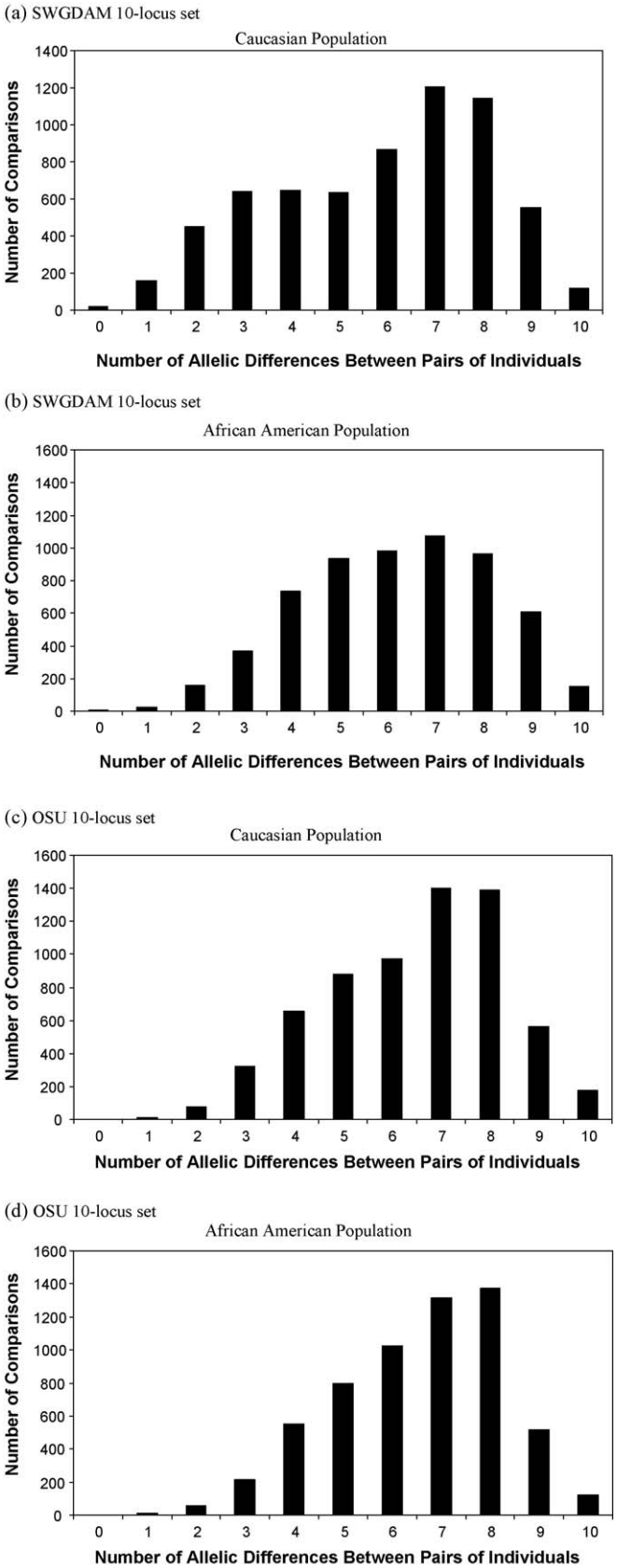

Fig. 2. Distribution of number of pairwise allelic differences between haplotypes. (a) SWGDAM set for the Caucasian population, (b) SWGDAM set for the African American population, (c) OSU 10-locus set for the Caucasian population and (d) OSU 10-locus set for the African American population.

more variability (Maybruck et al., submitted for publication) than is evident from this study. Without DYS488 the average gene diversity value is 11.8% higher for the OSU 10-locus set. Five loci in the OSU 10-locus set, DYS688, DYS471, DYS685, DYS576, and DYS504, had higher gene diversities than the most diverse locus, DYS390, in the SWGDAM set for the whole population and the Caucasian population. The African American population showed DYS19 as the most diverse locus for the SWGDAM set, while the same five loci from the OSU set, DYS688, DYS471, DYS685, DYS576, and DYS504, showed equivalent or greater diversity.

3.2. Haplotype diversity and comparisons

Due to the higher allelic variability and gene diversity for the OSU 10-locus set, the haplotype discrimination power for both sets was evaluated. A comparative analysis of haplotype diversity was conducted between the SWGDAM and OSU 10-locus sets; although, due to the DYS385 marker, the SWGDAM set has 11 loci. A match for the DYS385 marker was recorded only if both alleles were identical in a pair of individuals.

