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Biostatistics for mixed stains: the case of tested relatives of a non-tested suspect

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Abstract Analysis of a mixed biological stain by means of highly polymorphic VNTR systems usually reveals a profile composed of multiple markers. If the victim and one or several suspects match the profile, the evidential strength of the matches has to be very carefully analysed. The appropriate methods for the statistical analysis of DNA profiles advanced recently were limited to cases with no relationship between the tested and non-tested persons. The present paper extends the theory beyond this limitation.

Key words DNA · Forensic statistics · Mixed stains · Identification · Kinship

Introduction

DNA profiling of biological stains is now a central analytical method in forensic science which aims to identify the assailants out of the group of tested persons i.e. victims and suspects. The general formula for the statistical evaluation of DNA profiles with more than one contributor has been proposed by Weir and co-authors [1]. This basic approach comprises the entire statistical analysis of DNA profiles, but suffers from the following limitations:

1. All unknown persons should belong to the same ethnic group.
2. There should be no relationship between the non-tested persons (unknowns) subjected to the statistical analysis or between the unknowns and tested persons, irrespective of whether these tested persons have contributed to the stain or not.

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Thus, two cases not infrequent in the forensic practice, i.e. contributions to the stains by members of different ethnic groups and inclusion of information from relatives in the course of the statistical analysis, are not dealt with by this approach. Therefore the recent development was aimed at extending the basic methods beyond these limitations. In a recent paper [2] we have extended the theory to the case where some unknown contributors may belong to different ethnic groups. A further paper [3] described the extension of the theory to the case where two unknown contributors may be related. The present paper extends the theory to the case where one suspect who is unavailable for testing, turns out to be not abstract and nameless, but is a definite person whose relatives are available for the genetic test.

Formulation of the problem and general expressions

We recall first the general formulation of the statistical analysis of forensic DNA evidence in terms of the theory of hypotheses testing [4] whereby we introduce all the variables and specifications. A DNA mixture from a crime scene – the stain – can contain contributions from a number of persons e.g. victims and/or assailants. A comparison of the stain profile with the single person's DNA profiles taken from a group of tested persons (e.g. victims and suspects) is performed with the aim of identifying the assailants. However, such a comparison usually leaves room for more than one alternative, i.e. more than one hypothesis concerning the circumstances of the crime can be advanced. Each of the mutually excluding hypotheses H_1, $H_2,\ldots,H_N$ is a statement specifying members of a group of persons, among them all tested persons and if necessary, non-tested persons (unknowns) as either contributors or non-contributors to the crime sample. The aim of the statistical analysis, i.e. testing of the hypotheses, is achieved on the basis of the following considerations.

Let us assume that according to a current hypothesis the contributors of a stain which shows m alleles A_1, $A_2,\ldots,A_m$ are n unknowns and a number of tested persons.

The group of n unknown contributors can have only alleles from the stain and must have k "required alleles" $A_1,A_2,\ldots,Ak$ ($\leq$ m, $k \leq 2n$) which are absent in the genotypes of the tested contributors. The probability of this event is designated $p(n,k)$. The number of unknown persons, n, the number k and the composition of the "required alleles" and therefore the probabilities $p(n,k)$ vary for different hypotheses. It has been shown [4] that the set of probabilities $p(n,k)$ ($p(0,0) = 1$) comprises the entire information necessary to perform the statistical analysis. Therefore, the whole analysis is based on the expressions for the probability $p(n,k)$.

The general expression for $p(n,k)$ has the form [1, 4]:

$$p(n,k) = T_0(n) - \sum_{i=1}^{k} T_i(n) + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} T_{ij}(n) - \ldots (-1)^k T_{12..k}(n) \quad (1)$$

Here the items $T_0(n)$, $T_i(n)$, $T_{ij}(n)\ldots$ are the composite probabilities for n unknown persons to present alleles from the stain according to the following rule: $T_0(n)$ means that all alleles of the stain are presented, $T_i(n)$ means that all alleles of the stain except the "required allele" A_i are presented, $T_{ij}(n)$ means that all alleles of the stain except the "required alleles" A_i and A_j are presented etc.

