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SHORT COMMUNICATION

Paternity calculations in a di-spermy case

Bruce Budowle[1,2] · **Christina Capt**[1] · **Ranajit Chakraborty**[1] · **Jianye Ge**[3]

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Abstract In a criminal paternity case, which involved analysis of the product of conception, a rare circumstance was observed. The product of conception was triploidy, apparently due to an egg fertilized by two sperm. Since there is little guidance on how to calculate the probability of the DNA evidence given some basic hypotheses, the formulae were derived and are presented herein. These approaches could provide guidance for similar situations if they arise.

Keywords Paternity test · Polyspermy · Triploid · Product of conception · Paternity index · Partial molar pregnancy

Introduction

In some cases of rape, the victim becomes pregnant. Identity of the biological father in such cases can be highly informative in identifying the perpetrator of the sexual assault. A paternity test can be performed by typing the DNA from the child, fetus, or products of conception; the mother; and alleged father. The statistical calculations for determining the paternity index (PI; i.e., the likelihood ratio) for the standard trio cases are well described and straight forward. However, relatively infrequently one member of the assumed trio presents a profile that is atypical. Thus, calculating the PI can be challenging, although the principles of Mendelian inheritance still hold.

The Center for Human Identification of the University of North Texas Health Science Center was presented with a case in which an under-aged female became pregnant and an adult male was accused of rape. The products of conception were analyzed by STR typing, and the profile presented a triploid pattern. Other than the mother's genetic contribution to the initial embryo, contamination of the mother's DNA from comingled maternal tissue was ruled out as contributing to the triploid profile. Background level contributions from contaminating maternal tissue were low and approximately 5–10 times less than the alleles of the "fetal" tissue. After subtracting out the mother's genetic contribution to the fetal material and based on peak heights, the results supported that the egg was fertilized by two sperm. Polyspermy, rarely encountered in criminal cases, was first described by Boveri [1] over a century ago. An egg fertilized by two sperm does not yield a viable offspring and often result in what is called a partial molar pregnancy [2, 3].

A paternity test in a criminal case in which di-spermy is the best explanation to describe the evidence is rare. To the best of our knowledge, there is no guidance in the peer-reviewed literature on how to calculate a PI involving a di-spermy case.

✉ Bruce Budowle
bruce.budowle@unthsc.edu

[1] Institute of Applied Genetics, Center for Human Identification, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX, USA

[2] Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia

[3] Human Identification, Thermo Fisher Scientific, South San Francisco, CA, USA

Buckleton et al [4] in a chapter in their book briefly describe calculating a PI for four genotype scenarios. However, their description is not exhaustive and only considers one scenario (although a likely scenario). Therefore, in this technical note, formulae are presented for calculating the probability of the genetic evidence for all genotype combinations of an alleged father (AF), mother (M), and di-spermic child (C) for different plausible hypotheses that may be proposed.

Methods

In a typical paternity test, two hypotheses are considered:

H_1: AF is the biological father of C

H_2: A random man (RM) is the biological father of C.

In both hypotheses, M is assumed to be the biological parent of C and unrelated to the biological father. The ratio of probabilities of observing the genetic evidence under each hypothesis is then expressed as a PI, in which

$$\mathrm{PI} = \frac{\Pr\left(G_C, G_M, G_{AF} \middle| H_1\right)}{\Pr\left(G_C, G_M, G_{AF} \middle| H_2\right)},$$

with G_C, G_M, and G_{AF} representing the observed genotypes of C, M, and AF, respectively. Using the same logic, one can compute the PI for di-spermy scenarios. These hypotheses can entertain that the AF/RM contributed the two sperm, that the AF and RM each contributed a sperm, or that two RM each contributed a sperm. The various

Table 1 Probability of genetic evidence under hypotheses in which two sperm are donated by the biological father

