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TECHNICAL NOTE

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Biostatistical evaluation of mixed stains with contributors of different ethnic origin

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Abstract The analysis of a mixed biological stain by means of highly polymorphic VNTR systems usually reveals a profile composed of multiple alleles. 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 by Weir with coauthors and by the present authors are limited to cases where all non-tested persons involved in the analysis belong to the same ethnical group. The present paper extends the theory beyond this limitation.

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

Introduction

DNA profiling of biological stains is now a central analytical method in forensic science, the aim of which is 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 coauthors [1]. The present authors have then shown that this formula is within the scope of the hypotheses testing approach and derived an equivalent recursive formula which is especially useful in cases where computations with the Weir-formula become cumbersome [2].

These results comprising the entire statistical analysis of DNA profiles underly two following restrictions:

1. 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.
2. All unknown persons should belong to the same ethnic group.

Situations with mixed stains involving contributors of different populations are becoming increasingly more common. The forensic expert should be able to provide scientific mathematical procedures to evaluate such cases. The determination of the ethnicity of an individual is usually a difficult matter [3], but this is not primarily the concern of the legal expert who should only emphasize that differences in population frequency data can effect probability estimations of DNA evidence. Inserting inadequate frequencies in probability calculations however, may or may not effect the interpretation. Therefore each case has to be treated separately under well formulated hypotheses.

In the present paper we extend the theory to the case where some unknown persons may belong to different ethnic groups, i.e. the second restriction is removed.

Extension of the theory

Let us first recall the general formulation of the statistical analysis of forensic DNA evidence in terms of the theory of hypotheses testing [2]. Thereby we introduce all the variables and specifications.

A DNA mixture from a crime scence – the stain – can contain contributions from a number of persons e.g victims, assailants. A comparison of the stain profile with the single persons's DNA profiles taken from a group of tested persons (victims, suspects) is performed with the aim to identify 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.

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The aim of the statistical analysis - 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 $A_1, A_2, \dots, A_m$ 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” $A_1, A_2, \dots, A_k$ ($k \le m$, $k \le 2n$) which are not contained in the genotypes of the contributors tested. 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 [2] 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 expression for the probability $p(n, k)$.

When all n unknown persons belong to the same population with the allele frequencies $a_1, a_2, \dots, a_m$, two expressions can be used to calculate $p(n, k)$. The first one is the Weir formula [1, 2]:

$$\begin{aligned} p(n,k) = {} & s^{2n} - \sum_{i=1}^{k} (s - a_i)^{2n} \\ & + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \left(s - a_i - a_j\right)^{2n} - \dots \\ & + (-1)^k (s - a_1 - \dots - a_k)^{2n} \end{aligned} \tag{1}$$

where s is the sum of the frequencies of all alleles contained in the stain

$$s = a_1 + a_2 + \dots + a_m$$

The second expression is [2]:

$$p(n,k) = (2n)!\, a_1 a_2 \dots a_k \sum_{j=0}^{2n-k} \frac{q^{2n-k-j}\, Q(k,j)}{(2n - k - j)!}, \tag{2}$$

Where q is the sum of the frequencies of all alleles contained in the stain except the “required alleles”

$$q = a_{k+1} + a_{k+2} + \dots + a_m.$$

The quantity $Q(k, j)$ is calculated in a recursive way:

$$\begin{aligned} Q(k,j) &= \sum_{i=0}^{j} \frac{a_k^i}{(i+1)!} Q(k-1, j-i) \\ Q(k,0) &= 1; \\ Q(0,j) &= 0 \text{ for } j > 0. \end{aligned} \tag{3}$$

The recursive formulae (2, 3) yield the same results as the Weir formula (1) and are especially appropriate for large values of k where calculations with (1) become rather cumbersome. In particular

$$\begin{aligned} p(n, 2n) &= (2n)!\, a_1 a_2 \dots a_{2n} \\ p(n, 2n-1) &= (2n)!\, a_1 \dots a_{2n-1} (s + q)/2. \end{aligned} \tag{4}$$