Each of the 224 individuals, 114 Caucasian and 110 African Americans, was compared with every other individual within their respective sample population, in a pairwise fashion, to determine the number of allelic differences per locus between each pair of individuals for each set of loci (Fig. 2). The OSU 10-locus set shows a higher average number of differences between individuals as compared to the SWGDAM loci: Caucasians, 6.477 versus 5.841, and African Americans, 6.548 versus 6.193, average differences per comparison, respectively. To test the significance of the differences between the two groups of loci, the entire set of pairwise differences was combined, values randomized and resampled to make up two new groups. This was repeated 5000 times. Differences as great as that observed in the data were observed less than 4% of the time, indicating that the OSU 10-locus set had a significantly greater number of pairwise differences. The distribution of pairwise differences is shown in Fig. 2a–d for the two sets of loci in both populations. For the SWGDAM loci, 630 Caucasian pairs and 184 African American pairs of individuals have 0–2 differences (Fig. 2a and b) whereas only 85 Caucasian and 71 African American pairs of individuals differ at two or fewer loci, using the OSU set

Table 3
Non-unique OSU 10-locus set haplotypes differentiated using the SWGDAM loci.

Ethnicity of individual	SWGDAM loci DYS393–DYS392–DYS391–DYS389I–DYS389II–DYS438–DYS385–DYS390–DYS439–DYS19	OSU loci DYS487–DYS504–DYS576–DYS707–DYS688–DYS703–DYS448–DYS488–DYS685–DYS471
Af Am	13–13–11–13–16–12–11, 15–24–12–14	13–25–16–23–80–10–21–14–41–29
Af Am	13–11–09–14–16–10–13, 14–23–11–13	13–25–16–23–80–10–21–14–41–29
Af Am	13–11–10–13–18–11–16, 16–21–11–15	13–22–13–24–80–12–23–14–44–36
Cauc	13–13–10–13–17–12–11, 15–24–12–14	13–22–13–24–80–12–23–14–44–36

Table 4
Non-unique SWGDAM Haplotypes differentiated using the OSU 10-locus set.