The set of contributors to a stain comprises two subsets, the tested persons and the unknowns. Each tested person is specified by the known genotype while the unknowns can show alleles from the stain with the probabilities which the corresponding alleles have in the population. The genotype frequencies of the unknowns are then calculated according to the formula:

$$p(A_l A_r) = \begin{cases} a_l^2 & \text{if} \quad l = r, \\ 2a_l a_r & \text{if} \quad l \neq r, \end{cases} \quad (2)$$

where the allele frequencies are designated by the lowercase letters.

Thus, for one unknown:

$$T_0(1) = \sum_{l=1}^{m} \sum_{r=1}^{m} p(A_l A_r) = \sum_{l=1}^{m} a_l^2 + 2 \sum_{l=1}^{m-1} \sum_{r=l+1}^{m} a_l a_r = s^2$$
$$T_i(1) = \sum_{\substack{l=1 \\ l \neq i}}^{m} \sum_{\substack{r=1 \\ r \neq i}}^{m} p(A_l A_r) = (s - a_i)^2, \ldots \quad (3)$$

which in the case of n independent (non-related) unknowns results in the well known Weir expression [1, 4]

$$p(n,k) = T_0^n(1) - \sum_{i=1}^{k} T_i^n(1) + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} T_{ij}^n(1) - \ldots$$
$$= s^{2n} - \sum_{i=1}^{k} (s - a_i)^{2n} + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} (s - a_i - a_j)^{2n} \quad (4)$$
$$- \ldots + (-1)^k (s - a_1 - \ldots - a_k)^{2n},$$

where s is the sum of the frequencies of the alleles of the stain

$$s = a_1 + a_2 + \ldots + a_m \quad (5)$$

Usually an unknown contributor of a stain is an abstract nameless person. However, sometimes this is not the case and exactly such situations are subject to the present study. Consider a case where some definitely known person suspected to be a contributor of a stain cannot be tested. Nonetheless, some information about the genotype can be gained from the tests applied to the relatives. It can happen that this information will exclude this suspect from the contributors of the stain, for example, when both alleles of one of his parents do not belong to the stain.

This specified suspect with tested relatives (we will designate him as U_R) is a member of a pedigree E, which contains, among others, all his tested relatives. The frequency of the genotype G of the U_R, cannot be calculated with the general expression (Eq. 2), but is defined by the conditional probability

$$p_E(G/R) = p_E(G,R)/p_E(R) \quad (6)$$

Here R is the set of genotypes of all tested relatives of the U_R; $p_E(R)$ is the probability of the phenotypic pedigree E, where the genotypes correspond to each tested relative while any genotype which is compatible with the pedigree corresponds to each of the non-tested members of the pedigree (including the U_R); $p_E(G,R)$ is identical to $p_E(R)$ with the exception that the genotype of the U_R is specified as G.

The probability of the phenotypic pedigree is the sum of the probabilities of all genotypic pedigrees which are compatible with the phenotypic pedigree in question [5].

Now we can construct the special case of the general expression (Eq. 4) for a stain containing U_R and n–1 usual unknowns:

$$p(n,k) = T_0(U_R) s^{2(n-1)} - \sum_{i=1}^{k} T_i(U_R)(s - a_i)^{2(n-1)} + \ldots, \quad (7)$$

where the items $T_0(U_R)$, $T_i(U_R),\ldots$ are described by the expressions analogous to the formulae (Eq. 3):

$$T_0(U_R) = \sum_{l=1}^{m} \sum_{r=1}^{m} p_E(A_l A_r / R),$$
$$T_i(U_R) = \sum_{\substack{l=1 \\ l \neq i}}^{m} \sum_{\substack{r=1 \\ r \neq i}}^{m} p_E(A_l A_r / R), \ldots \quad (8)$$

Working out a corresponding computer program for any pedigree E and an arbitrary set R is straightforward. An example of computations according to the formulae (Eqs. 7, 8) will be given at the end of the paper (numerical example 2).

It turns out that in the case of a single tested relative all the calculations are essentially simplified so that $p(n,k)$ can be presented by a rather simple formula. Therefore, we consider in the following section this case for three typical relationships i.e. child-parent, siblings, half-siblings (child-grandparent).

