When no biological material is available, how accurate is DNA-based identification?

Essen-Möller and Identification Based on DNA

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The *MS Rocknes* capsized in Vatlestraumen on the Norwegian coast January 19, 2004. The photograph in Figure 1 was taken shortly after the accident. Twelve crew members were rescued, while 18—almost all of Asian origin—were either deceased (5) or missing (13). The lowest point of the capsized vessel was 25 meters under sea level. Three weeks later, divers managed to bring up 11 deceased crew members from inside the boat. One more deceased crew member was found inside the ship when it was turned. One crew member is still missing.

Most of the deceased crew members were identified using traditional methods based on odontological criteria or information from the medical examination. However, one of the deceased—designated I—could not be identified using traditional methods, so DNA analysis was used. Reference samples were received from two males (II and III). For various good reasons, identification cases such as this often are performed under great time pressure. There was only time for limited statistical consideration and calculation in connection with the actual case work. A more detailed account is now presented for the first time.

Figure 1. The capsized *MS Rocknes* shortly after the disaster. Photograph reproduced with permission from Gisle Mellum.

Sometimes, it is hard to get all relevant information quickly. For instance, we were informed that II, III, or neither was the brother of the deceased. However, it was not possible to establish whether "brother" was to be taken literally or also could mean half brother. This latter possibility is included throughout.

There have been many papers on DNA-based identification. If there is

biological material from the deceased available—for instance, from a previous hospital visit—identification can be simplified greatly. This was not the case for the present study, so it was necessary to approach presumed relatives to obtain DNA samples. The present case was further complicated because the traditional analysis, based on so-called autosomal markers, proved essentially inconclusive and further work was required.

Box 1. Basic Forensic Genetics

Figure 2 shows a simple pedigree. There are parents, a female (F_1), a male (M_1), and a son (M_2). Females are depicted conventionally by circles, men by squares. Consider first the data available for one locus (i.e., one specific location of the human genome). We prefer locus to gene as the latter term could indicate there is some coding involved; forensic loci are selected deliberately to reside in regions of no known importance.

As shown in Figure 2, F_1 has two copies of the A allele, M_1 is {B,C}, and M_2 is {A,B}. The precise definition of an allele will be of no importance for this paper and will vary depending on the type of DNA involved. M_2 has inherited one allele A from his mother and the other allele B from his father. According to Mendel, each of the parents' alleles is equally likely to be passed to the child.

Certainly, the data presents evidence in favor of the hypothesis of Figure 2 (i.e., H_1: *M_1 is the father of M_2*). To assess the evidence, we calculate the likelihood of the data as

$$\Pr\left(F_1=\{A,A\}, M_1=\{B,C\}, M_2=\{A,B\}\,\middle|\,H_1\right)=$$

$$\Pr\left(M_2=\{A,B\}\,\middle|\,F_1=\{A,A\}, M_1=\{B,C\}, H_1\right)P\left(F_1=\{A,A\}, M_1=\{B,C\}\right)=0.5p_A^2\,2p_Bp_C$$

The assumptions underlying these calculation types are based on the pedigree structure, no shared coancestry (implying Hardy-Weinberg equilibrium), no mutations, and mendelian inheritance.

Considering next the hypothesis H_2 (i.e., some other man is the father), we find a likelihood of $2p_A^2p_B^2p_C$. The paternity index is the ratio of these likelihood ratios. In modern parlance, the term likelihood ratio (LR) is preferred, and so for this locus, LR=P(data|H_1)/P(data|H_2)=$1/(2p_B)$, which corresponds to Essen-Möller's $W=LR/(LR+1)=1/(1+2p_B)$. Observe that the resulting evidence will be strong if the paternal allele B is rare. To obtain stronger evidence, the analysis in real cases is based on several unlinked (independent) loci, and the overall LR is obtained by multiplication.

Figure 2. The figure shows a standard paternity case with genotypes for one marker. The child M_2 has inherited an A from the mother F_1 and a B from the father. If the B allele is rare, then this marker will provide strong evidence in favor of paternity.