Let us assume now that only $n - 1$ out of n unknown persons belong to the basic population with the allele frequencies a_i and the corresponding sums $s = \sum_{i=1}^{m} a_i$, $q = \sum_{i=k+1}^{m} a_i$. We designate this basic population as the population 0. One of the n unknown persons is assumed now to be a member of some other population with the allele frequencies $\bar{a}_i$ and the corresponding sums

$$\bar{s} = \sum_{i=1}^{m} \bar{a}_i \qquad \bar{q} = \sum_{i=k+1}^{m} \bar{a}_i.$$

We specify this population as the population 1 and will call the unknown person belonging to the population 1 simply unknown 1.

The unknown 1 can show in principle 0, 1 or 2 “required alleles”. Consider the probabilities of these three events.

If the unknown 1 shows only one “required allele” A_i ($i = 1, 2, \dots, k$) from the stain, then he is either homozygous with respect to this allele – A_iA_i – or heterozygous A_iA_j, ($j = k + 1, \dots, m$) where A_j is outside the set of the “required alleles”. The probability for the unknown 1 to have one “required allele” A_i is

$$\bar{a}_i^2 + 2\bar{a}_i \sum_{j=k+1}^{m} \bar{a}_j = \bar{a}_i^2 + 2\bar{a}_i\bar{q}.$$

The rest of $n - 1$ unknowns (all of them belong to the population 0) should contribute the $k - 1$ out of k “required alleles” (A_i is lacking here). We designate the corresponding probability as $p_i(n - 1, k - 1)$.

Thus the probability that the unknown 1 shows some single “required allele” is

$$\sum_{i=1}^{k} (\bar{a}_i^2 + 2\bar{a}_i\,\bar{q})\, p_i(n - 1, k - 1)$$

If the unknown 1 shows two “required alleles” $A_i\,A_j$($i, j = 1, 2, \dots k$; $i \ne j$) (i.e. he is heterozygous with the probability $2\bar{a}_i\bar{a}_j$) then the corresponding probability of this event is

$$2\sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \bar{a}_i\,\bar{a}_j\,\bar{p}_{ij}(n - 1, k - 2)$$

where $p_{ij}(n - 1, k - 2)$ is the probability that $n - 1$ unknowns show $k - 2$ “required alleles” from the initial set of k “required alleles”, where now the alleles A_i and A_j are lacking.

Finally, the unknown 1 can shown no “required alleles”. In this case he is either homozygous A_iA_i ($i = k + 1, \dots, m$) or heterozygous A_iA_j ($i, j = k + 1, \dots, m$; $i \ne j$) with the probability

$$\sum_{i=k+1}^{m} \bar{a}_i^2 + 2\sum_{i=k+1}^{m-1} \sum_{j=i+1}^{m} \bar{a}_i\,\bar{a}_j = (\bar{a}_{i+1} + \dots + \bar{a}_m)^2 = \bar{q}^2$$

The rest of $n - 1$ unknowns should show in this case all the k “required alleles” with the probability $p(n - 1, k)$.

Summarizing the above arguments we can write the expression for $p(n, k)$ for the situation where one unknown person belongs to a different ethnic group:

$$\begin{aligned} p(n,k) = {} & \bar{q}^2\, p(n-1,k) \\ & + \sum_{i=1}^{k} (\bar{a}_i^2 + 2\bar{a}_i\,\bar{q})\, p_i(n-1,k-1) \\ & + 2\sum_{i=1}^{k-1}\sum_{j=i+1}^{k} \bar{a}_i\,\bar{a}_j\, p_{ij}(n-1,k-2) \end{aligned} \tag{5}$$

The last expression in principle allows us to calculate $p(n, k)$ using the formulae (1) or (2, 3) for the calculation of $p(n-1, k)$, $p_i(n-1, k-1)$ and $p_{ij}(n-1, k-2)$. Note that one should take $q + a_i$ instead of q when using the formulae (2, 3) for the calculation of $p_i(n-1, k-1)$ since the allele A_i is lacking in the set of "required alleles" in this situation. Similarly q should be substituted by $q + a_i + a_j$ when calculating $p_{ij}(n-1, k-2)$.