Ethnicity of individual	SWGDAM loci DYS393–DYS392–DYS391–DYS389I–DYS389II–DYS438–DYS385–DYS390–DYS439–DYS19	OSU loci DYS487–DYS504–DYS576–DYS707–DYS688–DYS703–DYS448–DYS488–DYS685–DYS471
Cauc	13–13–11–13–16–12–11, 14–23–12–14	13–26–16–23–81–10–21–14–42–29
Cauc	13–13–11–13–16–12–11, 14–23–12–14	13–23–15–23–81–11–21–14–43–30
Cauc	13-13–11–13–16–12–11, 14–23–12–14	13–27–16–23–80–10–21–14–41–30
Cauc	13–13–11–13–16–12–11, 14–23–12–14	13–25–14–23–86–10–21–14–44–31
Cauc	13–13–11–13–16–12–11, 14–23–12–14	13–26–17–22–82–10–21–14–42–33
Af Am	13–13–11–13–16–12–11, 14–23–12–14	12–25–16–23–80–10–21–14–42–30
Af Am	13–13–11–13–16–12–11, 14–23–12–14	13–23–15–23–81–10–21–14–42–31
Cauc	13–13–10–13–16–12–11, 14–24–12–14	13–27–17–23–82–10–21–14–42–29
Cauc	13–13–10–13–16–12–11, 14–24–12–14	13–26–17–23–81–10–20–14–43–30
Cauc	13–13–10–13–16–12–11, 14–24–12–14	14–25–16–23–77–10–21–14–41–30
Af Am	13–13–10–13–16–12–11, 14–24–12–14	13–25–16–23–79–10–21–14–41–30
Af Am	13–13–10–13–16–12–11, 14–24–12–14	13–26–16–22–77–10–21–14–41–31
Cauc	13–13–11–13–17–12–11, 14–24–11–14	13–25–16–23–84–10–21–14–42–32
Cauc	13–13–11–13–17–12–11, 14–24–11–14	13–26–17–23–84–10–21–14–42–29
Cauc	13–13–11–13–17–12–11, 14–24–12–14	13–26–16–23–82–10–21–14–43–30
Cauc	13–13–11–13–17–12–11, 14–24–12–14	14–26–18–23–81–10–21–14–42–29
Cauc	13–13–11–13–16–12–11, 14–24–12–14	13–27–16–23–82–10–21–14–43–29
Cauc	13–13–11–13–16–12–11, 14–24–12–14	13–26–17–23–82–10–21–14–42–32
Cauc	13–13–11–13–16–12–11, 14–24–12–14	13–25–16–23–79–10–21–14–43–31
Cauc	13–13–11–13–16–12–11, 14–24–11–14	13–24–15–23–81–10–21–14–43–32
Cauc	13–13–11–13–16–12–11, 14–24–11–14	13–25–15–23–78–10–21–14–40–30
Af Am	13–13–11–13–16–12–11, 14–24–11–14	13–26–16–22–79–10–22–14–44–29
Cauc	13–11–10–12–16–10–13, 14–22–11–14	13–27–14–23–81–13–22–15–45–27
Cauc	13–11–10–12–16–10–13, 14–22–11–14	12–26–15–22–79–12–22–15–45–29
Cauc	13–13–10–13–17–12–11, 14–24–12–14	13–23–17–24–82–10–21–14–41–31
Cauc	13–13–10–13–17–12–11, 14–24–12–14	13–26–16–23–82–10–21–14–39–30
Af Am	14–11–10–13–17–11–17, 18–21–13–16	13–22–16–24–73–13–22–14–46–33
Af Am	14–11–10–13–17–11–17, 18–21–13–16	14–22–14–24–74–12–23–14–45–33
Af Am	13–11–10–13–18–11–16, 17–21–12–15	14–22–14–24–78–12–22–14–46–35
Af Am	13–11–10–13–18–11–16, 17–21–12–15	13–22–14–24–79–12–23–14–45–35
Af Am	13–13–11–14–16–12–11, 14–24–12–14	13–25–17–23–80–10–20–14–37–31
Cauc	13–13–11–14–16–12–11, 14–24–12–14	13–26–15–23–78–10–21–14–39–29
Cauc	13–13–11–13–16–12–11, 15–24–12–14	13–25–14–23–85–10–21–14–42–31
Af Am	13–13–11–13–16–12–11, 15–24–12–14	13–25–16–23–80–10–21–14–41–29

(Fig. 2c and d). On the other end of the spectrum, 8–10 differences, approximately 300 additional pairs were observed for both populations with the OSU 10-locus set (Fig. 2). Within each population, all 114 Caucasian individuals and all but two of the 110 African American individuals have haplotypes that are unique according to the OSU 10-locus set (Table 3). In contrast, 21 Caucasian individuals and four pairs of African American individuals do not have unique haplotypes using the SWGDAM loci (Table 4). With the SWGDAM loci, seven haplotypes are shared in the Caucasian population and four haplotypes are shared in the African American population. The most common haplotype in the Caucasian population is shared by five individuals and each non-unique haplotype in the African American population is shared by two individuals. The most common haplotype in the Caucasian population is identical to one of the shared haplotypes in the African American population and is thus shared by seven individuals. Four additional haplotypes are shared across both populations using the SWGDAM loci, two that are already shared within one or both populations and two haplotypes that are unique within their own populations. Only one haplotype is shared across both populations using the OSU 10-locus set.

The comparison of the OSU 10-locus set and the SWGDAM set is further shown in Fig. 3. This figure displays a comparison of the number of allelic differences observed between specific pairs of individuals, utilizing the OSU 10-locus set and the SWGDAM set. The data show a skew towards a greater number of differences observed with the OSU 10-locus set (points above the diagonal). A total of 12,436 pairwise comparisons were conducted: 6441 comparisons within the Caucasian population and 5995 comparisons within the African American population. The results are respectively as follows for Caucasian and African American populations. Approximately 50% (3308 (51.36%) and 2731 (45.55%)) of the pairs showed a greater number of differences using the OSU 10-locus set. Only 1234 (19.16%) and 2013 (33.58%) pairs showed the SWGDAM set with a greater number of differences. The remaining 1899 pairs (29.48%) and 1251 pairs (20.87%) displayed an equivalent number of differences (points on the diagonal) for both the SWGDAM and OSU 10-locus sets.

