Chapter 37C

DNA Identification Science

by
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SYNOPSIS

Introduction

Generally

Deoxyribonucleic acid (DNA) is the elixir of life, the forensic gold standard and the holy grail of identification evidence. Packed into a human genome of three billion DNA letters, this information molecule contains the instructions for building a baby, growing into adulthood, and maintaining a person's trillion cells.[1] DNA information can also be used to identify people, apprehend suspects, convict the guilty and exonerate the innocent. The forensic myth of DNA infallibility pervades popular culture, reinforced daily by government, news media and entertainment like the Crime Scene Investigation (CSI) television shows. Through the "CSI effect", juries may expect to see DNA evidence routinely presented in criminal trials.[2]

These unrealistic DNA expectations are built on a scientific sleight of hand. Each person has a unique DNA sequence (except for identical twins). Therefore, as the forensic story goes, the DNA features and biological evidence can uniquely identify the person who left it. This virtual uniqueness can indeed be statistically true for pristine biological samples obtained under controlled conditions or fortunate circumstances.

Origins

[1] Watson JD, Baker TA, Bell SP, Gann A, Levine M, Losick R. *Molecular Biology of the Gene*. Sixth ed. San Francisco: Benjamin Cummings; 2008.
[2] Toobin J. The CSI effect. *The New Yorker;* 2007.

The earliest DNA identification successes employed pristine data. Using his pioneering (though now obsolete) restriction fragment length polymorphism (RFLP) laboratory testing, Alec Jeffreys compared the DNA of British immigrants to establish maternity and help a child re-enter the country.[3] Continued commercial and governmental RFLP testing was conducted on "single source" evidence containing just one person that had large amounts of DNA. Kary Mullis' 1983 discovery of polymerase chain reaction (PCR) enabled recovery from smaller DNA quantities.[4] RFLP and early PCR genetic markers were used as DNA evidence in the 1995 O.J. Simpson trial.

The advent of short tandem repeat (STR) PCR-based testing in the early 1990s revolutionized forensic science.[5] Minute amounts of DNA extracted from just dozens of human cells found at a crime scene became identifying evidence. Mixtures of two (or more) individuals could definitively implicate a perpetrator as having commingled their DNA with that of a victim. DNA databases were built that reached out across time and space to solve cold cases.[6] The remains of mass disaster victims could be associated with missing persons.

Uncertainty and Information

The price paid for STR identification power was acknowledging DNA uncertainty.

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[3] Jeffreys AJ, Brookfield JFY, Semeonoff R. Positive identification of an immigration test-case using human DNA fingerprints. *Nature.* 1985;317:818-819.
[4] Mullis KB, Faloona FA, Scharf SJ, Saiki RK, Horn GT, Erlich HA. Specific enzymatic amplification of DNA *in vitro*: the polymerase chain reaction. *Cold Spring Harbor Symp. Quant. Biol.* 1986;51:263-273.
[5] Edwards A, Civitello A, Hammond H, Caskey C. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *Am. J. Hum. Genet.* 1991;49:746-756.
[6] Gill P, Werrett D. Interpretation of DNA profiles using a computerised database. *Electrophoresis.* 1990;11:444-448.

Evidence data might no longer definitively single out just one individual, but instead suggest a plurality of people. Different DNA interpretation methods applied to the same data could lead to different conclusions.[7] Subjective human review could discard informative DNA evidence, mislabeling it "inconclusive". DNA evidence examination might be biased, particularly in cases where a human analyst used a suspect's DNA information when analyzing evidence data.[8]

Modern information science has resolved these difficulties.[9] A computer can thoroughly and objectively interpret DNA evidence, and then quantify the match information to a suspect. Low-level DNA, mixtures, kinship questions and investigative databases can all share a solid scientific foundation. Sound scientific reasoning can measure the identification information of biological evidence, translating uncertain data into a reliable DNA match statistic. This statistic can be understandably explained in a court of law. This chapter shows how.

Overview of Chapter

We begin with the biology of DNA and STR typing. We discuss how laboratories generate DNA data, along with well-known data artifacts and uncertainties. The genotype is introduced as the core concept of DNA identification. We show how a

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[7] Gill P, Brenner CH, Buckleton JS, et al. DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci Int.* 2006;160(2-3):90-101.
[8] Risinger DM, Saks MJ, Thompson WC, Rosenthal R. The Daubert/Kumho implications of observer effects in forensic science: hidden problems of expectation and suggestion. *California Law Review.* 2002;90(1):1-56.
[9] MacKay DJ. *Information Theory, Inference and Learning Algorithms*. Cambridge, UK: Cambridge University Press; 2003.

match statistic measures the identification information contained in DNA evidence. We present DNA mixtures, and methods for separating them into their component genotypes. Kinship is described, both for paternity and more general situations. We show how DNA databases can be used in criminal and disaster investigations.

At some point, DNA evidence may enter the courtroom. How reliable is DNA, and how is that reliability established in science and the law? How does DNA expert testimony support the trial attorney's case? How can DNA help exonerate an innocent individual? We explore all these topics, and then conclude by discussing the power and promise of modern forensic DNA evidence.

Biology

Generally

A cell's genetic material is contained in its nucleus. The DNA text is packaged into 23 chromosomes. Chromosomes come in pairs, with an individual inheriting one maternal copy from their mother, and one paternal copy from their father. Most of the DNA content resides in the 22 non-gender chromosomes. These "autosomal" chromosomes are numbered sequentially by size from 1 (largest) to 22 (smallest). The female X and small male Y sex chromosomes determine gender, with women usually having an XX pair and men an XY pair.

The genetic book of life is written in a four-letter DNA alphabet[10] – A (for the nucleotide base adenine), C (cytosine), G (guanine) and T (thymine). Long DNA sentences of A, C, G and T record human genes, typically 100 to 10,000 letters long. A gene usually codes for some biological function. Constrained by their function, such coding genes are conserved over evolutionary time, having only a few viable DNA sentence variants called "alleles". Forensic identification, however, relies on relatively unique features that can distinguish between different people; conserved genes do not serve this purpose. Therefore, forensic scientists use non-coding DNA locations (or "loci"), to develop highly polymorphic markers that have many different alleles.

STR Genetic Markers

The ideal forensic DNA markers would be genetic loci that have many allele variants, are abundant throughout the genome, and are easy to measure in the laboratory. The STR markers satisfy these conditions.[11]

1. An STR locus is polymorphic, having ten to twenty different alleles in the human population.
2. There are an estimated hundred thousand STR loci scattered throughout the human genome, so scientists have many identification loci to choose from.
3. STR alleles vary only in the number of letters in their DNA sentence. This length variation is easy to measure on an automated lab machine (i.e., "DNA sequencer") that records the length and amount of DNA sequences.

[10] Watson JD, Crick FH. Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid. *Nature.* 1953;171:737-738.
[11] Weber J, May P. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet.* 1989;44:388-396.

A forensic STR allele is a *short* DNA word (four or five letters long) that *tandemly repeats* a fixed number (e.g., 10 to 20) of times. More repeat units in an allele give greater DNA sentence length. For example, the D5S818 STR locus has the four letter repeat unit "AGAT" (see Figure 1). A locus allele containing ten of these repeat units would have a repeat section 40 DNA letters long (10 units, times 4 letters per unit), and is designated "10". An "11" D5 allele has a repeat section 44 letters long. These different allele lengths serve no known biological purpose, and do not affect health or disease, but they can be used as distinguishing markers for human identification.

At the genetic locus, an individual has a genotype (or, "genetic type") comprised of two alleles, each inherited from one parent. Ten population alleles would provide 55 possible allele pairs (10 + 9 + ... + 1), while twenty alleles can form 210 different allele pairs. So one STR locus typically yields around a hundred population genotype possibilities. A person has just one of these allele pair possibilities, and so chances are that two people will have different genotypes.

To increase a genotype's relative uniqueness, more STR loci are used.[12] Standard STR panels contain ten to twenty-five different loci.[13,14,15] These loci are

[12] Kimpton CP, Gill P, Walton A, Urquhart A, Millican ES, Adams M. Automated DNA profiling employing multiplex amplification of short tandem repeat loci. *PCR Meth. Appl.* 1993;3:13-22.
[13] Krenke B, Tereba A, Anderson S, et al. Validation of a 16-locus fluorescent multiplex system. *J Forensic Sci* 2002;47(4):773-785.
[14] Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFlSTR Identifiler PCR Amplification Kit. *J Forensic Sci.* Nov 2004;49(6):1265-1277.

chosen to be genetically independent. The total number of multi-locus genotype possibilities is the product of multiplying together the individual locus possibilities. With even just 10 different allele pair choices at a locus, 15 loci produce a quadrillion (10^{15}, or a "1" followed by 15 zeros) possible genotypes. Clearly the STR loci provide tremendous DNA identification power, with far more possible genotypes than there are (or ever were) people.

Lineage Markers

Some forensic applications focus only on male DNA. Y-STR panels have been developed that provide STR loci residing solely on the Y chromosome. Because of close proximity and lack of recombination on the small male chromosome, these Y-STR loci are not genetically independent. Thus the probabilities of their possible allele choices cannot be multiplied. Instead, a Y-STR haplotype lists one allele at each locus (since there is just one unpaired Y-chromosome). Therefore, Y-STR systems have identification power limited to known haplotypes, measured in thousands (instead of autosomal STR sextillions).

In addition to the nuclear DNA of chromosomes found in the nucleus, the cell also contains mitochondrial organelles. These mitochondria are the power plants of the cell, turning glucose sugar into high-energy molecules that drive metabolism. Believed to be remnants of ancient bacteria that merged with our cells in the evolutionary past,

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[15] Hennessy LK, Mehendale N, Chear K, Jovanovich S, Williams S, Park C, Gangano S. Developmental validation of the GlobalFiler® express kit, a 24-marker STR assay, on the RapidHIT® System. *Forensic Science International: Genetics*. 2014;13:247-58.

these organelles have their own DNA. They are passed from mother to child through the egg's cellular material (or, "cytoplasm") outside the nucleus. Determining mitochondrial DNA sequences can help trace a person's maternal lineage.