Swedish Medical Association, forensic genetics is not mentioned. While his contribution to other areas may well be of even greater lasting importance, his seminal paper from 1938 is still worth reading. Essen-Möller died in 1992.

Methodology

The objective of the present case study is to determine if there is a relationship between individuals II or III and the deceased. Five pedigrees corresponding to five hypotheses are shown in Figure 3. The first pedigree corresponds to I, II, and III being unrelated. If this one comes out as the most likely, then the deceased would remain unidentified. In all other cases, a successful identification is obtained. Obviously, statistical methods are needed to measure the certainty of the identification. Prior to Essen-Möller's work at the end of the 1930s, biological markers had been used only to exclude certain claimed relations between individuals; typically, one could determine successfully that a man was not the father of a child. The basic message in Essen-Möller's writings was to show that sometimes paternity could be proved. He also devised a framework for the statistical proof. His measure, W (German: Wahrscheinlichkeit; English: probability), still is used widely.

We first will consider a modern approach to the identification problem, and then subsequently show how this relates to W.

Essen-Möller

Erik Essen-Möller was born in 1901 in Lund, Sweden, as the son of Elis Essen-Möller, a professor of obstetrics and gynecology. Raised in an academic environment, Essen-Möller grew up in a well-situated and locally well-known family. After studies of natural sciences and medicine in Lund, he was awarded a PhD in genetics in 1935 and in psychiatry in 1939. He visited the Institute of Anthropology in Wien, the Kaiser-Wilhelm Institute of Anthropology and Eugenics, and the Genetic-Demographic Department at the Deutsche Forschungsanstalt für Psychiatrie in Munich, Germany. Following these stays, he finished his paper about the theoretical basics of the evidence of paternity in 1938. The German title is given in the reference list, while an English translation reads "The Evidential Value of Similarity as Proof of Paternity, Fundamental Principles."

In 1943, he became a professor—initially at Karolinska Institutet in Stockholm—then, in 1944, at Lund University. He became the longest professor of psychiatry in Sweden (both in time and body height). His main interest was genetic psychiatry, which was a new field at the time. These studies demanded a deep understanding of statistics, and he eventually published a book of statistics for physicians. His students characterized him as a professor who was too abstract for the medical students and too medical for the mathematical students.

During his career, he was responsible for a number of large-scale studies. He founded a registry for twin research in Lund that eventually led to classic studies in epidemiological psychiatry. For a large part of his career, he was engaged in population studies. In a short biography in *The Journal of the*
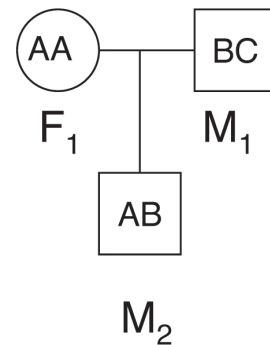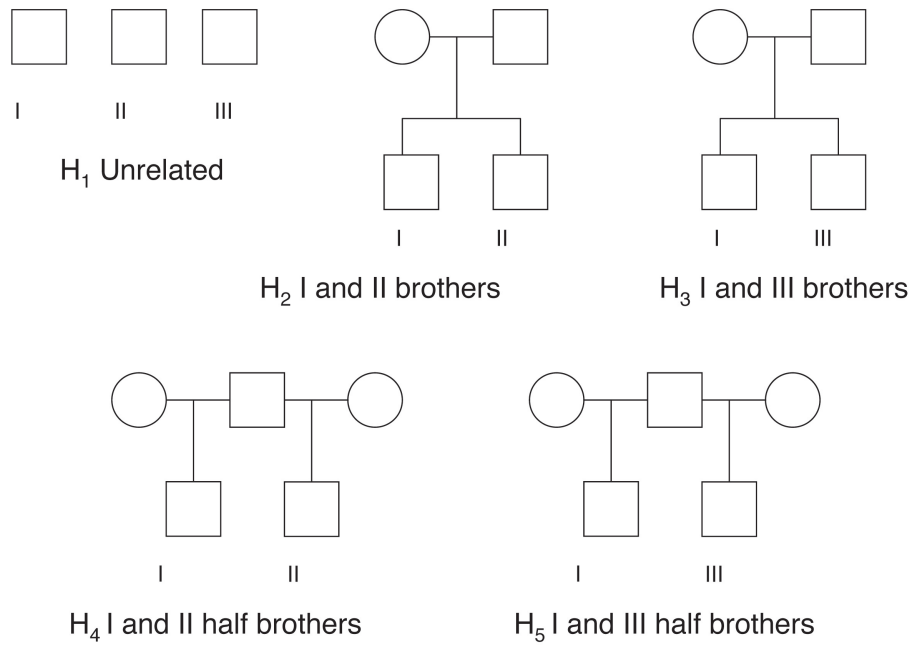
Figure 3. Five pedigrees are shown corresponding to the five hypotheses considered.