The case of one tested relative

Here the entire set R is reduced to the genotype of one tested person and $p_E(R)$ is just the frequency of this genotype R, i.e. $p(R)$, while the joint probability $p_E(G,R)$ is the probability of the corresponding phenotypic pedigree, where two persons – the U_R and his tested relative – are specified by the two genotypes, G and R, respectively.

The first step is to compute the match probabilities $p_E(G/R)$ [5]. As an example we consider a child-parent duo under the condition that the parent (tested relative) shows genotype AA. In this case

$$\begin{aligned} p_E(AA/AA) &= P_E(AA,AA)/p(AA) = a^3/a^2 = a, \\ p_E(AX/AA) &= P_E(AX,AA)/p(AA) = a^2 \cdot x/a^2 = x, \\ p_E(XY/AA) &= P_E(XY,AA)/p(AA) = 0 \ (\textit{exclusion}). \end{aligned} \tag{9}$$

Here any alleles not identical to those of the tested relative are specified as X, Y. The same specification holds in Table 1 where the magnitude $p_E(G/R)$ is calculated for the three relationships – child-parent, siblings, half-siblings (child-grandparent).

The next step is to compute the items $T_0(U_R)$, $T_i(U_R)$, $T_{ij}(U_R)$,… for the formula (Eq. 7). The computation depends on the type of the alleles found in the test of the relative. These alleles can generally belong to three different types:

1. The set of required alleles of the stain: $A_1, A_2,\ldots,A_k$
2. The set of non-required alleles of the stain: $A_{k+1},\ldots,A_m$
3. The set of alleles of the considered system which are not contained in the stain.

As will be seen later, it is necessary only for the set 1 to distinguish what concrete allele A_i out of the corresponding set $\{A_i\}$, $i = 1,\ldots,k$ has been found in the test. For the sets 2 and 3 it is not the precise specification of the alleles which is important but solely being part of the set. Therefore we designate all the alleles from the sets 2 and 3 as B and Z, respectively. Thus, for example, $R = A_iB$ means that the tested relative shows allele A_i from the set 1 and some allele from the set 2.

Altogether seven different cases can be distinguished

1. $R = A_iA_i$
2. $R = A_iA_j$ $(i \neq j)$
3. $R = A_iB$
4. $R = A_iZ$
5. $R = BB$
6. $R = BZ$
7. $R = ZZ$

It turns out that for each kind of relationship an explicit system of recurrent correlations can be constructed which provides direct and simple formulae yielding $p(n,k)$ for any given n and k. To this end we introduce the following function $L(r,k,s)$ of the frequencies of required alleles a_i $(i = 1,\ldots,k)$, $\sum_{i=1}^{m} a_i$ and an integer-valued parameter r:

$$\begin{aligned} L(r,k,s) = s^r - \sum_{i=1}^{k}(s-a_i)^r + \sum_{i=1}^{k-1}\sum_{j=i+1}^{k}(s-a_i-a_j)^r \\ -\ldots+(-1)^k(s-a_1-\ldots-a_k)^r. \end{aligned} \tag{10}$$

In the special case $r = 2n$ this function, $L(2n,k,s)$, is nothing else then Weir's formula (Eq. 4) for $p(n,k)$ applicable in the situation where all the unknowns are unrelated and have no tested relatives. Further, we designate expressions arising from (Eq. 10) upon elimination of all items containing a_i and both a_i and a_j as $L_i(r,k–1,s)$ and $L_{ij}(r,k–2,s)$, respectively.

We exemplify all the considerations leading to the explicit recurrent formulae using the case of siblings and under the condition that the tested sibling is homozygous with respect to the allele in question. We consider two possibilities arising when the allele found in the test belongs to the sets 1 and 2.