Data

Generally

The forensic laboratory transforms biological evidence into DNA data. The evidence contains genotypes from one or more individuals, and the task is to determine these genotypes. Through a series of successive separations, the lab refines the DNA molecules in the evidence into electronic signals. Following data generation, these DNA signals can be interpreted to infer genotypes.

In the extraction step, the DNA molecules are separated out from the biological material. This separation is done by breaking open the cell membranes to release DNA, and then concentrating the DNA by physical centrifugation or chemical affinity.[16] Rape kits undergo a differential extraction that can more specifically separate an assailant's sperm cells from a victim's epithelial cells.[17] Small quantities of DNA can be enhanced by further physical concentration or by removing impurities.[18] To a great extent, DNA can be extracted automatically by robotic machinery in the modern DNA laboratory,

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[16] Butler JM. *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers*. Second ed. New York: Academic Press; 2005.
[17] Gill P, Jeffreys AJ, Werrett DJ. Forensic application of DNA 'fingerprints'. *Nature.* Dec 12-18 1985;318(6046):577-579.
[18] Smith PJ, Ballantyne J. Simplified low-copy-number DNA analysis by post-PCR purification. *J Forensic Sci.* Jul 2007;52(4):820-829.

yielding more precise separations with less human involvement.[19]

Polymerase Chain Reaction

In 1983, Cetus scientist Kary Mullis was driving down California's Highway 1 when he had a vision that would forever change forensics and medicine.[20] In the everyday world, bacteria double every hour, producing millions of copies of their DNA in one day. What if this exponential chain reaction could be harnessed in a test tube, amplifying a few gene copies into virtually unlimited quantities for easy detection? The Cetus scientists worked with a heat-stable DNA copying enzyme (*Taq* polymerase). This *polymerase chain reaction* revolutionized molecular biology, netting Dr. Mullis a 1993 Nobel Prize. With PCR amplification, forensic scientists now have the power to easily and reliably develop STR genotype data from very small biological samples.

PCR amplification of an STR locus is done in a tube that contains the extracted template DNA (e.g., from evidence), the polymerase copying enzyme, other chemicals, and an abundance of fluorescently labeled DNA primers. These primers (about 20 DNA letters long) lie outside the STR repeat region, and, through the specificity of DNA double helix pairing, isolate the locus region within the genome. Twenty-eight (or so) rounds of copying then ensue, heating to separate DNA strands and cooling to initiate copying. Each cycle doubles the number of fluorescently labeled DNA molecules. For efficiency, 10 to 25 different STR loci, each assigned their own fluorescent dye and size

[19] Varlaro J, Duceman B. Dealing with increasing casework demands for DNA analysis. *Profiles in DNA.* 2002;5(2):3-6.
[20] Mullis KB. The unusual origin of the polymerase chain reaction. *Scientific American.* Apr 1990; 262(4):56-61, 64-55.

range, are amplified together in a single multiplex reaction tube.

However, PCR amplification is an imperfect copying mechanism that introduces a random element into the DNA data. At each of the 28 PCR cycles, with some probability, an allele may be copied or not.[21] Thus, repeated PCR experiments on the same DNA material produce different allele amounts. This copying variability results in different EPG allele patterns. The "variance" of PCR-amplified allele products is an important variable in accurate DNA evidence interpretation.[22]

The tube's amplified DNA is injected into an automated DNA sequencer to measure sequence length and amount.[23] Capillary electrophoresis separates the DNA molecules by their length, while a laser detects fluorescence intensity.[24] The resulting electropherogram (EPG) signal contains data peaks (see Figure 2). A peak has a DNA length that corresponds to an allele – more repeats in the allele make for a longer molecule that appears farther to the right on a length scale. A peak's height reflects the quantity of DNA – more DNA starting template amplifies into more fluorescently labeled PCR copies, which produce a stronger fluorescence signal for a taller peak.

DNA Data Complexity

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[21] Stolovitzky G, Cecchi G. Efficiency of DNA replication in the polymerase chain reaction. *Proc Natl Acad Sci USA*. 1996;93(23):12947–52.
[22] Perlin MW, Sinelnikov A, "An information gap in DNA evidence interpretation." *PLoS ONE*, 4(12):e8327, 2009.
[23] Smith LM, Sanders JZ, Kaiser RJ, et al. Fluorescence detection in automated DNA sequence analysis. *Nature.* Jun 12-18 1986;321(6071):674-679.
[24] Ziegle JS, Su Y, Corcoran KP, et al. Application of automated DNA sizing technology for genotyping microsatellite loci. *Genomics.* 1992;14:1026-1031.

Simple DNA yields simple STR EPG signals. A hundred cells from one individual are amplified into a signal having one or two tall peaks at locus. When the person's genotype is a homozygote (the same allele inherited from both parents) there will be one peak (see Figure 2a), and with a heterozygote (two different alleles) we see two peaks (see Figure 2b). A single source reference sample from one individual usually has such simple data that only one possible genotype can be inferred.

DNA evidence that is mixed (two or more contributors), low-level (far fewer than 100 cells) or degraded (larger DNA molecules broken) produces more complex EPG peak patterns. The mixture shown comes from two contributors in different amounts, producing two lower peaks from one person and two higher peaks from someone else (see Figure 3). Mixtures (and other complex DNA) can have multiple genotype explanations that account for the data.[25] Therefore, it no longer becomes possible (as with simple single source DNA) to just "look" at the data and state a genotype conclusion. Instead, some statistical inference method is needed to properly interpret or "unmix" the data.

The random PCR process generates a different chain reaction each time. Therefore, replicated STR experiments on the same DNA template will yield different EPG peak heights and patterns with each PCR test. Greater quantities of DNA generally form more reproducible peak patterns. Small DNA amounts exhibit randomness – dubbed "stochastic effects" – with more pronounced peak variation.

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[25] Ladd C, Lee HC, Yang N, Bieber FR. Interpretation of complex forensic DNA mixtures. *Croat Med J.* 2001;42(3):244-246.

These expected data variations can be accounted for with a PCR-variance parameter by statistically modeling the PCR process.[26]

Like most real-world amplifiers, PCR does not have perfect fidelity. The two peaks of a heterozygote individual at a locus will have different heights, with shorter alleles amplifying more efficiently (i.e., taller peaks) relative to larger alleles that have more repeat units. STRs exhibit "PCR stutter", where amplifying an allele may also produce a PCR product that is one repeat shorter, seen as a shadow peak next to the main peak (see Figure 4). Stutter artifact results from the polymerase enzyme occasionally skipping over a repeat unit during DNA replication.[27] With simple DNA, such DNA artifacts are easily noted and accounted for. Complex DNA evidence, however, often requires computer modeling for a more complete interpretation of the STR data.

Genotype

Generally

The genotype is the central concept in genetic identification. Biologically distinct individuals have statistically unique DNA barcodes, or "genotypes". Genotyping starts by taking a buccal swab or blood sample from a person, and generating STR data in the

[26] Perlin MW. Method and system for DNA mixture analysis, United States patent application 09/776,096. October 17, 2002.
[27] Hauge XY, Litt M. A study of the origin of 'shadow bands' seen when typing dinucleotide repeat polymorphisms by the PCR. *Hum. Molec. Genet.* 1993;2(4):411-415.

laboratory. Interpretation of the resulting pristine single source DNA is easy and produces a genotype having one definite allele pair at each locus.

An STR reference genotype is digital information, a list of two allele numbers (possibly the same) at fifteen or so loci that genetically identifies an individual. We can compare this genotype with an evidence genotype, use it to assess paternity, store it in a computerized database, or make matches that find missing people. A person's genotype is their genetic fingerprint, stamped into almost every cell of their body.

Biological evidence may contain DNA from one or more people. Each of these people contributes their genotype to the mix. The forensic identification task is to determine the separate contributor genotypes. Unlike reference samples, evidence is collected under less controlled circumstances, which can introduce uncertainty. Identification uncertainty is mathematically characterized using probability.

Probability

The science of probability is relatively new, originating from a series of letters exchanged between Pierre de Fermat and Blaise Pascal in 17th century France. Pierre-Simon Laplace codified basic probability in its modern form in his 1812 treatise.[28] When there is more than one possibility for an event, a non-negative probability number is assigned to each potential outcome, expressing our belief in that outcome's realization. Probability numbers always add up to one (i.e., 100%).

[28] Laplace PS. *Theorie analytique des probabilites*. Paris: Ve. Courcier; 1812.

The mathematical foundation of genetics is probability. Austrian friar Gregor Mendel was the first to write down the laws of genetic inheritance in the language of probability.[29] He predicted the genotypes of pea plant progeny as a probability distribution (i.e., the chance of each outcome occurring), confirming his theory by measuring the frequency of observed physical traits. Population genetics, gene discovery, genetic counseling, and evolutionary theory are all premised on probability and probabilistic genotypes. Sometimes the probability arises from chance events in nature (e.g., mating, gene transmission, survival of the fittest), and other times from uncertainty in the observed data.

Consider the two-person mixture data shown (see Figure 5a), with one allele dose each of 10 and 11, and a double dose of allele 12. One explanation of the data is that half of the DNA comes from a 10,11 genotype and the other half from a 12,12 genotype (see Figure 5b). Another good data explanation is that one person contributed a 10,12 genotype, while an equal amount of DNA was contributed by an 11,12 genotype individual (see Figure 5c). These (and other) allele pair combinations can explain the observed quantitative peak height data. Thus, there is inherent genotype uncertainty, since different genotype explanations can account for the DNA evidence. In the presence of explanatory uncertainty, an inferred evidence genotype becomes a probability distribution. Allele pairs that better explain the data, and are more common in the population, have higher probability.

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[29] Mendel G. Versuche über plflanzenhybriden (Experiments in plant hybridization). *Verhandlungen des naturforschenden Vereines in Brünn.* 1865;Bd. IV für das Jahr(Abhandlungen):3-47.