Box 2. Some Analytical Calculations

We need to calculate the likelihood of the data corresponding to the five pedigrees of Figure 3 (i.e., $\Pr(G_I, G_{II}, G_{III}|H_i)$, where G with subscripts denotes genotypes). The first hypothesis is the easiest, and $\Pr(G_I, G_{II}, G_{III}|H_1) = \Pr(G_I)\Pr(G_{II})\Pr(G_{III})$ as the individuals are unrelated and shared coancestry is disregarded. The required probabilities are obtained according to the Hardy-Weinberg equilibrium, as described in Box 1.

Next, consider hypothesis 3 (hypothesis 2 is treated similarly and is omitted). Obviously, $\Pr(G_I, G_{II}, G_{III}|H_3) = \Pr(G_{II})$ $\Pr(G_I, G_{III}|H_3)$. The last term is obtained most easily by conditioning on Z, the number of alleles shared, "identical by descent" (IBD): an allele in one brother is IBD to an allele in the brother if it derives from the same allele of the considered pedigree. In this case (again, based on the mendelian law), $P(Z=0)=P(Z=2)=1/4$, while $P(Z=1)=1/2$ and

$$\Pr(G_I, G_{III}|H_3) = \frac{1}{4}\Pr(G_I, G_{III}|Z=0) + \frac{1}{2}\Pr(G_I, G_{III}|Z=1) + \frac{1}{4}\Pr(G_I, G_{III}|Z=2).$$

Observe that the conditioning on the H_3 is removed because the relevant information is contained in IBD status. Further, calculations depend on the genotype of the marker. If I and III share no alleles, as for marker vWA,

$$\Pr(G_I, G_{III}|H_3) = \frac{1}{4}\Pr(G_I)\Pr(G_{III}).$$

Observe that a locus with such marker observations provides maximal evidence in favor of I and III being unrelated, the LR comparing H_3 to H_1 is in this case 1/4 independently of allele frequencies. On the contrary, locus D16S539 provides evidence in favor of the full sibling hypothesis. In this case,

$$\Pr(G_I, G_{III}|H_3) = \frac{1}{4}p_{10}^4 + \frac{1}{2}p_{10}^3 + \frac{1}{4}p_{10}^2 \text{ and}$$

$$LR = \frac{P(data|H_3)}{P(data|H_1)} = \frac{1}{4} + \frac{1}{2p_{10}} + \frac{1}{4p_{10}^2}$$

as $P(data|H_1) = p_{10}^4$, and so there will be strong support for H_3 if 10 is a rare allele. The calculations required for the remaining alternatives are quite similar and are omitted. Complete computations valid more generally are presented at *http://folk.uio.no/thoree/chance*.

Table 1—Results from Typing of the Autosomal STR Markers in the SGM+ Multiplex Kit

(All individuals are seen to be male because the "Amelo" marker shows XY.
The remaining 10 markers are used to try to establish the identity of I.)