Set 1 Assume that the allele found in the test belongs to the set 1. Let us put for the sake of simplicity $R = A_1A_1$. Using the expression (Eq. 8) and singling out all the items containing the allele A_1 we compute $T_0(U_R)$:

$$\begin{aligned} T_0(U_R) = p_E(A_1A_1/A_1A_1) + \sum_{l=2}^{m} p_E(A_1A_l/A_1A_1) \\ + \sum_{l=2}^{m} p_E \sum_{r=2}^{m} p_E(A_lA_r/A_1A_1) \end{aligned} \tag{11}$$

Applying Table 1 we obtain

$$\begin{aligned} T_0(U_R) = \Bigg[(1+a_1)^2 + 2(1+a_1)\sum_{l=2}^{m} a_l + \sum_{l=2}^{m} a_l^2 \\ + 2\sum_{l=2}^{m-1}\sum_{r=l+1}^{m} a_l a_r\Bigg]/4 = (s^2+2s+1)/4 \end{aligned} \tag{12}$$

By analogy formulae for $T_i(U_R)$, $T_{ij}(U_R)$,… arise for $i, j \neq 1$:

$$\begin{aligned} T_i(U_R) &= [(s-a_i)^2 + 2(s-a_i) + 1]/4, \\ T_{ij}(U_R) &= [(s-a_i-a_j)^2 + 2(s-a_i-a_j) + 1]/4,\ldots \end{aligned} \tag{13}$$

Table 1 The probabilities $p_E(G/R)$ where G is the genotype of the U_R and R is the genotype of his tested relative (Capital letters represent the alleles, the corresponding lower-case letters represent the frequencies in the population)

R	G	Child-parent	Siblings	Half-siblings (Child-grandparent)
AA	*AA*	a	$(1+a)^2/4$	$a(1+a)/2$
	AX	x	$(1+a)x/2$	$(1+2a)x/2$
	XX	0	$x^2/4$	$x^2/2$
	XY	0	$xy/2$	xy
AB	*AA*	$a/2$	$a(1+a)/4$	$a(1+2a)/4$
	BB	$b/2$	$b(1+b)/4$	$b(1+2b)/4$
	AB	$(a+b)/2$	$(1+a+b+2ab)/4$	$(a+b+4ab)/4$
	AX	$x/2$	$x(1+2a)/4$	$x(1+4a)/4$
	BX	$x/2$	$x(1+2b)/4$	$x(1+4b)/4$
	XX	0	$x^2/4$	$x^2/2$
	XY	0	$xy/2$	xy

When computing $T_1(U_R)$, $T_{1i}(U_R)$,… characters of the expression (Eq. 11) containing A_1 vanish

$$T_1(U_R) = \sum_{l=2}^{m}\sum_{r=2}^{m} p_E\left(A_iA_j/A_1A_1\right) = \left[\sum_{l=2}^{m} a_l^2 + 2\sum_{l=2}^{m-1}\sum_{r=l+1}^{m} a_ia_j\right]/4 = (s-a_1)^2/4 \quad (14)$$

$$T_{1i}(U_R) = \sum_{\substack{l=2\\ l\neq i}}^{m}\sum_{\substack{r=2\\ r\neq i}}^{m} p_E\left(A_lA_r/A_1A_1\right) = (s-a_1-a_i)^2/4,...$$

Substituting the above expressions into formula (Eq. 7) and using the definitions of $L(r,k,s)$ and $L_1(r,k–1,s)$ we obtain

$$p(n,k) = [L(2n,k,s) + 2L_1(2n-1,k-1,s) + L_1(2n-2,k-1,s)]/4 \quad (15)$$

Set 2 Assume that the allele found in the test belongs to the set 2. Let us take $R = A_mA_m$. By analogy to the previous case we use (Eq. 8) and Table 1. This yields:

$$T_0(U_R) = \left[(1+a_m)^2 + 2(1+a_m)\sum_{l=1}^{m-1} a_l + \sum_{l=1}^{m-1} a_l^2 + 2\sum_{l=1}^{m-2}\sum_{r=l+1}^{m-1} a_la_r\right]/4 = (s^2+2s+1)/4, \quad (16)$$

$$T_i(U_R) = \left[(s-a_i)^2 + 2(s-a_i)+1\right]/4,$$

$$T_{ij}(U_R) = \left[(s-a_i-a_j)^2 + 2(s-a_i-a_j)+1\right]/4,...$$

which leads to the following expression for $p(n,k)$

$$p(n,k) = [L(2n,k,s) + 2L(2n-1,k,s) + L(2n-2,k,s)]/4 \quad (17)$$

Thus one can see that $p(n,k)$ does not depend of a_m, i.e. that solely the fact that the allele shown by the relative belongs to the set 2, but not the concrete nature of this allele, is of importance.