Probabilistic Genotypes

In general, an evidence genotype for a DNA contributor at a locus is a probability distribution over allele pairs. The probabilities reflect the constraints imposed by the evidence data, with greater probability mass placed on those allele pair possibilities that better explain the data. The genotype distribution can be visualized as a bar chart (see Figure 6), with each feasible allele pair value listed on one axis and the pair's probability number shown as a proportional bar length on another axis. Often, most of the probability resides in only a few allele pairs. Just these more probable values (out of a hundred possibilities) are shown in the figure. Since the bars represent probability, their lengths add up to one.

Probabilistic genotyping (PG) methods can account for data variation arising from the PCR amplification experiment. PG mathematically measures variation in EPG data, summarizes the variation as a variance statistic, and uses that variance value to calculate accurate genotype probability. PG software can also handle low-level DNA mixtures and other PCR artifacts, such as PCR stutter or heterozygote allele imbalance.

In addition to the definite *reference* and uncertain *evidence* genotypes, there is also a *population* genotype. A human population has a genotype that includes all the allele pair choices that nature can assign to an individual.[30] A typical STR locus has

[30] Hartl DL, Clark AG. *Principles of Population Genetics*. Fourth ed. Sunderland, MA: Sinauer Associates; 2006.

about a hundred possible allele pairs, each with a probability reflecting its prevalence in the population (see Figure 7). A higher population genotype probability means that an allele pair is more prevalent, so there is a greater chance of a person having that genotype value. In the absence of any informative STR data, an evidence genotype would simply be the diffuse population genotype.

Forensic DNA science sees a close interaction between population, evidence, and reference genotypes. A *population* genotype is estimated by measuring a few hundred individual genotypes.[31] DNA evidence data reshapes an initial population genotype into an *evidence* genotype, shifting probability away from less likely allele pairs and concentrating probability mass onto genotype values that better explain the data. To statistically assess whether an individual contributed their DNA to evidence, their *reference* genotype is compared with an evidence genotype, relative to a population genotype, as discussed next.

Match

Generally

When is a person's DNA present in biological evidence? The DNA match statistic answers this question numerically, quantifying the degree of match. Large numbers (e.g., a million) scientifically support a match, while small numbers (e.g., a millionth, or

[31] Chakraborty R. Sample size requirements for addressing the population genetic issues of forensic use of DNA typing. *Hum Biol.* 1992;64(2):141-159.

one over a million) can suggest otherwise. A neutral value (e.g., around one) means that, whether or not the person is present, there is no statistical support in the data either way.

Match Statistics

All valid DNA match statistics, or "likelihood ratios" (LR), are an assessment of the evidence data under two competing hypotheses.[32] The identification (or "prosecutor") hypothesis H is that a person contributed their DNA to the evidence, while the alternative (or "defense") hypothesis $\overline{H}$ is that they did not, i.e., someone else left their DNA. In a criminal trial, the identification hypothesis can be related to a hypothesis regarding a defendant's guilt.

Before hearing DNA evidence, a trier of fact has a belief about guilt or innocence, and, by extension, whether or not a person contributed their DNA. This prior belief can be expressed by the *prior odds*, a ratio

$$O(H) = \frac{\Pr(H)}{\Pr(\overline{H})}$$

that compares the probability $\Pr(H)$ of the identification hypothesis with its alternative $\Pr(\overline{H})$. After having heard the DNA evidence, the judge or juror forms an updated identification belief, expressed as the conditional probability $\Pr(H \mid data)$ of the identification hypothesis as moderated by having seen the data. Dividing this posterior probability $\Pr(H \mid data)$ by the alternative $\Pr(\overline{H} \mid data)$ gives the *posterior odds*, the ratio

[32] Good IJ. *Probability and the Weighing of Evidence*. London: Griffin; 1950.

$$O(H \mid data) = \frac{\Pr(H \mid data)}{\Pr(\overline{H} \mid data)}$$

The DNA match statistic quantifies by how much the DNA evidence changes our belief in the identification hypothesis as the posterior odds divided by the prior odds

$$LR = \frac{O(H \mid data)}{O(H)}$$

This ratio shows that the DNA match statistic satisfies two important factors for the relevance of expert testimony[33]. First, the LR is *probative* since the posterior odds numerator $O(H \mid data)$ addresses how the evidence data affects some hypothesis H about a particular person. Second, the LR is *not prejudicial* since the prior beliefs and prejudices $O(H)$ are factored out through division in the denominator. The match statistic quantifies the nonprejudicial probative force of DNA evidence.

Statisticians define "likelihood" as the conditional probability of observing fixed evidence data under varying hypotheses.[34] The likelihood ratio derives its name from being the ratio of two likelihoods,

$$LR = \frac{\Pr(data \mid H)}{\Pr(data \mid \overline{H})}$$

under the competing hypotheses of identification (H) and nonidentification ($\overline{H}$). The ratio tells us which hypothesis (H or $\overline{H}$) better explains the data. This likelihood ratio form is derived from the odds ratio form above using Bayes theorem,[35] and gives the same numerical answer. However, likelihood is a specialized concept that many people

[33] Fed. R. Evid. 403.
[34] Lindgren BW. *Statistical Theory*. Fourth ed. New York, NY: Chapman & Hall; 1993.
[35] Bayes T, Price R. An essay towards solving a problem in the doctrine of chances. *Phil. Trans.* 1763;53:370-418.

(who are not statisticians) may not readily understand, and so this likelihood ratio expression can potentially confuse a jury.

Explaining the Match Statistic

There are many equivalent ways of mathematically, verbally or visually expressing the LR.[36] For non-specialist understanding and courtroom presentation, the most intuitive formulation is based on genotypes. The LR focuses on a reference (e.g., suspect's) genotype, since the other allele pairs are not probative for that comparison.

For one locus, at the suspect's allele pair, consider the evidence and population genotypes. The *population* genotype probability at the suspect's allele pair describes the coincidental occurrence of this genotype, the chance that a person picked at random from a crowd would have the suspect's genetic type. The *evidence* genotype probability at the suspect's allele pair, determined *after* having seen the DNA data, is the chance of an evidentiary match between the evidence and suspect genotypes.

The genotype form of the DNA match statistic is the probability ratio of the evidence genotype to the population genotype, evaluated at the suspect's allele pair (see Figure 8). For the suspect, the statistic gives the DNA identification information change based on the evidence, relative to coincidence. All of these forms calculate the same numerical LR value.

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[36] Perlin MW. Explaining the likelihood ratio in DNA mixture interpretation. *Promega's Twenty First International Symposium on Human Identification*. San Antonio, TX, 2010.

In the mathematically equivalent match LR form, the ratio

$$LR = \frac{\Pr\{evidence\ match\}}{\Pr\{coincidental\ match\}}$$

tells us the probability of an evidence match to the suspect (numerator), accounting for the chance of coincidental suspect match (denominator). A report can state that "a match between the evidence and the suspect is (some number of times) more probable than coincidence."

Population Considerations

Matches are customarily reported relative to particular ethnic populations.[37] These population genotypes are determined by allele frequencies in a subpopulation database developed from individuals belonging to the same ethnic group. In the United States, DNA match statistics are typically reported for appropriate regional ethnicities such as African-American, Asian, Caucasian or Hispanic. Each ethnic match statistic can be more specifically stated as "a match between the evidence and the suspect is (some number of times) more probable a coincidental match to an unrelated [Caucasian] person." Since the ethnic sample size is relatively small (typically a few hundred individuals), there is about a factor of ten variation in the reported match statistic value.[38]

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[37] Budowle B, Moretti T, Baumstark A, Defenbaugh D, Keys K. Population data on the thirteen CODIS core short tandem repeat loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians. *J Forensic Sci.* 1999;44(6):1277–1286.

[38] Chakraborty R. Sample size requirements for addressing the population genetic issues of forensic use of DNA typing. *Hum Biol.* 1992;64(2):141-159.

The DNA match number can be adjusted for co-ancestry.[39] Since all people are ultimately related through a common ancestor, a genotype match may appear rarer when ancestors are not considered.[40] The match statistic can be adjusted to account for the fact that a rare allele, once observed, has become less rare. The co-ancestry coefficient (or "theta") that measures population relatedness is typically small (theta less than 1%), and so the conservatively adjusted match statistic is usually within a factor of ten of the unadjusted value.

Adding Up DNA Match Information

Information is a standard scientific concept that works on an additive scale.[41] LR factors multiply, but their information logarithms add. The base ten logarithm of a number is its "power of ten" exponent, i.e., the number of zeros after the one. For example, the log of a million is 6, since there are 6 zeros in 1,000,000, and a million is 10 multiplied by itself 6 times, or 10^6. Instead of multiplying together the locus LRs, we can add up their (positive or negative) log(LR) information values to determine the weight of evidence.[42]

Mixtures

[39] Balding DJ, Nichols RA. DNA profile match probability calculation: how to allow for population stratification, relatedness, database selection and single bands. *Forensic Sci Int.* 1994;64(2-3):125-140.
[40] Weir BS. The coancestry coefficient in forensic science. In: Promega, ed. *Eighth International Symposium on Human Identification*. Scottsdale, Arizona,1997:87-91.
[41] Shannon CE. A Mathematical Theory of Communication. *The Bell System Technical Journal.* 1948;27:379–423.
[42] Good IJ. *Probability and the Weighing of Evidence*. London: Griffin; 1950.

Generally

Mixtures arise when more than one person contributes their DNA to biological evidence. Mixtures are found in rape (victim plus assailant), homicide, touch (handgun, clothing, surfaces) and other DNA evidence. In many crime labs, mixed samples form the majority of processed DNA items.

Separating DNA Mixtures

Instead of inferring a single genotype from mixture data, the forensic task is to "unmix" the data by separating out the contributing genotypes. Mixture interpretation is usually more involved than single source analysis – there are multiple ways contributor allele pairs could combine to account for the data. These multiple genotype choices introduce uncertainty, and so probability enters the equation. Although a mixture evidence genotype typically has probability less than 100% at a matching allele pair, the DNA match statistic is designed to handle this less certain situation.