Sample ID	D3S1356	vWA	D16S539	D2S1338	Amelo	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
I (deceased)	18, 17	16, 19	10, 10	24, 20	XY	16, 13	30, 30.2	12, 20	15.2, 14	6, 7	23, 26
II, putative brother of I	16, 17	14, 19	9, 10	17, 20	XY	11, 13	30, 30.2	14, 15	13, 14	6, 8	22, 25
III, putative brother of I	16, 17	17, 17	10, 10	25, 20	XY	13, 13	29, 29	14, 16	15.2, 14	6, 9	23, 21

Consider therefore n hypotheses $H_1,...,H_n$, each corresponding to a pedigree relating people. Let $L_i=P(\text{data}|H_i)$ be the likelihood of the data, given hypothesis H_i. This likelihood can be computed in a large number of software packages. Analytical examples based on some simplifying assumptions are presented in Boxes 1 and 2, along with a brief review of the required genetics. The likelihood can be used to form likelihood ratios, such as $LR = L_i/L_j$, corresponding to two hypotheses H_i and H_j. If the numerator corresponds to a hypothesis where a specified man is the father of a child, as in Figure 2, whereas the denominator is the likelihood assuming some other man is the father, then this would be called a paternity index (PI) in forensic genetics. The greater the PI, the stronger the evidence is in favor of paternity. By introducing prior probabilities for the hypotheses, Bayes Theorem can be used to calculate the posterior probabilities $P(H_i|\text{data})$.

What Does Essen-Möller Have to Do with It?

Consider two hypotheses, and, as Essen-Möller did, denote the likelihoods $L_1=X$ and $L_2=Y$, where the former is calculated assuming paternity (denoted H_1 below) and the latter nonpaternity. Furthermore, equal priors are assigned to these hypotheses, corresponding to the following German statement (quotes are from Essen-Möller, 1938, unless stated otherwise): "Wir wollen nun die weitere Annahme machen, daß wahre und falsche Väter gleich häufig zur Begutachtung kommen." From Bayes Theorem, it then follows directly that $P(H_1|\text{data})=X/(X+Y)$. This is Essen-Möller's probability

(Wahrscheinlichkeit) $W=P(H_1|\text{data})$, "die Wahrscheinlichkeit der wahren Vaterschaft." W may be rewritten in several ways, for instance $W=PI/(PI+1)$, which explicitly relates the two measures used for paternity.

Hummel (1982) reviews Essen-Möller's work, credits Hans Gurtler for introducing the PI in 1956, and comments on varying international practice: "The W value is used in the German-speaking countries (Germany, Switzerland, Austria). Some Eastern European countries and Nordic countries (Norway, Sweden, and Denmark) prefer the PI. Many countries—including England, the United States, France, and Spain—have as yet not shown much interest in positive serostatistical proof of paternity." This statement is no longer valid in its entirety. For one thing, the situation in, say, England and the United States is much changed. Furthermore, practice no longer necessarily follows national borders, but rather to some extent on laboratories, some of which are web-based.

Essen-Möller also introduced a threshold for the interpretation of W, and considered paternity practically proved ("Vaterschaft praktisch erwiesen") if W exceeds 99.73%. He states statisticians conclude certainty about a difference if this difference is more than three times the mean error. He was unable to provide similar support for the quoted threshold, and he rather chose this threshold pragmatically because of technical and evaluation reasons ("Die Grenze wurde aus technisch-rechnerischen Gründen willkürlich gewählt und hat sich gut bewährt"). This value corresponds to the upper threshold of a 99.73%-confidence interval according

to a standard normal distribution. That implies, strangely, that W is assumed to be normally distributed.

It is remarkable that this threshold remains in use and has proved its value, as can be confirmed by "googling" *paternity 99.73*. For instance, stated at *www.vanhosp.bc.ca/paternity/faqs.html* is: "A probability of paternity of 99.73% is accepted as being 'practically proven' according to international standards."

Other suggested thresholds for W are 95.5% (German: sehr wahrscheinlich; English: very probable) and 68.2% (German: wahrscheinlich; English: probable). This was the first time someone suggested a stepladder of probabilities for paternity cases. Hummel has refined these verbal predicates, and a complete table is reproduced by Charles H. Brenner—along with comments and much else of interest—at *http://dna-view.com*.