As seen in Fig. 3, the identities of the 21 Caucasian individuals and four pairs of African American individuals were resolved by an average of five differences per haplotype using the OSU 10-locus set. Additionally, five shared haplotypes (19 individuals) seen with the SWGDAM loci across the two populations (data not shown) were also resolved by an average of five differences per haplotype utilizing the OSU 10-locus set. Out of all 224 individuals only two unresolved pairs were observed using the OSU 10-locus set, one within the African American population and one identical pair between the two populations, which were distinguished with the SWGDAM loci.

The power of discrimination for the OSU 10-locus set is apparent when direct comparisons are made between the two sets for the same individuals. For every identical pair observed with the SWGDAM loci a unique pattern was observed with the OSU 10-locus set. Five of the loci in the OSU 10-locus set, DYS471, DYS504, DYS576, DYS685, and DYS688, were the most useful to distinguish the non-unique haplotypes seen in the SWGDAM set. One or more of the five aforementioned loci helped to differentiate each of the 45 pairs that were identical for the SWGDAM haplotypes.

3.3. Linkage disequilibrium

These loci reside on the Y-chromosome where little or no recombination occurs and therefore are expected to demonstrate linkage disequilibrium with each other. However, because of reasonably high mutation rates, some loci pairs may not show detectable levels of linkage disequilibrium. For both sets of loci,

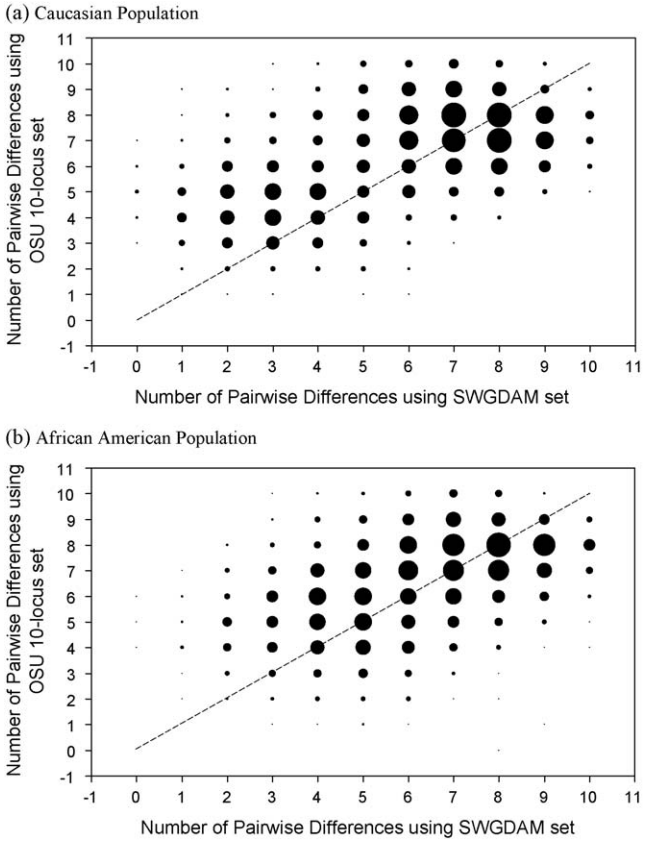

Fig. 3. Bubble plots of pairwise haplotype comparisons within both populations utilizing either the SWGDAM set or the OSU 10-locus set. *X*-axis and *Y*-axis show the number of allelic differences between pairs of individuals for the SWGDAM set and OSU 10-locus set, respectively. The smallest dot represents one pair of individuals; for example, one pair of Caucasian individuals had zero differences, using the SWGDAM set whereas the same pair had seven differences, using the OSU 10-locus set. The largest dot observed in the Caucasian population represents 360 pairs of individuals. This occurred when there were eight differences for both the SWGDAM and OSU 10-locus sets. (a) Caucasian population and (b) African American population.

linkage disequilibrium was tested for the African American and Caucasian populations (Figs. 4 and 5). In the Caucasian population, highly significant linkage disequilibrium, $p < 0.01$, was observed more often between loci within the SWGDAM set (locus pairwise comparisons shown on the bottom right quadrant of Fig. 4) than between loci within the OSU 10-locus set (locus pairwise comparisons shown on the top left quadrant of Fig. 4). For the African American population the values were similar without DYS703 which showed a much higher level of non-random association than the other OSU loci. Since DYS385 is a duplicated locus and the alleles could not be attributed to their locus of origin without the use of additional assays, DYS385 was not included in this analysis.