When DNA from two or more individuals is mixed together in evidence, there is some definite (though unknown to us) number of copies left by each person. The "mixture weight" is the relative proportion of the contributor amounts. This proportion helps shape the data, since the genotype weighting forms a particular allele pattern. We illustrate how three different weighted combinations (25%+75%, 50%+50%, and 75%+25%) of two allele pairs 10,12 and 11,12 add up to different allele amounts (see Figure 9). A PCR experiment produces a data peak pattern that is a random variant of

the underlying allele quantities.

DNA Mixture Interpretation

Accurate DNA mixture interpretation starts with an assumed number of contributors. Over many thousands of iterations, a computer considers weighted combinations of contributor allele pairs at every locus. These hypothetical mixed locus patterns are compared with the STR locus data. Proposed patterns that better explain the observed data confer a higher probability to the hypothesized genotypes and mixture weights (see Figure 10). The result is a mixture weight (probability distribution) for each contributor (see Figure 11), and a genotype (probability distribution) at every contributor's locus (see Figure 6). For accurate probability, PCR variance (variability probability distribution) is also determined. These genotypes, mixture weights, and variance parameters are objectively inferred (without using a suspect reference) by a thorough examination of all the data, considering essentially all feasible pattern explanations.

The DNA match statistic compares an inferred evidence contributor genotype with a reference, relative to population. With single source DNA producing a definite genotype, the evidence match numerator is 1. With a mixture, the genotype probability at the reference allele pair is generally less than 1, and so the numerator is lower, thereby reducing the weight of evidence. The LR mathematics works perfectly well for mixtures, balancing the evidence strength of match (numerator) with chance of coincidence (denominator). No other allele pair is relevant in the DNA match statistic

determination – only that of the suspect, or some other reference genotype.

Mixture calculations that are done manually preserve less DNA match information.[43] While statistical computing can accurately determine how much probability to place on each evidence genotype solution, people can only approximate an answer. Human review of DNA evidence applies "thresholds" that crudely truncate STR peaks into all-or-none "allele" events that may be incorrect or discard the quantitative data (see Figure 12). The result usually is a diffusion of probability mass away from the true evidence genotype, dispersing it onto allele pairs that have little or no data support (see Figure 13). This diffusion tends to lower evidence genotype probability at a contributing suspect's allele pair, which artificially *deflates* the DNA match statistic.[44] Examination bias, [45] or other circumstances,[46] can conversely *inflate* the statistic.

Using Mixtures as Evidence

DNA mixtures can strengthen a prosecutor's case. In a sexual assault, the presence of both victim and defendant DNA in the same mixture item provides strong evidence for physical contact. Similarly, a mixture of deceased and assailant DNA at a homicide crime scene can implicate the accused. The presence of additional (possibly unidentified) DNA contributors in a mixture does not change the fact that the suspect is

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[43] Gill P, Brenner CH, Buckleton JS, et al. DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci Int.* 2006;160(2-3):90-101.
[44] Perlin MW, Legler MM, Spencer CE, et al. Validating TrueAllele® DNA mixture interpretation. *J Forensic Sci.* 2011;56(6):1430-1447.
[45] Thompson WC. Painting the target around the matching profile: the Texas sharpshooter fallacy in forensic DNA interpretation. *Law, Probability and Risk.* 2009;8(3):257-276.
[46] Curran JM, Buckleton J. Inclusion probabilities and dropout. *J Forensic Sci.* 2010;55(5):1171–1173.

in the evidence.

Mixtures can also help the defense. Multiple contributors in blood stains may suggest signs of a struggle, making first-degree homicidal intent less likely. DNA mixtures on airbags can indicate from which car door – driver or passenger – the occupants exited. In some cases, the presence of defendant DNA on too many evidence items, mixed in with DNA from too many unidentified people, may reduce the probative value of crime scene DNA.

Defense attorneys can raise legitimate questions about mixture evidence.[47,48] The presence of individuals other than the defendant can raise doubt about who committed a crime, affecting sentencing, if not conviction. Has the mixture interpretation method applied to the data been scientifically tested for reliability? If human mixture review was performed, was a thorough interpretation done that considered all possibilities? Was the interpretation done objectively, without any knowledge of the suspect's genotype that might bias the answer? Was all the STR data used? Did thresholds eliminate potentially exculpatory data that could have suggested someone else (e.g., an unreported minor contributor) was present in the DNA evidence?

DNA mixtures are a common form of forensic evidence. Proper data interpretation can produce compelling DNA match results that complete and corroborate

[47] Thompson WC, Ford S, Doom T, Raymer M, Krane DE. Evaluating forensic DNA evidence: Essential elements of a competent defense review. Part 1. *The Champion.* 2003;27(3):16-25.
[48] Thompson WC, Ford S, Doom T, Raymer M, Krane DE. Evaluating forensic DNA evidence: Essential elements of a competent defense review. Part 2. *The Champion.* 2003;27(4):24-28.

a case narrative. Mixture misinterpretation offers many avenues for effectively attacking DNA evidence. Quite often, highly probative DNA evidence is incorrectly called "inconclusive", due to people's inability to interpret informative mixture data without computer assistance. Greater prosecution and defense awareness of the promise and pitfalls of DNA mixture evidence can have a major impact on criminal justice.

Kinship

Generally

People share physical traits (phenotypes) with their relatives because they have DNA in common. This DNA is transmitted genetically from parent to child, with half of a person's genotype coming from each parent. Molecular biology lets a scientist directly examine a person's DNA through STR experiments. The hitherto unobservable genotype inside the cell can now become a new phenotype, just one more measurable physical characteristic. The probability laws of genetic inheritance let us reconstruct a person's genotype from their relatives. Such kinship genotype inference and comparison is useful in forensic identification.

Probabilistic prediction of offspring genotypes from known parents dates back 150 years to Mendel.[49] Consider an example where, at a given locus, a mother has a 10,10 genotype and a father an 11,12 (see Figure 14). Then the child must inherit a 10

[49] Mendel G. Versuche über plflanzenhybriden (Experiments in plant hybridization). *Verhandlungen des naturforschenden Vereines in Brünn.* 1865;Bd. IV für das Jahr(Abhandlungen):3-47.

allele from its mother (that is all she has), and either an 11 or 12 paternal allele (with equal probability from one of the two father's chromosomes). Therefore, the genotype of the child is the probability distribution shown, with 10,11 at 50% and 10,12 at 50%.

Paternity

Paternity testing is the most widely performed DNA identification assay. Given a mother and a child, how likely is it that an alleged father is the biological parent of the child? The fundamentals of forensic inference and match were described almost a century ago for ABO blood groups,[50] and extended naturally to DNA and STR loci.[51] Consider a situation where at some STR locus a mother has a 10,10 genotype and her child has a 10,12 genotype (see Figure 15). Since the child inherited the 10 allele from its mother, the 12 allele must have come from its biological father. The father's genotype must be a 12 allele, combined with any other possible allele at that locus.

The inferred genotype of the biological father is a list of allele pairs containing a 12 allele. The father inherited the other (unknown) allele x from a population where allele x has probability p_x of occurring. Therefore, the father's genotype probabilities are proportional to the population alleles, as shown in Figure 16; these numbers add up to one, as required. This genotype determination is made objectively, without considering any alleged father. The inference uses all available data – the mother, child and population genotypes.

[50] Essen-Möller E. Die Biesweiskraft der Ähnlichkeit im Vater Schaftsnachweis; Theoretische Grundlagen. *Mitteilungen der anthropologischen Gesellschaft in Wien.* 1938;68(9-53).
[51] Evett IW, Weir BS. *Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists*. Sunderland, MA: Sinauer Assoc; 1998.

To compute a parentage match statistic (or "paternity index" likelihood ratio), the biological father's inferred evidence genotype must be compared with the alleged father's reference genotype, relative to a population. Suppose the alleged father's genotype at the locus is 11,12. Then the statistic's numerator is the inferred father's genotype probability p_{11}, while the denominator has the coincidental population probability $2p_{11}p_{12}$. The ratio of these two genotype probabilities is $p_{11}/2p_{11}p_{12}$. The common p_{11} factor cancels, giving a DNA match statistic of $1/2p_{12}$. The rarer a child's non-maternal allele, the higher the paternity index. Multiplying together the individual locus statistics gives the full DNA match statistic for paternity.

Reconstructing Genotypes from Relatives

The basic principles of objectively inferring genotypes (up to probability) from all available evidence data, and then making a comparison to determine a DNA match statistic, applies to all kinship situations. Any number of relatives can be used, with more data generally producing a more informative genotype probability distribution. For example, with the pedigree shown (see Figure 17), having spouse, parents and children available as DNA references can allow essentially complete reconstruction of a person's genotype.

Uncertain kinship-inferred genotypes can be compared with uncertain DNA evidence genotypes (e.g., mixtures). In these more general situations, where there is no

definite reference genotype, a more general match formula is used that sums over the different DNA match possibilities. The match statistic principles, of course, remain the same. Kinship comparisons are also made in DNA database searches, as discussed next.

Database

Generally

When DNA evidence is collected from a crime scene, there may be no suspects available for comparison. However, the inferred evidence genotypes can be stored in a computerized database. Similarly, reference genotypes of likely suspects (say, convicted criminals) can also be stored in a separate DNA database table. The computer can then compare all the crime scene evidence against the many potential perpetrators through their respective genotypes, and find DNA matches. The computed DNA match score estimates the weight of evidence that can be used in court. A DNA database thus provides a way to collect evidence from different cases and locations, persisting indefinitely to solve cold cases through genetic identification.

Early DNA Databases

DNA databases were used early on to solve criminal cases. In 1987, the British police collected blood from over 4,000 villagers in Leicestershire to compare with DNA

evidence collected from two young rape homicide victims.[52] Through these RFLP comparisons, Colin Pitchfork was identified and ultimately convicted of the crimes.