Arguing by analogy one can derive the corresponding formulae for all three modi of relationship and for all seven types of the genotype of the tested relative. All these expressions are presented in Table 2. The three modi of relationship – child-parent, siblings, half-siblings (child-grandparent) – are designated as E_1, E_2 and E_3 respectively.

Numerical examples

Example 1

Consider a stain showing five alleles A_1, A_2,…A_5 which is produced by three persons: the victim (V) with the genotype A_4A_5 and two assailants (Table 3). One suspect (U_R) is known but unavailable for testing. The information on his genotype is given by one of his relatives with the genotype R. The problem to be solved is whether U_R is a contributor of the stain.

Two hypotheses settle the matter.

H_1: V, U_R and one unknown are the contributors of the stain.

H_2: V and two unknowns are the contributors of the stain.

Table 2 The expressions for $p(n,k)$ for seven types of the genotypes (R) of the tested relative (The three modi of relationship – child-parent, siblings, half-siblings (child-grandparent) – are designated as E_1, E_2 and E_3,respectively)

	R	E	$p(n,k)$
1	A_iA_i	E_1	$L_i(2n-1,k-1,s)$
		E_2	$[L(2n,k,s) + 2\,L_i(2n-1,k-1,s) + L_i(2n-2,k-1,s)]/4$
		E_3	$[L(2n,k,s) + L_i(2n-1,k-1,s)]/2$
2	A_iA_j	E_1	$[L_i(2n-1,k-1,s) + L_j(2n-1,k-1,s)]/2$
		E_2	$[L(2n,k,s) + L_i(2n-1,k-1,s) + L_j(2n-1,k-1,s) + L_{ij}(2n-2,k-2,s)]/4$
		E_3	$[2\,L(2n,k,s) + L_i(2n-1,k-1,s) + L_j(2n-1,k-1,s)]/4$
3	A_iB	E_1	$[L(2n-1,k,s) + L_i(2n-1,k-1,s)]/2$
		E_2	$[L(2n,k,s) + L(2n-1,k,s) + L_i(2n-2,k-1,s)]/4$
		E_3	$[2L(2n,k,s) + L(2n-1,k,s) + L_i(2n-1,k-1,s)]/4$
4	A_iZ	E_1	$L_i(2n-1,k-1,s)/2$
		E_2	$[L(2n,k,s) + L_i(2n-1,k-1,s)]/4$
		E_3	$[2L(2n,k,s) + L_i(2n-1,k-1,s)]/4$
5	BB	E_1	$L(2n-1,k,s)$
		E_2	$[L(2n,k,s) + 2L(2n-1,k,s) + L(2n-2,k,s)]/4$
		E_3	$[L(2n,k,s) + L(2n-1,k,s)]/2$
6	BZ	E_1	$L(2n-1,k,s)/2$
		E_2	$[L(2n,k,s) + L(2n-1,k,s)]/4$
		E_3	$[2L(2n,k,s) + L(2n-1,k,s)]/4$
7	ZZ	E_1	0
		E_2	$L(2n,k,s)/4$
		E_3	$L(2n,k,s)/2$

Table 3 The stain. Three required alleles A_1, A_2, A_3; $k = 3$

Allele	Frequency	Victim
A_1	0.05	
A_2	0.22	
A_3	0.14	
A_4	0.18	+
A_5	0.21	+
s	0.80	

Table 4 The likelihood quotients for seven types of the genotypes (R) of the tested relative (The four modi of relationship – child-parent, siblings, half-siblings (child-grandparent), not related – are designated E_1, E_2, E_3 and E_4, respectively)

Genotype	Mode of relationship			
R	E_1	E_2	E_3	E_4
A_1A_1	5.2101	3.5553	3.1050	1.0000
A_1A_2	3.2783	4.2128	2.1391	1.0000
A_1B	2.8151	2.3578	1.9075	1.0000
A_1Z	2.6050	1.5525	1.8025	1.0000
BB	0.4202	0.4601	0.7101	1.0000
BZ	0.2101	0.3550	0.6050	1.0000
ZZ	0.0000	0.2500	0.5000	1.0000

The likelihood quotients X_1/X_2 for three modi of relationship and for seven types of the genotype of the tested relative are given in Table 4. Here $X_2 = L(4,3,s)$ and X_1 is computed according to Table 2 ($n = 2$; $k = 3$).