A Case Study

Samples from the deceased and the two males (II and III) were typed initially using the STR markers in the SGM®Plus multiplex kit. Genotypes of the persons are shown in Table 1. Individuals I and III share no alleles for some markers, such as vWA, and this supports the unrelated hypothesis corresponding to hypothesis 1 of Figure 3. On the other hand, I and III have identical genotypes for, say, marker D16S539, which strengthens the brother hypothesis. If the individuals share rare alleles, the evidence generally will be strong. The allele frequencies are estimated from databases. Databases sampled from different populations will differ. However, for the autosomal markers shown in

Table 2—Results from Typing of the Y-STR Markers in the Powerplex-Y-System

(In DYS385 a/b, two loci are typed simultaneously, and DYS385 thus represents results from two markers (a and b).
The data indicate that I and II may well have the same father, whereas this is highly unlikely for I and III.)

Sample ID	DYS 391	DYS 389I	DYS 439	DYS 389II	DYS 438	DYS 437	DYS 19	DYS 392	DYS 393	DYS 390	DYS 385a/b
I (deceased)	10	12	12	28	10	14	16	12	15	23	12,14
II, putative brother of I	10	12	12	28	10	14	16	12	15	23	12,14
III, putative brother of I	10	12	13	28	10	14	15	13	12	24	13,15

Table 1, differences are expected to be relatively small. This is in contrast to the Y-chromosome markers of the next section, where frequencies in populations not sharing a recent common ancestry might vary considerably.

It is instructive to obtain analytical results, as shown in Boxes 1 and 2. However, removing the simplifying assumptions renders analytical calculations impractical, and, hereafter, we use the freeware program Familias (*www. nr.no/familias*). In particular, allowing for mutations complicates calculations. We use a mutation model published by Dawid, Mortera, and Pascali in 2001. Complete details on all aspects of the calculations are available at *http://folk. uio.no/thoree/chance*.

We assigned a flat prior to the five hypotheses of Figure 3. Alternative 4— where II and I are half brothers—came out as the most likely, with a posterior probability of 0.38, whereas the unrelated hypothesis was the least likely, with a posterior probability of 0.06. The evidence is far from conclusive. More markers are needed to discriminate between relations of the type considered. There are several possible sources of additional DNA data. It was not practical to obtain the required extra number of autosomal markers. Moreover, the lab analysis of mitochondrial DNA proved unsuccessful. Fortunately, there remains a valuable data source, the Y chromosome.

Results for Y-Chromosome Markers

The Y-analysis was successful, and data for the three individuals are presented in Table 2. If two boys have the same father, they will share the same Y-chro-mosome markers, provided there are no mutations. In our case, I and II share all 12 markers on the Y chromosome, while there are discrepancies for more than half of the markers comparing I and III. Thus, the latter boys cannot have the same father; the probability of the required number of mutations can be set to 0 for practical purposes.

The Y haplotype (i.e., collection of all markers on one chromosome) of the deceased was not previously observed in our population database of 1,760 Norwegian males. Using the Y-STR haplotype reference database (*www.yhrd. org/index.html*), we searched for a match in the Filipino population database (211 males) and the pooled Southeast Asian population database (3,900 males). No match was found in these populations or in a worldwide database search (33,000 males in 273 populations). Based on the above, it is intuitively unlikely that sharing should occur by chance. Let q_1 be the frequency of the Y-chromosome haplotype shared by I and II, while q_2 is the corresponding frequency for individual III. Based on the above, it is hard to estimate q_1 and q_2 beyond stating that these probabilities must be very small.

Formal calculations can be done again using Bayes Theorem, and the posteriors of the previous section would serve now as priors when the Y-chromosome data is introduced. The resulting posterior probability for II being the brother (half with same father or full brother) of the deceased will depend on q_1 and q_2. If, for instance, $q_1=q_2=0.01$, this probability is 0.999, and it will be even higher for smaller and more realistic values for q_1 and q_2. Again, the alternative non-Bayesian approach is to multiply the likelihood ratio obtained for the markers of the previous section by the one based on Y-markers.

In conclusion, the required identification of the deceased has been achieved. **C**

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