Examination of the comparisons between loci within the SWGDAM set showed 11 (31%) of the Caucasian and 6 (17%) of the African American population pairs of loci in linkage disequilibrium at $p < 0.01$, while 64% of the Caucasian and 33% of the African American population pairs of loci showed linkage disequilibrium at $p < 0.05$. DYS438 identified the most notable level of linkage disequilibrium within the Caucasian population with six of eight loci in the SWGDAM set at $p < 0.01$ and all of the loci at $p < 0.05$. Within the African American population DYS390 revealed the most considerable level of linkage disequilibrium with four of eight loci in the SWGDAM set at $p < 0.01$. In the African American population two loci, DYS389I and DYS439, did not show any detectable linkage

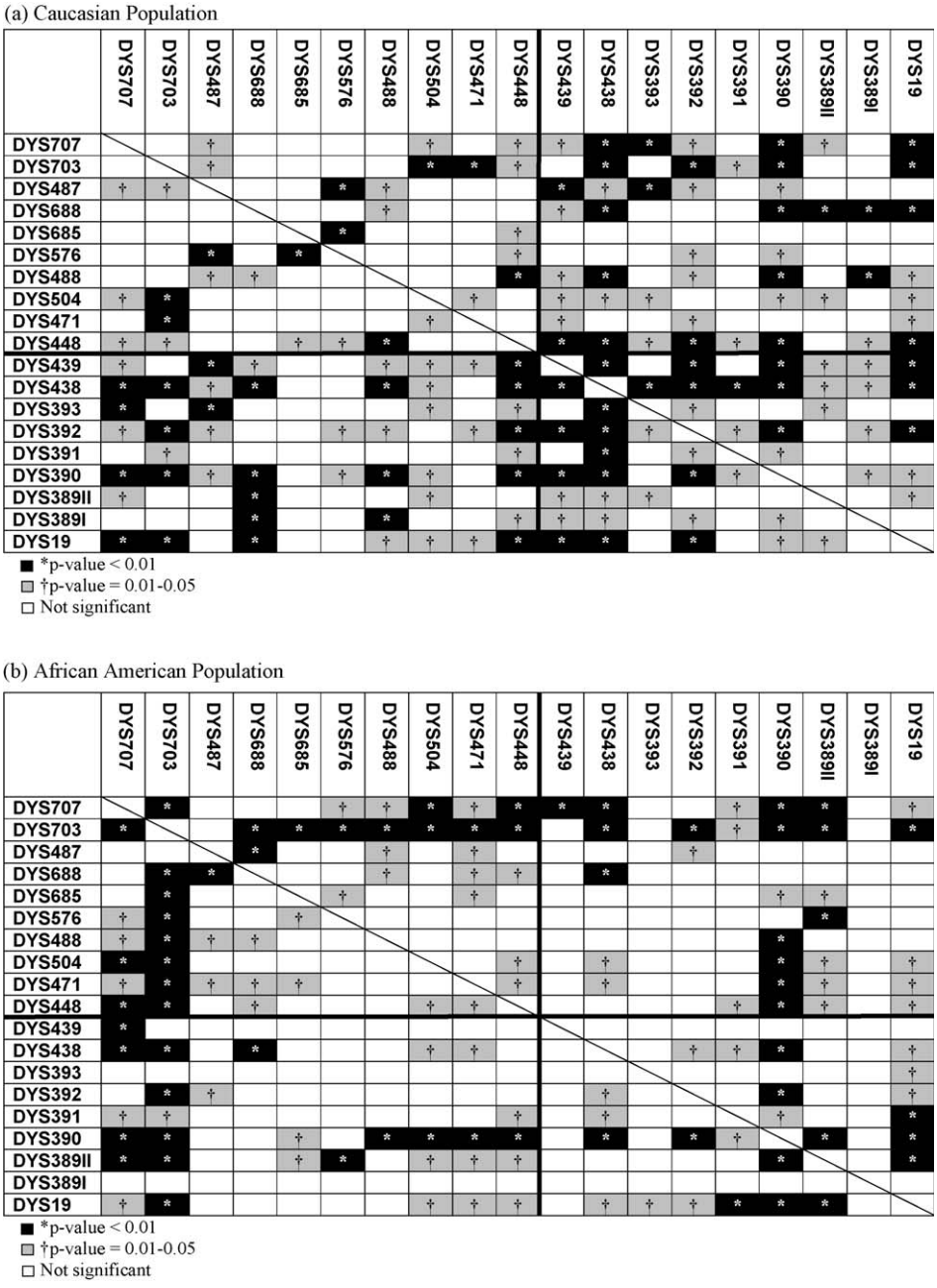

Fig. 4. Linkage disequilibrium results for all 19 single copy loci in both populations. The results can be read horizontally or vertically. The points above the diagonal line are a mirror image of the points below the diagonal line. (a) Caucasian population and (b) African American population.

disequilibrium with other loci of the SWGDAM set. However, these same two loci were in linkage disequilibrium at $p < 0.05$ in the Caucasian population with four and six loci, respectively.

Assessment of non-random association between loci within the OSU set on the same population identified only five (11%) Caucasian and 11 (24%) African American pairs of loci in linkage disequilibrium at $p < 0.01$. DYS703 was non-randomly associated with eight loci in the African American population and accounted for 73% of the linkage disequilibrium observed at $p < 0.01$. At $p < 0.05$, 33% of the Caucasian and 51% of the African American pairs of loci were in linkage disequilibrium. DYS703 contributed to 35% of the significant level of linkage disequilibrium observed in the African American population.