Example 2

Consider the preceding example but in a more general formulation where U_R has not one but two tested relatives. Assume that these relatives are: a parent with the homozygous genotype AA and a child with the genotype AB where $A \neq B$.

The pedigree E is shown in Fig. 1. It is a child-grandparent duo with

$$R = \{AA,AB\} \text{ and } p_E(R) = a^2b(1+2a)/2 \tag{18}$$

The frequencies of the possible genotypes of U_R are computed according to formula (Eq. 6), which yields

$$\begin{aligned} &p_E(AA/R) = 2a/(1 + 2a)\\ &p_E(AB/R) = (a + b)/(1 + 2a)\\ &p_E(AX/R) = x/(1 + 2a) \quad X \neq A,B \end{aligned} \tag{19}$$

Let us apply the above formulae under the assumption that A belongs to the set 1 ($A = A_1$) and B does not belong to the stain ($B = Z$).

Using the expression (Eq. 8) and singling out all the items containing the allele A_1 we compute $T_0(U_R)$:

$$\begin{aligned} T_0(U_R) &= p_E(A_1A_1/R) + \sum_{l=2}^{m} p_E(A_1A_l/R)\\ &= (2a_1 + a_2 + \ldots a_m)/(1 + 2a_1)\\ &= (a_1 + s)/(1 + 2a_1). \end{aligned} \tag{20}$$

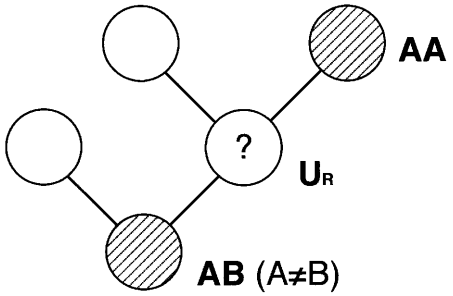

Fig. 1 Pedigree E with two tested relatives of the unavailable suspect U_R: a parent with genotype AA and a child with genotype AB ($A \neq B$). See also numerical example 2

Furthermore:

$$\begin{aligned} &T_1(U_R) = T_{12}(U_R) = T_{13}(U_R) = T_{123}(U_R) = 0;\\ &T_2(U_R) = (a_1 + s - a_2)/(1 + 2a_1);\\ &T_3(U_R) = (a_1 + s - a_3)/(1 + 2a_1);\\ &T_{23}(U_R) = (a_1 + s - a_2 - a_3)/(1 + 2a_1) \end{aligned} \tag{21}$$

Substituting these expressions into formula (Eq. 7) ($n = 2$, $k = 3$) we obtain an expression for X_1, which can be presented in a convenient form using the functions $L(r,k,s)$ and $L_i(r,k-1,s)$:

$$\begin{aligned} &X_1 = [a_1L_1(2,2,s) + L_1(3,2,s)]/(1 + 2a_1);\\ &X_2 = L(4,3,s) \end{aligned} \tag{22}$$

Thus we obtain the likelihood quotient:

$$X_1/X_2 = 4.8637 \tag{23}$$

Note that for the situation with a single tested relative we have pointed out, when the allele of this relative belongs to the set 2, then solely the fact of belonging to this set but not the precise specification of the allele is of importance. In the general situation with more than one tested relative this is not the case. For example, in our case when $A = A_5$, then

$$\begin{aligned} &T_0(U_R) = (a_5 + s)/(1 + 2a_5) \text{ and}\\ &X_1 = [a_5L(2,3,s) + L(3,3,s)]/(1 + 2a_5) \end{aligned} \tag{24}$$

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