However, calculations with the formula (5) appear rather cumbersome. Therefore, in the next section we will derive a more convenient expression which turns out to be a generalization of (1).

Computational formula for the calculation of *p (n, k)* in the case of one unknown belonging to a different ethnic group

Let us consider the cases of $k = 0,1$ on the basis of the formula (5). In this way we will approach a general expression.

1. For $k = 0$ one has $\bar{q} = \bar{s}$ and the formula (5) is reduced to one term

   $$p(n,0) = \bar{q}^2 p(n-1,0)$$

   According to the formula (1) $p(n-1,0) = s^{2(n-1)}$. Thus

   $$p(n,0) = s^{2(n-1)}\bar{s}^2$$

2. For $k = 1$ the formula (5) contains two terms:

   $$p(n,1) = \bar{q}^2 p(n-1,1) + (\bar{a}_1^2 + 2\bar{a}_1\bar{q})\, p_1(n-1,0)$$

   In this case $\bar{q} = \bar{s} - \bar{a}_1$ and on the basis of the formula (1):

   $$p_1(n-1,0) = s^{2(n-1)}$$
   $$p(n-1,1) = s^{2(n-1)} - (s-a_1)^{2(n-1)}$$

   Therefore

   $$\begin{aligned} p(n,1) = {} & s^{2(n-1)}(\bar{q}^2 + \bar{a}_1^2 + 2\bar{q}\,\bar{a}_1) \\ & - (s-a_1)^{2(n-1)}\bar{q}^2 = s^{2(n-1)}\bar{s}^2 \\ & - (s-a_1)^{2(n-1)}(\bar{s}-\bar{a}_1)^2 \end{aligned}$$

On the basis of the above expression a general formula for $p(n, k)$ for any k can be suggested:

$$\begin{aligned} p(n,k) = {} & s^{2(n-1)}\,\bar{s}^2 - \sum_{i=1}^{k} (\bar{s}-\bar{a}_i)^{2(n-1)}(\bar{s}-\bar{a}_i)^2 \\ & + \sum_{i=1}^{k-1}\sum_{j=i+1}^{k} \left(s - a_1 - a_j\right)^{2(n-1)}\left(\bar{s} - \bar{a}_i - \bar{a}_j\right)^2 \\ & - \ldots + (-1)^k (s - a_1 - \ldots - a_k)^{2(n-1)}(\bar{s} - \bar{\mathrm{a}}_1 - \ldots - \bar{a}_k)^2 \end{aligned} \tag{6}$$

The proof of this formula is given in the Appendix.

The general case: groups of unknown persons from different populations

The formula (6) can be easily extended to the case where two unknown persons (thereafter called unknown 1 and unknown 2) belong to two different populations (population 1 and population 2 respectively) which are not identical to population 0. Let the allele frequencies in the population of the unknown 1 be as specified above $\bar{a}_1, \bar{a}_2, \ldots, \bar{a}_m$ with ($\bar{s} = \sum_{i=1}^{\mathrm{m}} \bar{a}_i$, $\bar{q} = \sum_{i=k+1}^{\mathrm{m}} \bar{a}_i$), while in the population 2 they are $\tilde{a}_1, \tilde{a}_2, \ldots, \tilde{a}_m$ with

$$\tilde{s} = \sum_{i=1}^{m} \tilde{a}_i \qquad \tilde{q} = \sum_{i=k+1}^{m} \tilde{a}_i$$

First we consider the case $k = 0$. Accordingly to the formula (5) we have

$$p(n,0) = \bar{q}^2 p(n-1,0)$$

Since in the set of $n-1$ unknown persons (where the unknown 1 is lacking) contains the unknown 2 who belongs to the population 2

$$p(n-1,0) = \tilde{q}^2 p(n-2,0) = \tilde{q}^2 s^{2(n-2)}$$

Thus

$$p(n,0) = s^{2(n-2)}\bar{q}^2\tilde{q}^2 = s^{2(n-2)}\bar{s}^2\tilde{s}^2$$

because in this case $\bar{q} = \bar{s}$ and $\tilde{q} = \tilde{s}$.