In our examination of the entire set of 19 single copy loci for both populations (Figs. 4 and 5), on average the SWGDAM loci are in linkage disequilibrium with a greater number of loci than are loci from the OSU set for the Caucasian population (Fig. 4). In the African American population with DYS703 the OSU set exhibited a more significant level of non-random association however without DYS703 the values for both sets are similar. The highest proportion of significant linkage disequilibrium was seen for DYS438 in the Caucasian population and DYS703 in the African American population. The aforementioned loci were each in linkage disequilibrium with 15 and 14 other loci, respectively at $p < 0.05$. In the Caucasian population, 10 loci exhibited linkage disequilibrium with four or more loci at $p < 0.01$. Of the six loci showing the most significant levels of non-random association, five were from the SWGDAM set (Fig. 5a). The remaining nine loci were in linkage disequilibrium with three or fewer loci at $p < 0.01$, five loci were from the OSU 10-locus set. In the African American population, six loci showed four or more non-random associations with all nineteen loci at $p < 0.01$. Four of the six loci with the most

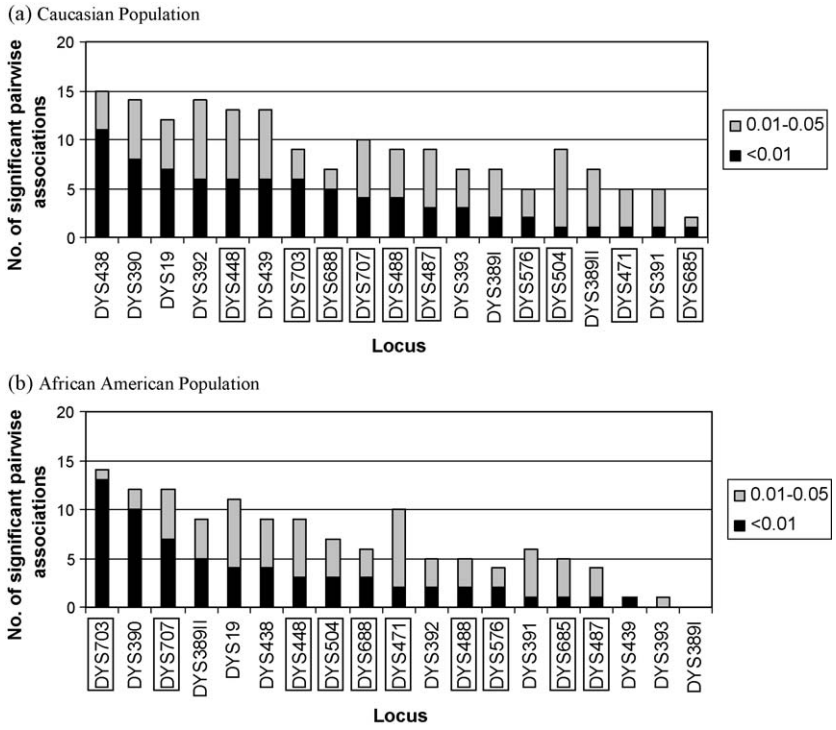

Fig. 5. Pairwise associations between all 19 single copy loci for both populations. The results show the overall trend of the linkage disequilibrium results for comparisons conducted between all 19 single copy loci. The loci are sorted in descending order according to the $p < 0.01$ value and then according to the $0.01 < p < 0.05$ value. The loci from the OSU 10-locus set are denoted with a rectangular border. (a) Caucasian population and (b) African American population.