The United Kingdom extended this approach to build a national DNA database (NDNAD) of forensic evidence and criminal offender genotypes.[53] By aggressively genotyping property crimes and obtaining DNA from all arrestees, the UK home office achieved a NDNAD hit rate of over 50%,[54] putting an early end to many criminal careers.[55] Other countries soon developed their own DNA databases, such as the FBI's National DNA Index System (NDIS) in the United States.[56]

These early investigational databases were designed for single-source DNA samples. Some, such as NDIS, try to accommodate DNA mixtures by using allele lists[57] to approximate genotype probability distributions. However, these lists lose much identification information, and can thus generate many false leads. This artificially high false hit rate restricts genotype uploads to just the simplest mixtures.

Newer Technology

The latest DNA database technology uses genotypes (probabilities, not allele

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[52] Wambaugh J. *The Blooding*. New York: Perigord Press; 1989.
[53] Gill P, Werrett D. Interpretation of DNA profiles using a computerised database. *Electrophoresis.* 1990;11:444-448.
[54] Howitt T. Maximising the value of DNA evidence through a service approach. Paper presented at: *15th International Symposium on Human Identification*, 2004; Phoenix, AZ.
[55] Blumstein A, Cohen J, Das S, Moitra SD. Specialization and seriousness during adult criminal careers. *J. of Quantitative Criminology.* 1988;4(4):303-345.
[56] Niezgoda SJ, Brown B. The FBI Laboratory's COmbined DNA Index System Program. *Sixth International Symposium on Human Identification*. Scottsdale, AZ, 1995.
[57] Scientific Working Group on DNA Analysis Methods (SWGDAM). Short Tandem Repeat (STR) interpretation guidelines. *Forensic Sci Commun (FBI).* July 2000;2(3).

lists) to better preserve all the match information present in biological evidence.[58] Unlike older government systems, informative investigative DNA databases have high sensitivity, detecting the criminals who contributed their DNA to crime scene evidence (see Figure 18a). These improved databases are also highly specific, and rarely make false hits (see Figure 18b). Since much (if not most) current DNA evidence items are mixtures, these more informative approaches to preserving and matching genotypes can help solve additional crimes.

DNA databases are primarily used for solving cold cases. Typically, evidence genotypes (from many cases) are compared with reference types (from many suspects) to associate criminals with cases. However, other investigative comparisons can be made. For example, a computer can compare evidence to evidence (rather than to suspects) to find links between serial crimes. The mechanism is the same – the computer compares evidence genotypes, calculates DNA match statistics, and reports on positive matches.

People can be identified through the DNA of their relatives, as we saw with paternity. This kinship genetic identification can be extended from a single case to an investigative DNA database.[59] In disaster victim identification (DVI), victim remains are collected, analyzed by a DNA lab, and stored as inferred genotypes on a DNA database. Separately, DNA is collected from relatives of missing people, so that computers can

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[58] Perlin MW. Investigative DNA databases that preserve identification information. *Forensic Science International: Genetics Supplement Series.* December 2011;3(1):e484–e485.
[59] Perlin MW. Mass casualty identification through DNA analysis: overview, problems and pitfalls. In: Okoye MI, Wecht CH, eds. *Forensic Investigation and Management of Mass Disasters*. Tucson, AZ: Lawyers & Judges Publishing Co; 2007:23-30.

reconstruct genotypes of the missing, and record them on the database. (Personal effects, such as clothing and toothbrushes, of missing people are also analyzed.) A DVI database then compares the genotypes of victim remains with those of missing people in order to find matches, and associate biological remains with actual people.[60] A TrueAllele® probabilistic genotyping matching database was used this way to help identify victim remains in the World Trade Center disaster.[61]

Familial search is a way to connect crime scene evidence to suspects *through their relatives* by using a DNA database.[62] This is best done by comparing an evidence genotype with a database of genotypes inferred from convicted offender relatives (e.g., parent, child or sibling). These person-to-relative kinship matches are less informative (i.e., have lower statistics) than person-to-person genotype matches. Therefore, additional genetic testing (such as Y-STR paternal lineage) is often done to confirm a familial database hit.[63] Dozens of familial searches have been successfully conducted to identify criminal suspects.

Ethical Issues

DNA databases can raise interesting ethical questions. Is the state justified in

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[60] Perlin MW. Identifying human remains using TrueAllele® technology. In: Okoye MI, Wecht CH, eds. *Forensic Investigation and Management of Mass Disasters*. Tucson, AZ: Lawyers & Judges Publishing Co; 2007:31-38.
[61] Dickerson TM, Gajewski C, Ishii A, Desire M, Prinz MK. Renewed efforts to identify the victims of the World Trade Center disaster via DNA testing (A81). *American Academy of Forensic Sciences 64th Annual Meeting*, Atlanta, GA: AAFS; 2012.
[62] Bieber FR, Brenner CH, Lazer D. Finding criminals through DNA of their relatives. *Science.* June 2 2006;312(5778):1315-1316.
[63] Myers SP, Timken MD, Piucci ML, et al. Searching for first-degree familial relationships in California's offender DNA database: validation of a likelihood ratio-based approach. *Forensic Sci Int Genet.* Nov 2011;5(5):493-500.

finding criminals through the DNA of their relatives? Since certain groups may be overrepresented on DNA databases, is there a potential racial bias when evidence comparisons are made? Should DNA databases be made more racially "fair" by obtaining reference DNA from *all* Americans, and uploading everyone's genotype onto the FBI's DNA database?

The answer to this last question is clearly "no", at least at the present time. The older database architectures still in use represent mixture evidence through "allele lists". Thus national DNA database searches with complex mixtures have an unacceptably high false hit error rate. Putting all Americans onto NDIS might soon make everyone a suspect in some crime. The question may arise again once information-preserving DNA databases that use probabilistic genotypes for greater specificity are used.

Governments maintain proprietary DNA databases (e.g., CODIS) hidden from public scrutiny or independent scientific searching. Since most old cases used ineffective mixture analysis methods, which labs can't re-examine, these closed databases seal off the DNA past from informative computer analysis. Therefore, defendants and the wrongfully convicted – as well as police and prosecutors – cannot use DNA databases to prove innocence or find criminals. In 2019, TrueAllele reanalysis of a 2010 homicide led to an unprecedented CODIS search that exonerated innocent Lydell Grant, freed him from prison, and found the true killer.[64] With PG computer automation, open data access could help rectify thousands of past injustices.

[64] Ortiz E. Texas man close to exoneration after computer algorithm leads to new suspect. *NBC News*. Feb 16, 2020.

Reliability

Generally

In American courts, the Federal Rules of Evidence, state rules, or common law rules govern the admissibility of scientific (and other) expert testimony. Federal Rule 702 states that a qualified expert with specialized knowledge that can help the trier of fact may testify when their testimony is based on (a) sufficient data, (b) reliable methods, and (c) the methods have been reliably applied to the data.[65] This rule codifies three major opinions of the Supreme Court, and opinions of many lower federal courts, demanding "evidentiary reliability" for scientific and other expert evidence.

Frye Standard

When a party proffers scientific evidence, the opposing party may request a pretrial admissibility hearing. The trial court normally has discretion as to whether to hold such a hearing. These hearings are often called *Frye* or *Daubert* hearings, depending on the evidence code or common law in the jurisdiction.

The older *Frye* test arose in a civil case involving the admissibility of a systolic blood pressure lie detector.[66] The Court of Appeals for the District of Columbia Circuit ruled that in order to be admissible, a novel scientific principle should be "sufficiently

[65] Fed. R. Evid. 702.
[66] *Frye v. United States,* 293 F. 1013 (Court of Appeals of District of Columbia 1923).

established to have gained general acceptance in the particular field in which it belongs." Lie detection via blood pressure changes failed to meet this test. This criterion does not speak to the underlying science, but instead looks to the cultural question of community acceptance. For example, a new method (such as Einstein's theory of relativity) might be perfectly valid, but too new and untested to be considered admissible. Subsequent advances in the philosophy of science, particularly Karl Popper's "falsifiability"[67] and Thomas Kuhn's sociological insights,[68] eventually led to the development of a new reliability standard.

Daubert Standard

In the decades after the original decision in *Frye*, most federal courts and many state courts adopted this general-acceptance test. The perception that it was unduly restrictive, and the adoption of the Federal Rules of Evidence in 1974 (rules that made no mention of *Frye*), led to a strong minority view that it would be more appropriate for the courts to inquire directly into the validity and reliability of scientific methods. In response to the division of authority, the Supreme Court, in *Daubert v. Merrell Dow Pharmaceuticals*,[69] addressed the issue in connection with the admissibility of plaintiff's evidence in a civil case about birth defects allegedly caused by the drug Bendectin.

The Supreme Court held that the federal rules jettisoned the requirement of general acceptance. In its place, the Court called on judges to determine whether there

[67] Popper K. *The Logic of Scientific Discovery*. Vienna, Austria: Verlag von Julius Springer; 1935.
[68] Kuhn TS. *The structure of scientific revolutions*. Chicago: University of Chicago Press; 1962.
[69] *Daubert v. Merrill Dow Pharmaceuticals, Inc.,* 509 U.S. 579 (Supreme Court of the United States 1993).

were "good grounds based on what is known" to rely on the scientific technique or theory. The majority *Daubert* opinion suggested that courts could consider, among other things, "(1) whether the theory or technique can be (and has been) tested, (2) whether it has been subjected to peer review and publication, (3) its known or potential error rate, and the existence and maintenance of standards controlling its operation, and (4) whether it has attracted widespread acceptance in a relevant scientific community".