For the case $k = 1$ we have

$$p(n,1) = \bar{q}^2 p(n-1,1) + (\bar{a}_1^2 + \bar{a}_1\bar{q})\, p_1(n-1,0) \tag{7}$$

A set of $n-1$ unknown contains the unknown 2, therefore, according to the formula (6):

$$p(n-1,1) = s^{2(n-2)}\tilde{s}^2 - (s-a_1)^{2(n-2)}(\tilde{s}-\tilde{a}_1)^2$$
$$p_1(n-1,0) = s^{2(n-2)}\tilde{s}^2$$

Substitution of the last expressions into (7) yields:

$$\begin{aligned} p(n,1) = {} & s^{2(n-2)}\tilde{s}^2(\bar{q}^2 + \bar{a}_1^2 + 2\bar{a}_1\bar{q}) \\ & - \bar{q}^2(s-a_1)^{2(n-2)}(\tilde{s}-\tilde{a}_1)^2 = s^{2(n-2)}\bar{s}^2\tilde{s}^2 \\ & - (s-a_1)^{2(n-2)}(\tilde{s}-\tilde{a}_1)^2(\bar{s}-\bar{a}_1)^2 \end{aligned}$$

since in this case $\bar{q} + \bar{a}_1 = \bar{s}$.

The general form of the formula for $p(n, k)$ in the case of two foreigners appears to be

$$\begin{aligned} p(n,k) = {} & s^{2(n-2)}\,\bar{s}^2\,\tilde{s}^2 \\ & - \sum_{i=1}^{k} (s-a_i)^{2(n-2)}(\bar{s}-\bar{a}_i)^2(\tilde{s}-\tilde{a}_i)^2 \\ & + \sum_{i=1}^{k-1}\sum_{j=i+1}^{k} \left(s - a_i - a_j\right)^{2(n-2)}\left(\bar{s} - \bar{a}_i - \bar{a}_j\right)^2 \\ & \left(\tilde{s} - \tilde{a}_i - \tilde{a}_j\right)^2 - \ldots + (-1)^k \\ & (s - a_1 - \ldots - a_k)^{2(n-2)}(\bar{s} - \bar{a}_1 - \ldots - \bar{a}_k)^2 \\ & (\tilde{s} - \tilde{a}_1 - \ldots - \tilde{a}_k)^2 \end{aligned} \tag{8}$$

The proof of this final expression is also given in the Appendix.

Repeating the above arguments for the additional unknowns from different populations we arrive at the following obvious generalization. Let us assume that n unknown persons subdivided into r groups with $n_1, n_2, \dots, n_r$ persons in a group ($n_1 + n_2 + \dots + n_r = n$) are involved in a statistical analysis of a stain. All the unknowns from the group i belong to the same population with the allele frequencies a_{ij} ($j = 1, 2, \dots, m$) so that

$$s_i = \sum_{j=1}^{m} a_{ij}$$

Then the generalized formula (8) reads

$$\begin{aligned} p(n,k) = {} & s_1^{2n_1} s_2^{2n_2} \dots s_r^{2n_r} - \sum_{i=1}^{k} (s_1 - a_{1i})^{2n_1} (s_2 - a_{2i})^{2n_2} \\ & \dots (s_r - a_{ri})^{2n_r} + \dots \\ & + (-1)^k (s_1 - a_{11} - \dots - a_{1k})^{2n_1} \qquad (9) \\ & (s_2 - a_{21} - \dots - a_{2k})^{2n_2} \\ & \dots (s_r - a_{r1} - \dots - a_{rk})^{2n_r} \end{aligned}$$

Numerical example

As shown earlier [2] it is of no importance for the statistical analysis which populations the tested persons belong to. In contrast, the specification of the population of the unknowns is crucial.