significant levels of linkage disequilibrium were from the SWGDAM set (Fig. 5b). For the 13 loci in the African American population demonstrating a lesser amount of non-random association, three or fewer, eight were from the OSU set.

Of the loci with the lowest levels of linkage disequilibrium, the most informative loci would also have high gene diversity values. This observation is consistent with that of Budowle et al. [24] and Beleza et al. [40] who reported that gene diversity alone is not sufficient for predicting the most informative loci for increasing haplotype diversity. The five loci in the Caucasian population with low levels of linkage disequilibrium at $p < 0.01$ and higher gene diversities are DYS576, DYS504, DYS389II, DYS471, and DYS685. The five loci in the African American population that meet the same criteria are DYS471, DYS393, DYS576, DYS685, and DYS439. Across both populations, the loci, DYS471, DYS576, and DYS685, have low levels of non-random association at $p < 0.01$ as well as high gene diversities. Two of the remaining four loci, DYS439 in the Caucasian population and DYS389II in the African American population, are in the lower one-third of the 19 loci, displaying higher levels of linkage disequilibrium at $p < 0.01$. While the level of non-random association for DYS393 is in the middle one-third of the 19 loci in the Caucasian population, the gene diversity value is very low, 0.322. Locus DYS688 in the Caucasian population and loci DYS504 and DYS688 in the African American population are in the middle one-third of all the loci with respect to linkage disequilibrium at $p < 0.01$. Additionally, DYS504 and DYS688 have high gene diversity values in both populations.

4. Discussion and conclusions

The most appropriate Y-STR loci for forensic purposes are those that are male-specific, show locus specific amplification, and provide sufficient discriminate power in concert with other loci comprising the haplotype. Other evaluations are additionally important such as sensitivity and specificity of the loci, which are included in a validation study of the OSU 10-locus set that has been completed and is the topic of a manuscript in preparation. The current study served as an initial screen of the discrimination power of the OSU 10-locus set. Several comparative analyses were made, and the OSU 10-locus set was more informative than the SWGDAM set of loci.

The number of alleles found in the population samples of 114 Caucasian and 110 African American individuals using the SWGDAM set is consistent with the number of alleles detected in population samples ranging from 103 to 4558 individuals from different ethnic groups, including African populations [34,41–43]. The loci of the OSU 10-locus set had higher numbers of alleles per locus on average than the loci of the SWGDAM set (Fig. 1). Five loci, DYS471, DYS504, DYS576, DYS685, and DYS688, had nine or more alleles (seven or more alleles in the Caucasian population and eight or more alleles in the African American population). Even if we included the multiple copy locus, DYS385, as a single locus two of the OSU loci, DYS471 and DYS688, each had a larger number of alleles.

Estimates of relative gene diversity for the SWGDAM loci are consistent with those obtained for several populations reported in the literature [24,25]. Five of the OSU loci, DYS471, DYS504, DYS576, DYS685, and DYS688, had higher gene diversities than the most diverse single copy locus in the SWGDAM set, DYS390 (Table 2). This suggests that the OSU 10-locus set should have a higher power of discrimination, although haplotype diversity is a better indication of discrimination power.

Haplotype comparisons, revealed a greater proportion of unique haplotypes in the OSU 10-locus set. Among 224 individuals, 190 (84.8%) unique haplotypes were observed for the SWGDAM set whereas 220 (98.2%) unique haplotypes were revealed with the OSU 10-locus set. Nearly all of the shared haplotypes for the SWGDAM loci observed in this study were also seen as shared haplotypes in a previous study of nearly 3000 Caucasian and African American individuals [33]. In the current study, when

multiple individuals shared identical SWGDAM set haplotypes, the OSU 10-locus set disclosed an average of five differences between the individuals. The resolution of every non-unique SWGDAM haplotype included at least one of the following five OSU loci: DYS471, DYS504, DYS576, DYS685, and DYS688. Analysis of pairwise differences between haplotypes showed that in approximately 50% of pairwise comparisons in both population samples there were a larger number of loci that discriminated two individuals with the OSU 10-locus set, whereas approximately 25% of comparisons showed a greater number of SWGDAM loci able to differentiate a pair of individuals (Fig. 3).