Challenges to DNA Evidence

Early admissibility challenges to DNA identification concerned laboratory procedures for RFLP testing of single-source DNA. The general methodology was generally ruled admissible in the early *Frye* cases, although defendants sometimes prevailed in excluding evidence when its application to DNA in a particular case was not.[70] Challenge to the methods for computing the probability of random match followed, focusing on population statistics and the interpretation of DNA data.[71]

After many courtroom battles[72] and two National Research Council reports,[73] DNA testing emerged as the forensic gold standard. O.J. Simpson's defense dream team decided not to challenge DNA admissibility in his 1995 trial.[74] The modern STR

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[70] *People of the State of New York v. Joseph Castro,* 545 N.Y.S.2d 985 (Bronx County Supreme Court 1989).
[71] *United States of America v. Stephen Wayne Yee, et al.,* 134 F.R.D.161 (U.S. District Court for Northern District of Ohio 1991).
[72] *United States of America v. John Ray Bonds, Mark Verdi and Stephen Wayne Yee,* 12 F.2d 540 (U.S. Court of Appeals, 6th Circuit 1993).
[73] *National Research Council (NRC)*. Evaluation of Forensic DNA Evidence: Update on Evaluating DNA Evidence. Washington, DC: National Academies Press, 1996.
[74] *People of the State of California v. Orenthal James Simpson,* BA097211 (Los Angeles County Superior Court 1995).

systems were introduced with the benefit of "DNA wars" legal hindsight, and were rapidly accepted in both Frye and Daubert[75] jurisdictions.

DNA Mixture Interpretation

Early challenges to the admissibility of manual DNA mixture failed. That is, the courts generally allowed testimony about matches to evidence mixtures, stating that differences of scientific opinions could be used to attack the weight of the testimony.[76] But how reliable are these interpretation methods as applied to the data from mixed, degraded and low-level DNA? Conventional human review of STR mixture data can be biased,[77] inaccurate,[78] nonreproducible[79] or a random locus count uncorrelated with DNA identification information.[80] The prevalent Combined Probability of Inclusion (or, "CPI") mixture interpretation method has not been thoroughly tested by its proponents,[81] and does not have a known error rate or peer-reviewed validation studies.[82] However, newer objective computer-intensive interpretation methods have been more fully validated.

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[75] *People of the State of Colorado v. Michael Eugene Shreck,* 22 P.3d 68 (Supreme Court, State of Colorado 2001).
[76] *Orlando Roberts v. United States,* 03-CF-853 (District of Columbia Court of Appeals 2007).
[77] Dror IE, Hampikian G. Subjectivity and bias in forensic DNA mixture interpretation. *Science & Justice.* 2011;51(4):204-208.
[78] Gill P, Brenner CH, Buckleton JS, et al. DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci Int.* 2006;160(2-3):90-101.
[79] Butler JM, Kline MC. NIST Mixture Interpretation Interlaboratory Study 2005 (MIX05), Poster #56. *Promega's Sixteenth International Symposium on Human Identification*. Grapevine, TX2005.
[80] Perlin MW. Inclusion probability for DNA mixtures is a subjective one-sided match statistic unrelated to identification information. *J Pathol Inform*. 2015;6(1):59.
[81] President's Council of Advisors on Science and Technology (PCAST). Forensic Science in criminal courts: ensuring scientific validity of feature-comparison methods. Washington, DC: *Executive Office of the President*; 2016.
[82] Perlin MW, When DNA is not a gold standard: failing to interpret mixture evidence. *The Champion,* 2018;42(4):50-56.

A DNA mixture interpretation method should be **tested**, both on casework items[83,84,85] and on laboratory synthesized samples of known composition,[86,87,88,89,90] with the results published in peer-reviewed scientific journals. The method's error rate can be measured using DNA match information (i.e., the logarithm of the match statistic). For example, the sensitivity, specificity and reproducibility of the TrueAllele® computer system's interpretation method has been determined, as shown in Figure 19 for N = 20 two and three person DNA mixtures of typical complexity.[91] *Sensitivity* (averaging 1.12 trillion, or $10^{12.05}$) characterizes how informative a method is when detecting true DNA matches, while *specificity* (averaging one over 288 quintillion, or $10^{-20.46}$) describes how well it rejects false DNA matches. *Reproducibility* (within-group standard deviation of 1.77, or $10^{0.249}$) measures information deviations with repeated application of a method to the same data (see Figure 20).

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[83] Perlin MW, Legler MM, Spencer CE, et al. Validating TrueAllele® DNA mixture interpretation. *J Forensic Sci.* 2011;56(6):1430-1447.
[84] Perlin MW, Belrose JL, Duceman BW. New York State TrueAllele® Casework validation study. *J Forensic Sci*. 2013;58(6):1458-66.
[85] Perlin MW, Dormer K, Hornyak J, Schiermeier-Wood L, Greenspoon S. TrueAllele® Casework on Virginia DNA mixture evidence: computer and manual interpretation in 72 reported criminal cases. *PLoS ONE*. 2014 March 25;9(3):e92837.
[86] Perlin MW, Sinelnikov A. An information gap in DNA evidence interpretation. *PLoS ONE.* 2009;4(12):e8327.
[87] Ballantyne J, Hanson EK, Perlin MW. DNA mixture genotyping by probabilistic computer interpretation of binomially-sampled laser captured cell populations: Combining quantitative data for greater identification information. *Sci Justice*. 2013;53(2):103-14.
[88] Perlin MW, Hornyak J, Sugimoto G, Miller K. TrueAllele® genotype identification on DNA mixtures containing up to five unknown contributors. *J Forensic Sci*. 2015;60(4):857-68.
[89] Greenspoon SA, Schiermeier-Wood L, Jenkins BA. Establishing the limits of TrueAllele® Casework: a validation study. *J Forensic Sci*. 2015;60(5):1263-76.
[90] Bauer DW, Butt N, Hornyak J, Perlin MW. Validating TrueAllele® interpretation of DNA mixtures containing up to ten unknown contributors. *J Forensic Sci.* 2020;65(2):380-398.
[91] Perlin MW, Belrose JL, Duceman BW. New York State TrueAllele® Casework validation study. *J Forensic Sci*. 2013;58(6):1458-66.

Modern statistical computing can determine an **error rate** derived from the DNA evidence within a case.[92] A defendant enters a trial cloaked in innocence. To convict, a juror must be persuaded of guilt by evidence beyond a reasonable doubt. An innocent person who did not contribute their DNA to a mixture is a "non-contributor." An evidence genotype's "non-contributor distribution" describes the frequency of (primarily exclusionary) match statistics (see Figure 21).

The chance that an innocent non-contributor's genotype has a match statistic at least as large as the defendant's LR is the *probability of misleading evidence* (PME).[93] The PME provides an error rate for the DNA evidence against the defendant. The PME is a tail probability – the area under the non-contributor distribution curve to the right of the defendant's LR value. The PME error value is always less than 1/LR, but can be much smaller. In a Southampton (England) rape case, the LR was 67,890, while the PME error rate was one in 1,087,000, which is less than one in 67,890.

The Daubert prong for **standards** and controls can be addressed by the issuance of DNA interpretation guidelines,[94] compliance with software validation guidelines[95] and standards,[96,97] and standards for the exchange of genotype results[98]

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[92] Perlin MW. Efficient construction of match strength distributions for uncertain multi-locus genotypes. *Heliyon*, 2018;4(10):e00824.
[93] Royall R. On the probability of observing misleading evidence. *J Am Stat Assoc.* 2000;95(451):760-8.
[94] Scientific Working Group on DNA Analysis Methods (SWGDAM). Interpretation guidelines for autosomal STR typing by forensic DNA testing laboratories; 2010.
[95] Scientific Working Group on DNA Analysis Methods (SWGDAM). Guidelines for the validation of probabilistic genotyping systems. FBI Laboratory; 2015.
[96] Academy Standards Board/American National Standards Institute. Standard for validation studies of DNA mixtures, and development and verification of a laboratory's mixture interpretation protocol (Standard 020). Colorado Springs, CO; 2018.

and regulatory approval by independent scientific bodies.[99] **General acceptance** can be supported by a scientific literature bibliography that shows how an interpretation method relies on established principles and procedures, and by a citation index that shows how other scientists rely on the method. Legal scholars and others write papers about forensic methods[100] and their admissibility,[101] describing preferred attributes such as scientific objectivity and absence of bias. In their admissibility decisions, judges can rely on legal precedents set by appellate[102,103,104] courts and refer to well-written rulings.[105,106,107,108,109]

Testimony

Generally

At trial, each side has a narrative, an explanation of the evidence that supports its conclusion about a defendant. The prosecution seeks to establish beyond reasonable doubt that the defendant is guilty, while the defense strives to undermine that

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[97] Academy Standards Board/American National Standards Institute. Standard for validation of probabilistic genotyping systems (Standard 018). Colorado Springs, CO; 2020.
[98] Carey S. Data format for the interchange of fingerprint, facial & other biometric information. In: Wing B, ed. Gaithersburg, MD: American National Standards Institute (ANSI) and National Institute for Standards and Technology (NIST); 2011.
[99] *Approval for the use of TrueAllele® technology for forensic casework,* (New York State Commission on Forensic Science, 2011).
[100] Bentley D, Lownds P. Low Template DNA. *Archbold Review.* 2011;1(1):6-9.
[101] Duffy SK. Challenging the admissibility of DNA evidence. *New York State Bar Association (NYSBA) New York Criminal Law Newsletter.* 2012;10(2):7-11.
[102] *Commonwealth of Pennsylvania v. Kevin James Foley,* 38 A.3d 882 (Superior Court of PA, 2012).
[103] *People of New York v. Casey Wilson*, 175 A.D.3d 158 (Supreme Court of NY, 3d Dep't 2019).
[104] *State of Nebraska v. Charles Simmer*, 302 Neb. 369, (Supreme Court of NE, 2019).
[105] *The Queen v. Colin Duffy and Brian Shivers,* NICC 37 (Crown Court in Northern Ireland, 2011).
[106] *Commonwealth of Virginia v. Matthew Brady*, CR11000494 (Colonial Heights County, 2013).
[107] *State of Ohio v. Maurice Shaw*, CR-575691 (Cuyahoga County, 2014).
[108] *People of New York v. John Wakefield*, A-812-29 (Schenectady County, 2015).
[109] *State of Washington v. Emanuel Fair*, 10-1-09274-5 SEA (King County, 2017).

conclusion. DNA plays a supporting role in this confrontation, helping to buttress the closing argument of one or both sides. Typically, an item's DNA match is not the sole basis for conviction or exoneration, but rather plays a supporting role to corroborate other witnesses or evidence.