Let us consider a stain showing six alleles $A_1, A_2, \dots, A_6$ and an hypothesis that three unknowns contribute to this stain together with a number of tested persons which combined show three alleles A_4, A_5, A_6. We assume that two unknowns belong to the population 1 with allele frequencies a_i while the third unknown belongs to the population 2 with the allele frequencies $\bar{a}_i$. The quantities a_i, $\bar{a}_i$ ($i = 1, \dots, 6$) and s, q, $\bar{s}$, $\bar{q}$ are given in the Table 1. Here s, $\bar{s}$ are the sums of the allele frequencies of the stain and q, $\bar{q}$ are the sums of the frequencies of the alleles shown by the tested persons.

If the computations would be performed under the assumption that all three unknowns belong to population 1 formula (1) yields

$$\begin{aligned} p(3,3) = {} & s^6 - \sum_{i=1}^{3} (a - a_i)^6 \\ & + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \left(s - a_i - a_j\right)^6 - \\ & (s - a_1 - a_2 - a_3)^6 = s^6 - \sum_{i=1}^{3} (s - a_i)^6 \\ & + \sum_{i=1}^{3} (q + a_i)^6 - q^6 = 0.00692 \end{aligned}$$

When it is taken into account that the third unknown belongs to population 2, formula (6) yields

$$\begin{aligned} \bar{p}(3,3) = {} & s^4 \bar{s}^2 - \sum_{i=1}^{3} (s - a_i)^4 (\bar{s} - \bar{a}_i)^2 \\ & + \sum_{i=1}^{3} (q + a_i)^4 (\bar{q} + \bar{a}_i)^2 \\ & - q^4 \bar{q}^2 = 0.00337 \end{aligned}$$

The ratio of the results obtained in the above two cases is

$$\bar{p}(3,3)/p(3,3) = 2.05$$

Thus neglecting the suitable population frequencies results in an error of 100%.

Table 1 The allele frequencies for the two populations used in the numerical example

	Allele	Frequency	
		Pop. 1 (a_i)	Pop. 2 ($\bar{a}_i$)
1	A_1	0.10	0.05
2	A_2	0.05	0.02
3	A_3	0.20	0.13
4	A_4	0.10	0.12
5	A_5	0.06	0.02
6	A_6	0.04	0.10
		$s = 0.55$	$\bar{s} = 0.44$
		$q = 0.20$	$\bar{q} = 0.24$

A Appendix

1. The starting point for the proof of the formula (6) is the expression (5):

$$\begin{aligned} p(n,k) = {} & \bar{q}^2 p(n-1, k) \\ & + \sum_{i=1}^{k} (\bar{a}_i^2 + 2\bar{a}_i \bar{q}) p_i (n-1, k-1) \qquad \text{(A1)} \\ & + 2 \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} a_i \bar{a}_j \, p_{ij} (n-1, k-2) \end{aligned}$$

On the basis of formula (1) and the relationship

$$s - a_1 - a_2 - \dots - a_k = q$$

we can write

$$\begin{aligned} p(n-1,k) = {} & s^{2(n-1)} - \sum_{i_1=1}^{k} \left(s - a_{i_1}\right)^{2(n-1)} \\ & + \sum_{i_1=1}^{k-1} \sum_{i_2=i_1+1}^{k} \left(s - a_{i_1} - a_{i_2}\right)^{2(n-1)} \\ & - \dots + (-1)^{k-2} \sum_{i_1=1}^{k-1} \sum_{i_2=i_1+1}^{k} \left(q + a_{i_1} + a_{i_2}\right)^{2(n-1)} \\ & + (-1)^{k-1} \sum_{i_1=1}^{k} \left(q + a_{i_1}\right)^{2(n-1)} + (-1)^k q^{2(n-1)} \end{aligned}$$

In the analogous development of the expression for p_i $(n - 1, k - 1)$ all terms containing a_i disappear and the last term will be $(-1)^{k-1}(q + a_i)$.