Genotypes			2-sperms from AF	2-sperms from RM	
AF	M	C	H_0	H_1	
AA	AA	AAA	1	aa + 0.25(2aq)	q = 1-a
AB	AA	AAA	0.25	aa + 0.25(2aq)	q = 1-a
AB	AA	ABB	0.25	bb + 0.25(2bq)	q = 1-b
AB	AA	AAB	0.5	0.5(2ab)	
AA	AB	AAA	0.5	0.5aa + 0.125(2aq)	q = 1-a
AA	AB	AAB	0.5	0.5aa + 0.125(2aq) + 0.25(2ab)	q = 1-a
AB	AB	AAA	0.125	0.5aa + 0.125(2aq)	q = 1-a
AB	AB	AAB	0.375	0.5aa + 0.125(2aq) + 0.25(2ab)	q = 1-a
AB	AB	ABB	0.375	0.5bb + 0.125(2bq) + 0.25(2ab)	q = 1-b
AB	AB	BBB	0.125	0.5bb + 0.125(2bq)	q = 1-b
AA	CC	AAC	1	aa + 0.25(2aq)	q = 1-a
AB	CC	AAC	0.25	aa + 0.25(2aq)	q = 1-a
AB	CC	BBC	0.25	bb + 0.25(2bq)	q = 1-b
AB	CC	ABC	0.5	0.5(2ab)	
AA	BC	AAB	0.5	0.5aa + 0.125(2aq)	q = 1-a
AA	BC	AAC	0.5	0.5aa + 0.125(2aq)	q = 1-a
AB	BC	AAB	0.125	0.5aa + 0.125(2aq)	q = 1-a
AB	BC	AAC	0.125	0.5aa + 0.125(2aq)	q = 1-a
AB	BC	BBB	0.125	0.5bb + 0.125(2bq)	q = 1-b
AB	BC	BBC	0.125	0.5bb + 0.125(2bq) + 0.25(2bc)	q = 1-b
AB	BC	ABB	0.25	0.25(2ab)	
AB	BC	ABC	0.25	0.25(2ab) + 0.25(2 ac)	
AB	CD	AAC	0.125	0.5aa + 0.125(2aq)	q = 1-a
AB	CD	AAD	0.125	0.5aa + 0.125(2aq)	q = 1-a
AB	CD	BBC	0.125	0.5bb + 0.125(2bq)	q = 1-b
AB	CD	BBD	0.125	0.5bb + 0.125(2bq)	q = 1-b
AB	CD	ABC	0.25	0.25(2ab)	
AB	CD	ABD	0.25	0.25(2ab)	

Capital letters (A,B,C,D) represent the alleles, and lower case letters (a,b,c,q) represent respective allele frequencies (with q varying depending on the context of the genotypes, represented in the last column of the table)

AF alleged father, *M* mother, C typically denotes child and in this case is product of conception resulting from an egg fertilized by two sperm, *RM* random man not related to AF and the RMs are unrelated

hypotheses considered are as follows:

H_0: AF is the biological father and contributed two sperm

H_1: RM is the biological father and contributed two sperm

H_2: AF and a RM are the biological fathers and each contributed a sperm

H_3: Two unrelated RMs are the biological fathers and each contributed a sperm

Other hypotheses may be considered, such as the AF and a biological relative are the biological fathers and each contributed a sperm. However, due to the complexity of that scenario and others similar to it, only the above four hypotheses were entertained.

The principles of genetic inheritance used in a typical paternity case (i.e., trio) also apply for a di-spermy case; the only difference is that independent contribution of two sperm from the biological father or fathers must be factored into the equations. Calculations for the probability of the genetic evidence for the first two hypotheses (H_0 and H_1) in which a single man contributes the two sperm are displayed in Table 1 (see Appendix for derivation example).

Results and discussion

The calculations in Table 1 apply to the propositions in which a single male contributes the two sperm. Although not as likely, another proposition is two men each contribute a sperm. Table 2 displays the

Table 2 Probability of genetic evidence under hypotheses in which two sperm are donated by two true biological fathers

Genotypes			1-sperm from AF + 1-sperm from RM	1-sperm from RM1 + 1-sperm from RM2
AF	M	C	H_2	H_3
AA	AA	AAA	a	aa
AB	AA	AAA	0.5a	aa
AB	AA	ABB	0.5b	bb
AB	AA	AAB	0.5(a + b)	2ab
AA	AB	AAA	0.5a	0.5aa
AA	AB	AAB	0.5(a + b)	ab + 0.5aa
AB	AB	AAA	0.25a	0.5aa
AB	AB	AAB	0.25b + 0.5a	ab + 0.5aa
AB	AB	ABB	0.25a + 0.5b	ab + 0.5bb
AB	AB	BBB	0.25b	0.5bb
AA	CC	AAC	a	aa
AB	CC	AAC	0.5a	aa
AB	CC	BBC	0.5b	bb
AB	CC	ABC	0.5(a + b)	2ab
AA	BC	AAB	0.5a	0.5aa
AA	BC	AAC	0.5a	0.5aa
AB	BC	AAB	0.25a	0.5aa
AB	BC	AAC	0.25a	0.5aa
AB	BC	BBB	0.25b	0.5bb
AB	BC	BBC	0.25(b + c)	bc + 0.5bb
AB	BC	ABB	0.25(a + b)	ab
AB	BC	ABC	0.25(a + b + c)	ab + ac
AB	CD	AAC	0.25a	0.5aa
AB	CD	AAD	0.25a	0.5aa
AB	CD	BBC	0.25b	0.5bb
AB	CD	BBD	0.25b	0.5bb
AB	CD	ABC	0.25(a + b)	ab
AB	CD	ABD	0.25(a + b)	ab