Role of DNA Evidence

DNA evidence can place a person at the scene of the crime. We may not know how or when the DNA got there, but the DNA helps show that biological material was present. In a sexual assault, an intimate (oral, vaginal or anal) mixture contains both victim and assailant DNA; a match between the assailant's genotype and a suspect requires him to explain why his DNA is there. Mixture DNA under the deceased fingernails can similarly associate a defendant with a homicide. In a child molestation case, defendant mixture DNA found on a girl's underwear can corroborate her accusation, strengthening a prosecutor's argument.[110]

Sometimes it is the victim's DNA that is found around the perpetrator. In a child abduction and assault case, a five-year old girl's blood was found on clothing in a bag at the defendant's home, while a mixture stain containing his and the girl's DNA on the same garment established his involvement.[111] Criminals often leave their DNA at a crime scene, such as a bank robber who drops his hat or cane, or a terrorist whose

[110] *Commonwealth of Virginia v. Michael Armin Gardner,* Cr11000771, 772, 773 (Arlington County, 2012).
[111] *Commonwealth of Virginia v. Jonathan Nathaniel Ramsey* (Fairfax County, 2012).

DNA is found on a matchstick used to burn a getaway car.[112] In all these cases, to paraphrase the Cat in the Hat, the defendant's DNA "should not be there if the person was not".[113]

DNA mixtures can be highly probative, providing physical evidence that multiple handled an item. A homeless man claimed he hadn't been near a murdered young woman, but finding their DNA mixed together on a water bottle where she died proved otherwise.[114] Finding the DNA of young woman who had been abducted from Florida and forced into prostitution, along with the DNA of two New Orleans pimps, inside the barrel of a pistol corroborated her story of having been raped with the gun.[115] Separating a four person mixture from a handgun used in a Pennsylvania shooting led to dropped charges against one defendant who was statistically absent, and a guilty plea from another defendant who was statistically present.[116]

Defending Against DNA Evidence

The best line of defense against DNA evidence is often to acknowledge that a defendant was at the crime scene, but provide an innocent explanation. Perhaps the crime occurred in the defendant's home or some other place he was expected to be, so that is why his DNA was found. An alleged sexual assault may have been consensual.

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[112] Perlin MW, Galloway J. Computer DNA evidence interpretation in the Real IRA Massereene terrorist attack. *Evidence Technology Magazine.* 2012;10(3):20-23.
[113] Seuss D. *The Cat in the Hat*. New York: Random House; 1957.
[114] *State of Louisiana v. Christopher Hutsell* (Orleans Parish, 2015).
[115] *State of Louisiana v. Willard Anthony* (Jefferson Parish, 2016).
[116] *Commonwealth of Pennsylvania v. Evan McBride* (Allegheny County, 2015).

By Locard's Exchange Principle,[117] items that come into contact can transfer DNA, such as sperm migrating between articles of clothing in the laundry.[118]

A DNA item may be non-probative, confirming that the defendant was present at the scene, but not speaking to his guilt or innocence in the crime. Other probative DNA evidence may suggest that someone else was there, perhaps the true perpetrator of the crime. Complex DNA evidence that a crime lab declares to be "inconclusive" may actually be informative or exculpatory, warranting a more accurate reinterpretation of the same data by an independent scientist or computer.[119]

DNA in the Courtroom

An attorney prepares for trial by first reviewing the case report and curriculum vitae of the DNA expert witness. At a pretrial meeting, the DNA scientist may provide a set of questions for qualifying as an expert and conducting the direct examination. The direct exam often includes a PowerPoint or other presentation about the DNA science and match results in the case. These presentation materials should be thoroughly reviewed at the pretrial meeting so that the attorney is comfortable with the evidence and the expert testimony. It is helpful to discuss the case particulars with the scientist, explaining how the DNA evidence fits in with the overall narrative and closing argument, as well as likely avenues of cross-examination by counsel from either side.

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[117] Morrish R. *The Police and Crime-Detection Today*. London: Oxford University Press; 1940.
[118] Kafarowski E, Lyon AM, Sloan MM. The retention and transfer of spermatozoa in clothing by machine washing. *Can Soc Forens Sci.* 1996;29(1):7-11.
[119] *People of California v. Manuel Lopez* (Santa Clara, 2020).

At trial, the order of witnesses for DNA evidence usually follows the chronological sequence of events. Police or other crime scene investigators testify about collecting, preserving and transporting the biological evidence to the crime laboratory. A lab analyst describes how DNA is extracted, amplified and detected to develop STR signal data. As learned from the O.J. Simpson case, establishing a clear chain of custody for DNA evidence is crucial. DNA match results are presented numerically for each relevant genotype comparison between an item of evidence and an individual, relative to one or more reference populations. Cross-examination of the scientists can further elucidate the DNA methodology and match conclusions.

The closing argument retells the attorney's narrative in the light of presented evidence. The DNA evidence supports key elements of the trial attorney's story, helping to establish which people were present at what locations and what they did there. The DNA can corroborate eyewitness and victim testimony, resolving "he said, she said" disagreements. The scientist's DNA evidence can be suggestive, but the prosecution or defense attorney's narrative must be compelling.

Who Should Testify?

In the modern forensic factory, where each DNA laboratory step is conducted by a different person on a batch of samples containing many cases, should the entire team stop work to testify in court or should just one scientist report on the group's findings? In

Crawford v. Washington,[120] the US Supreme Court applied the Sixth Amendment's Confrontation Clause. They decided that in a criminal case a "testimonial" statement from a person who does not testify at trial is inadmissible unless the person is unavailable to testify, and the defendant had a prior opportunity to cross-examine the individual.

In *Melendez-Diaz v. Massachusetts*,[121] the Court applied this holding to a "bare bones" sworn report from a state toxicology laboratory declaring that a substance was cocaine. Because no one from laboratory appeared at trial to present the report, its admission deprived the defendant of his right to confront his accusers. In *Bullcoming v. New Mexico*,[122] the Court held that "surrogate testimony" about a defendant's blood alcohol level was inadmissible. The forensic analyst who conducted the gas chromatography, wrote a report and signed a certificate of analysis did not testify. However, he was not shown to be unavailable to testify. Moreover, the witness who testified in his place worked at the same laboratory and was familiar with its procedures, but had not participated in the testing or supervised the original analyst.

On the other hand, in *Williams v. Illinois*,[123] the Court upheld testimony from a witness in a state DNA laboratory. This testimony essentially stated that a defendant was the source of semen in a vaginal swab, even though a private laboratory did the testing of the swab and a reference sample from the victim, and the witness neither

[120] *Crawford v. Washington,* 541 U.S. 36 (Supreme Court of the United States 2004).
[121] *Melendez-Diaz v. Massachusetts,* 07-591 (Supreme Court of the United States 2009).
[122] *Bullcoming v. New Mexico*, 131 S.Ct. 2705 (Supreme Court of the United States 2011)
[123] *Williams v. Illinois,* 10–8505 (Supreme Court of the United States 2012).

participated in nor observed the actual testing. But a majority of the Court could not agree on any theory that would explain this outcome. The Court is likely to revisit the related issues of the admissibility of laboratory reports, and of testimony from witnesses based on such reports in lieu of testimony from the scientists or technicians who produced the report. Justice Breyer, in particular, commented on the need for the Court to resolve the question of which findings must be presented through the testimony of which witnesses in a multi-step, multi-person procedure such as modern DNA analysis.

Exoneration

Generally

There is a long-held legal tradition that "better that ten guilty persons escape than that one innocent suffer".[124] The first DNA exoneration was in the 1986 Colin Pitchfork case, where early on in the investigation 17 year old Richard Buckland confessed to the murders. However, an RFLP comparison of his DNA with that of the rape evidence cleared him of the crimes; he was let go and the DNA manhunt continued.[125] Since 1989, DNA has excluded tens of thousands of prime suspects, preventing wrongful convictions.[126] To date, there have been over three hundred post-conviction DNA exonerations in the United States.[127]

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[124] Blackstone W. *Commentaries on the Laws of England*. Oxford: Clarendon Press; 1765.
[125] Wambaugh J. *The Blooding*. New York: Perigord Press; 1989.
[126] Connors E, Lundregan T, Miller N, McEwen T. Convicted by juries, exonerated by science: case studies in the use of DNA evidence to establish innocence after trial. Washington, DC: *National Institute of Justice*; 2006.
[127] Scheck B, Neufeld P. 250 exonerated: too many wrongfully convicted. *Innocence Project*. New York: Benjamin N. Cardozo School of Law, Yeshiva University; 2010.

DNA and Wrongful Convictions

At its most informative, "a DNA profile is evidence that tends to exculpate all but one of the more than 7 billion people in the world today".[128] A DNA match statistic can show that coincidence is far more probable than a suspect matching the evidence. At the very least, nonmatching DNA evidence (say, in a rape or homicide) can establish that someone other than the accused was present or involved in the crime. Often, through DNA comparison with other suspects or a database search, the true perpetrator can be found. Such DNA identification occurs in about half of US post-conviction DNA exonerations.

Why are innocent men wrongfully convicted? In most post-conviction DNA exonerations, an eyewitness misidentified the defendant.[129] While eyewitness testimony is highly persuasive to juries in court, it turns out to be incorrect much of the time.[130] False confessions occur in about 10% of post-conviction DNA exoneration cases, clearly more commonly than many believe. A 2009 National Academy of Science (NAS) report questioned the scientific validity of many non-DNA forensic techniques.[131] Indeed, unvalidated or improper forensic science appears in about half of DNA exonerations.