In the development of the expression for $p_{ij}(n-1, k-2)$ all the terms containing a_i or a_j disappear and the last term will be $(-1)^{k-2}(q + a_i + a_j)$.

We will now substitute the above developments into expression (A1) and collect all coefficients of the terms.

$$s^{2(n-1)},\ (s - a_{i_1})^{2(n-1)},\ \dots ,$$
$$(s - a_{i_1} - a_{i_2} - \dots - a_{i_r})^{2(n-1)},\ \dots$$
$$(q + a_{i_1})^{2(n-1)},\ q^{2(n-1)}$$

The formula (6) will be proven if these coefficients are

$$\overline{s}^2,\ (\overline{s} - \overline{a}_{i_1})^2,\ \dots ,\ (\overline{s} - \overline{a}_{i_1} - \overline{a}_{i_2} - \dots - \overline{a}_{i_r})^2,\ \dots ,$$
$$(\overline{q} + \overline{a}_{i_1})^2,\ \overline{q}^2$$

Indeed, the term $s^{2(n-1)}$ is contained in all three items of (A1) and its coefficient is

$$\overline{q}^2 + \sum_{i=1}^{k} \overline{a}_i^2 + 2\overline{q} \sum_{i=1}^{k} \overline{a}_i$$
$$+ 2\sum_{i_1}^{k-1} \sum_{j=i+1}^{k} \overline{a}_i\, \overline{a}_j = \left(\overline{q} + \sum_{i=1}^{k} \overline{a}_i\right)^2 = \overline{s}^2$$

The term $(s - a_{i_1})^{2(n-1)}$ disappears in the two last items of the expression (A1) as soon as the index i or j in the expressions $p_i(n-1, k-1)$ and $p_{ij}(n-1, k-2)$ acquires the value i_1. The coefficient of the term $(s - a_{i_1})^{2(n-1)}$ is

$$\overline{q}^2 + \sum_{\substack{i=1 \\ i \neq i_1}}^{k} \overline{a}_i^2 + 2\overline{q} \sum_{\substack{i=1 \\ i \neq i_1}}^{k} \overline{a}_i + 2\sum_{\substack{i=1 \\ i \neq i_1}}^{k-1} \sum_{\substack{j=i+1 \\ i \neq i_1}}^{k} \overline{a}_i\, \overline{a}_j$$
$$= \left(\overline{q} + \sum_{\substack{i=1 \\ i \neq i_1}}^{k} \overline{a}_i\right)^2 = \left(\overline{s} - \overline{a}_{i_1}\right)^2$$

Using analogous reasoning we can see that when $r < k - 1$ the term $(s - a_{i_1} - a_{i_2} - \dots - a_{i_r})^{2(n-1)}$ disappears in the last two items of (A1) as soon as i or j acquires one of the values $i_1, i_2, \dots , i_r$. The coefficient of this term is

$$(\overline{s} - \overline{a}_{i_1} - \overline{a}_{i_2} - \dots - \overline{a}_{i_r})^2$$

The last but one term $(s + a_{i_1})^{2(n-1)}$ is contained only in the first two items of (A1). The coefficient of this term is

$$\overline{q}^2 + \overline{a}^2_{i_1} + 2\overline{q}\,\overline{q}_1 = (\overline{q} + \overline{a}_{i_1})^2$$

Finally, the last term $q^{2(n-1)}$ is contained only in the first item and its coefficient is $\overline{q}^2$.

2. The proof of the formula (8) is completely analogous to the proof of formula (6). The expression for $p(n-1, k)$, $p_i(n-1, k-1)$ and $p_{ij}(n-1, k-2)$ in (A1) are developed into series according to the formula (6) and the coefficients of the terms

   $$s^{2(n-2)}\tilde{s}^2,\ (s - a_i)^{2(n-2)}(\tilde{s} - \tilde{a}_i)^2,\ \dots$$

   are elucidated.

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