Capital letters (A,B,C,D) represent the alleles, and lower case letters (a,b,c) represent respective allele frequencies

AF alleged father, *M* mother, C typically denotes child and in this case is product of conception resulting from an egg fertilized by two sperm, *RM* random man not related to AF

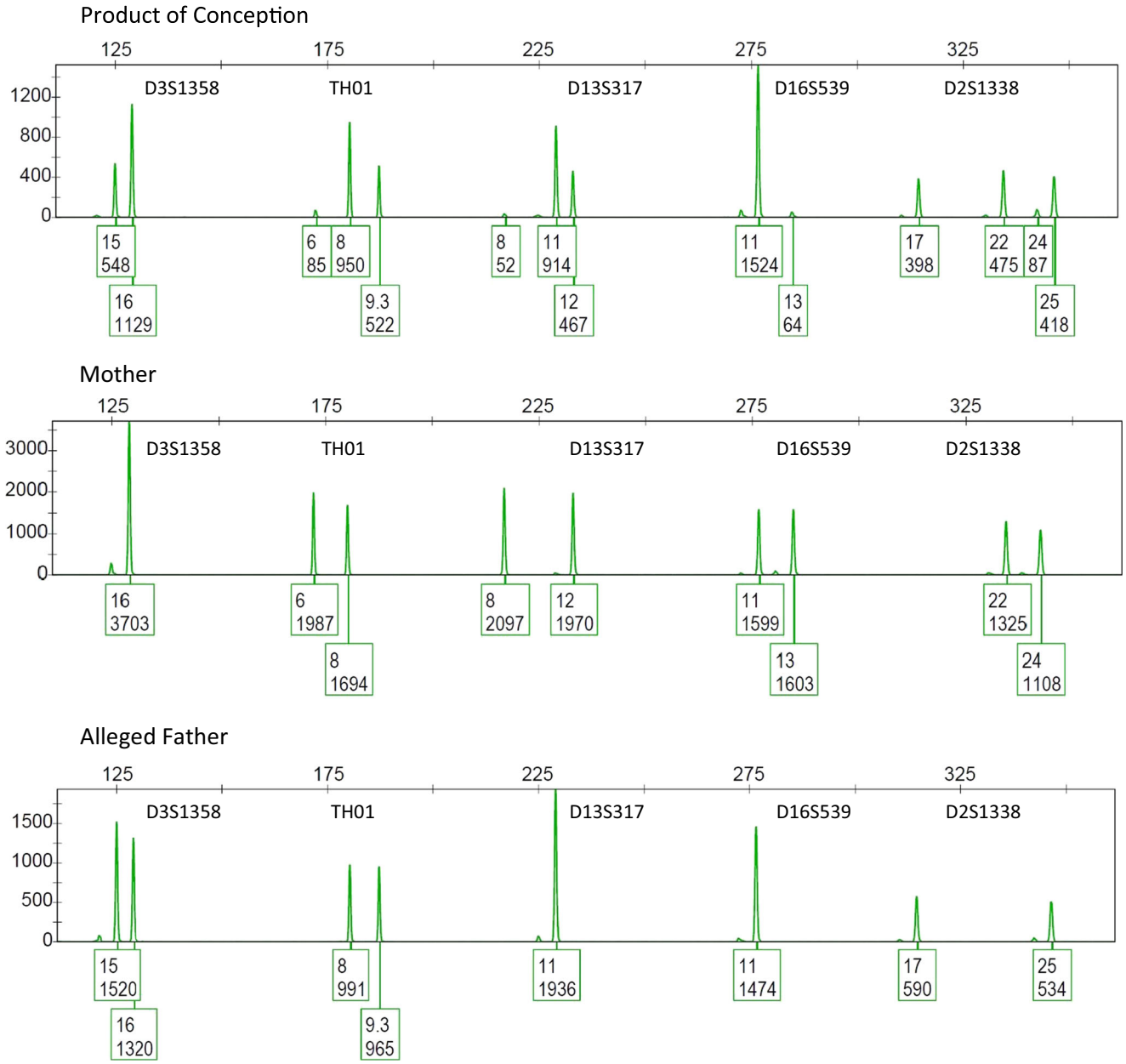

Fig. 1 The set of loci in the green dye channel of the AmpFlSTR® Identifiler® Plus PCR Amplification kit observed in the di-spermy paternity case. Only one dye channel is shown as an example of results obtained. The *top panel* is the profile of the product of conception; the *middle panel* is the profile of M; the *bottom panel* is the profile of AF

calculations for H_2 and H_3 (see Appendix for derivation example).