The levels of linkage disequilibrium between loci within the SWGDAM set are consistent with those reported by Budowle [44]. In an examination of 2443 unrelated individuals, DYS438 showed significant linkage disequilibrium with a number of loci [24]. Additionally, several other pairs of loci with significant linkage disequilibrium in Sinha et al. [34] and Budowle et al. [24] were also non-randomly associated in our sample population. In the Sinha et al. study, linkage disequilibrium among the six loci from the Y-PLEX™6 kit was determined in 226 unrelated individuals from Louisiana [34]. Eight pairs of loci in 111 Caucasian individuals and nine pairs of loci in 115 African American individuals showed linkage disequilibrium. This observation is similar to ours, that high levels of linkage disequilibrium are found among many of the SWGDAM loci.

The linkage disequilibrium analyses disclosed a much higher proportion of loci pairs in linkage disequilibrium within the SWGDAM set for the Caucasian population. However, differences between the SWGDAM and OSU sets for non-random association observed within the African American population were negligible, particularly when DYS703 was excluded from the analysis. The most informative loci to include in a set of loci for population analyses are those that have low levels of linkage disequilibrium and high gene diversities. The following loci from the OSU set meet these criteria for both Caucasian and African American population samples: DYS471, DYS504, DYS576, DYS685, and DYS688.

The higher levels of non-random association (linkage disequilibrium) for the SWGDAM set in the Caucasian population may be due to lower variability (and thus lower mutation rate) of the SWGDAM loci. Since recombination is expected to be absent for loci on the male-specific region of the Y-chromosome, decay of linkage disequilibrium will occur only by the accumulation of new mutations on pre-existing haplotypes. The observation that the loci of the OSU 10-locus set are more variable on average for the Caucasian population than the loci of the SWGDAM set is consistent with an assumption of a higher level of mutation for the loci that have been included in the OSU set. This would then lead to a more rapid decay of linkage disequilibrium between these loci. The similar patterns for linkage disequilibrium in the African American population for the two sets of loci, when excluding DYS703, are most likely due to the higher ancestral haplotype diversity in the African American population. Therefore, the higher mutation rates of the OSU loci would not seem to contribute as much to the decay of linkage disequilibrium between the loci.

Although a comparative analysis was conducted between the two sets, the intent is not to replace the SWGDAM loci but to identify additional loci that are useful for forensic identification purposes. The loci contained in the OSU set were deliberately chosen from regions outside of the regions of the SWGDAM loci, and primers were designed with annealing temperatures similar to the widely used loci. In light of the findings in this study the loci DYS471, DYS504, DYS576, DYS685, and DYS688 are likely to be the most informative of the OSU 10 loci to use in conjunction with the SWGDAM set to improve the discrimination power. Due to the results of this analysis, further comparative analyses of these two sets of loci may be warranted in larger and other population samples.

Acknowledgements

We would like to thank everyone in the States of Ohio and Virginia who provided samples for this study. We particularly appreciate the non-criminal samples provided by Dr. Roger Kahn, from the State of Ohio BCI laboratory. We value the contributions of Brian Mark who designed software for the pairwise comparison study and Dr. Laura Kubatko who conducted the statistical analysis which determined the significance of the pairwise comparison study. We are additionally grateful to Dr. Arthur HM Burghes, Dr. Thomas W Prior, and Dr. Amanda Simcox for discussion and editorial comments. We also appreciate the editorial comments of Dr. Lizhao Wu, Dr. Greg Booton, Dr. Roger Kahn and Judith S. Maybruck. We are thankful for the helpful comments and exchanges with the reviewers that improved the manuscript, especially with respect to DYS385. A research award from The Ohio State University supported this work. Portions of this work were supported under Award number 2005-MU-MU-K044 from the Office of Justice Programs, National Institute of Justice, Department of Justice. The points of view in this document are those of the authors and do not necessarily represent the official position of the U.S. Department of Justice.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fsigen.2009.03.004.

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