In the particular case, H_0 and H_1 were inferred as these hypotheses were the most likely to occur. Genotyping was performed using the AmpFlSTR® Identifiler® Plus PCR Amplification kit (Thermo Fisher Scientific, South San Francisco, CA, USA). As an example of the profiles for this case, Fig. 1 shows the green dye channel loci for the product of conception, M, and AF, and observed genotypes and various allele contributions are represented in Table 3. Using Caucasian allele frequencies [5, 6], the combined PI (for all 15 STRs) was 130 quadrillion, thus providing support for the AF being the biological father. While di-spermy is an unlikely occurrence to encounter in a criminal case, hopefully the information in this technical note could be helpful for similar case scenarios.

Table 3 Alleles observed for five STR loci, deduced allele contributions and genotypes, and formulae used for calculating the PI under hypotheses H_0 and H_1 in di-spermy paternity case

Loci	M	AF	PC[a]	Genotype PC[b]	Maternal allele	Obligate paternal alleles	H_0	H_1
D3S1358	16	15,16	15,16	15,16,16	16	15,16	0.5	$0.5(2p_{15}p_{16})$
TH01	6,8	8,9.3	[6],8,9.3	8,8,9.3	8	8,9.3	0.25	$0.25(2p_{8}p_{9.3})$
D13S317	8,12	11	[8],11,12	11,11,12	12	11,11	0.5	$p_{11}^2 + 0.125(2p_{11}\text{-}p_{11}^2)$
D16S539	11,13	11	11,[13]	11,11,11	11	11,11	0.5	$p_{11}^2 + 0.125(2p_{11}\text{-}p_{11}^2)$
D2S1338	22,24	17,25	17,22,[24],25	17,22,25	22	17,25	0.25	$0.25(2p_{17}p_{25})$

M mother, *AF* alleged father, *PC* product of conception

[a] Bracketed alleles in the PC profile column are attributable to low-level maternal background DNA and are not part of the genetic contribution of M to PC

[b] Assumed genotype of PC based on peak heights

Appendix: Example of formulae derivation

Table 4 One set of genotypes are shown as an example of how the formulae in Tables 1 and 2 were generated.

Genotypes	AF M C	AA AB AAB
2-sperm from AF	H_0	0.5
2-sperm from RM	H_1	0.5aa + 0.125(2aq) + 0.25(2ab) q = 1-a
1-sperm from AF + 1-sperm from RM	H_2	0.5(a + b)
1-sperm from RM1 + 1-sperm from RM2	H_3	ab + 0.5aa

H_0:

AF can only contribute A sperm with probability of 1. The mother must contribute B to the child with a chance of 0.5. Therefore, the probability of the scenario is 0.5.

H_1:

If the mother contributes A to the child (with 0.5 chance), then the biological father must be AB, and the probability of this scenario is 0.5* 2*0.5*0.5*(2ab) = 0.25*(2ab).

If the mother contributes B to the child (with 0.5 chance), then the biological father can be AA or AQ (Q ≠ A, and Q can be B). If he is AA, the probability is 0.5*(aa). If he is AQ, the chance is 0.5*0.5*0.5*(2aq), where q = 1-a. Here, q is NOT 1-a-b, because Q can be B.

Add these two scenarios together to derive the formula 0.5aa + 0.125(2aq) + 0.25(2ab), where q = 1-a.

H_2:

The biological father contributes A (with probability of 1).

If the mother contributes A to the child (with 0.5 chance), then the RM has to contribute B (with a probability of "b"). Therefore, the probability of this scenario is 0.5*(b).

If the mother contributes B to the child (with 0.5 chance), then the RM has to contribute A (with a probability of "a"). Therefore, the probability of this scenario is 0.5*(a).

Add these two scenarios together to derive the formula 0.5*(a + b).

H_3:

If the mother contributes A to the child (with 0.5 chance), then RM1 can contribute A (with a probability of "a") and RM2 can contribute B (with a probability of "b"), or RM1 can contribute B and RM2 can contribute A. Therefore, the probability of this scenario is 0.5(ab) + 0.5(ab) = ab.

If the mother contributes B to the child (with 0.5 chance), both RM1 and RM2 have to contribute A (each with a probability of "a"). Therefore, the probability of this scenario is 0.5*(aa).

Add these two scenarios together to derive the formula ab + 0.5aa.

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