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[128] *Williams v. Illinois,* 10–8505 (Supreme Court of the United States 2012).
[129] Scheck B, Neufeld P, Dwyer J. *Actual Innocence: Five Days to Execution, and Other Dispatches From the Wrongly Convicted*. New York: Doubleday; 2000.
[130] Wixted JT, Mickes L, Fisher RP. Rethinking the reliability of eyewitness memory. *Perspectives on Psychological Science*. 2018;13(3):324–35.
[131] National Research Council. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: National Academies Press; 2009.

Making Better Use of Exculpatory DNA

Much exculpatory DNA evidence is never used to help the innocent avoid false conviction. DNA evidence has become increasingly more complex, involving mixtures and other uncertainties. While human review of a two-person mixture can readily provide a major contributor match statistic, beyond these simple samples crime labs often misinterpret (potentially exculpatory) DNA as "inconclusive".

At a crime scene, there can be probative DNA evidence intimately related to the offense, as well as nonprobative items at innocent locations where someone would have naturally left their DNA. A laboratory may report matches for simple nonprobative DNA that is easier to interpret, but stay silent about more complex probative items they mistakenly call inconclusive. Such selective DNA interpretation bias can unintentionally steer juries toward a wrongful conviction. More informative computer reinterpretation of the same DNA evidence can overcome these human limitations, and reach accurate match conclusions that help exonerate the innocent.

In 1989, a gang of five men was committing "bump and rape" highway attacks on Indiana women. Misidentified through stolen work uniforms, Darryl Pinkins and Roosevelt Glenn were wrongfully convicted on rape and sent to prison.[132] In 2001, conventional interpretation of DNA mixture clothing evidence showed two unknown people who could be assailants. However, the post-conviction court reasoned that two-

[132] Glenn R. *Innocent Nightmare*: CreateSpace Independent Publishing, 2015.

unknown assailants, plus the three defendants, accounted for the gang of five, and so did not grant relief.

Reexamining the same DNA data in 2014, TrueAllele® computing separated out 5% and 10% contributors from the mixtures, producing five unknown people.[133] TrueAllele showed that three of the men were genetically related, probably brothers. In April 2016, after 24 years in prison, Pinkins was exonerated and released.[134] Later that year, a judge signed an order vacating Glenn's conviction. To date, TrueAllele re-analysis of old DNA crime scene evidence has helped exonerate ten innocent men.[135]

Conclusion

Generally

Early forensic DNA had a notion of "individualization", that biological evidence could be uniquely associated with only one person. This is untrue, of course[136] – even if every (genetically distinct) individual did have their own unique genotype, actual DNA evidence is often mixed or degraded, and so supports multiple genotype possibilities. Instead, science accounts for this uncertainty by identifying people within the mathematical confines of probability.

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[133] Perlin MW, Hidden DNA evidence: exonerating the innocent. *Forensic Magazine*, 15(1):10-12, 2018.
[134] *Darryl Pinkins v. State of Indiana* (Lake County, 2016).
[135] Cybergenetics, TrueAllele® Exonerations, https://www.cybgen.com/news/exoneration/page.shtml
[136] Saks MJ, Koehler JJ. The individualization fallacy in forensic science evidence. *Vanderbilt Law Review.* 2008;61(1):199-219.

Three Revolutions

Our generation has witnessed three great revolutions that have led to modern forensic DNA science. One was the PCR revolution in molecular biology. This scientific advance permitted the separation of small stretches of DNA, isolating sentences of a few hundred letters from within a three billion letter genomic background. Entire biomedical industries in research and diagnostics have developed around PCR, including the automated machinery of the forensic laboratory. The PCR process transforms biological material into electronic data, whose signals signify the genotypes of contributing individuals.

In law, the Daubert revolution[137] altered the admissibility landscape for scientific and technical[138] evidence. Previously, the Frye "general acceptance" standard limited evidence to older science. Under Daubert, new scientific advances that had been sufficiently established through rigorous testing could now be separated from junk science and used in evidence. DNA was a Daubert poster child, a novel form of forensic identification whose reliability was proven through laboratory experiment and courtroom precedent. Unlike its sister forensic disciplines, DNA was tested by Daubert courts, and soon emerged as the new gold standard for scientific evidence.[139]

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[137] Bernstein DE. The unfinished Daubert revolution. *Engage.* 2009;10(1):35-38.
[138] *Kumho Tire Co., Ltd. v. Carmichael,* 526 U.S. 137 (Supreme Court of the United States, 1999).
[139] Lynch M. God's signature: DNA profiling, the new gold standard in forensic science. *Endeavour.* Jun 2003;27(2):93-97.

The information revolution is a signature of the modern age.[140] Intelligent computers can analyze reams of data to separate out critical information from background noise. Eighteenth century Bayesian reasoning,[141] the mathematical way to update belief based on new data, was revitalized by the digital computer,[142] finding application in many areas of human inquiry.[143] Bayesian probability had been used to quantify paternity[144] and glass evidence[145] information, and proved to be perfectly suited to forensic DNA identification.[146]

Reliable DNA Identification Information

Throughout the twentieth century, before the emergence of ubiquitous computing, frequentist statistics dominated data analysis.[147] However, the frequentist reliance on many repeated experiments is not always suited to forensic analysis. For example, we can't statistically examine a homicide by repeating the event a hundred times. But, through Bayesian statistics, we can quantify how the observed evidence updates our beliefs about alternative scenarios. With DNA, Bayesian computing lets us thoroughly

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[140] Moore GE. Cramming more components onto integrated circuits. *Electronics.* April 19 1965;38(8):114-117.
[141] Jevons WS. *The Principles of Science: A Treatise on Logic and Scientific Method*. London: Macmillon & Co; 1874.
[142] McGrayne SB. *The Theory That Would Not Die: How Bayes' Rule Cracked the Enigma Code, Hunted Down Russian Submarines, and Emerged Triumphant from Two Centuries of Controversy*. New Haven: Yale University Press; 2011.
[143] Lindley DV. *Understanding Uncertainty*. Hoboken, NJ: John Wiley & Sons; 2006.
[144] Essen-Möller E. Die Biesweiskraft der Ähnlichkeit im Vater Schaftsnachweis; Theoretische Grundlagen. *Mitteilungen der anthropologischen Gesellschaft in Wien.* 1938;68(9-53).
[145] Lindley DV. A problem in forensic science. *Biometrika.* 1977;64(2):207-213.
[146] Foreman LA, Smith AFM, Evett IW. Bayesian analysis of deoxyribonucleic acid profiling data in forensic identification applications (with discussion). *Journal of the Royal Statistical Society, Series A.* 1997(160):429-469.
[147] Fisher RA. On the mathematical foundations of theoretical statistics. *Phil. Trans. R. Soc. A.* 1922;222:309–368.

infer genotypes (and their probabilities) objectively from the evidence.[148,149] Bayes Theorem then lets us compare these evidence genotypes to particular individuals, determining the change in identification information that produces a DNA match statistic.

These three revolutions – PCR in science, Daubert in law, and Bayesian computing of information – inaugurated modern forensic DNA science. Starting from biological evidence, scientists and their machines can produce reliable identification information.[150] This DNA match information is routinely used to identify suspects, convict criminals, exonerate the innocent, establish parentage, find missing people, and link human remains to disaster victims. An understanding of the power and limitations of DNA evidence can help attorneys better represent their clients in civil and criminal proceedings.

[148] Curran J. A MCMC method for resolving two person mixtures. *Sci Justice.* 2008;48(4):168-177.
[149] Perlin MW, Sinelnikov A. An information gap in DNA evidence interpretation. *PLoS ONE.* 2009;4(12):e8327.
[150] Perlin MW. Forensic science in the information age. *Forensic Magazine.* 2012;9(2):17-21.

Figures

Figure 1. An STR genotype is a pair of alleles, such as the 10,11 allele pair shown for locus D5S818. The maternal '10' allele repeats the 4 letters "AGAT" 10 times for a total length of 40 DNA letters, while the paternal '11' allele has a length of 44 letters.

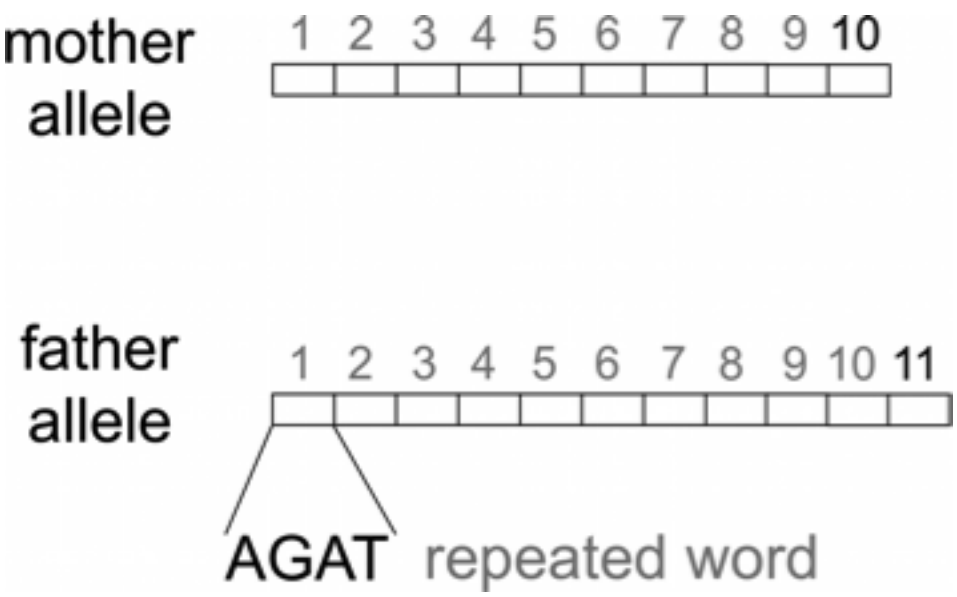

Figure 2. Single-source DNA produces STR data having one or two allele peaks. The x-axis indicates the size separation of alleles as the number of repeats, while the y-axis is measured in relative fluorescent units (RFU). The **(A)** homozygote 10,10 shows just a '10' allele peak, while a **(B)** heterozygote 10,11 allele pair has two peaks.

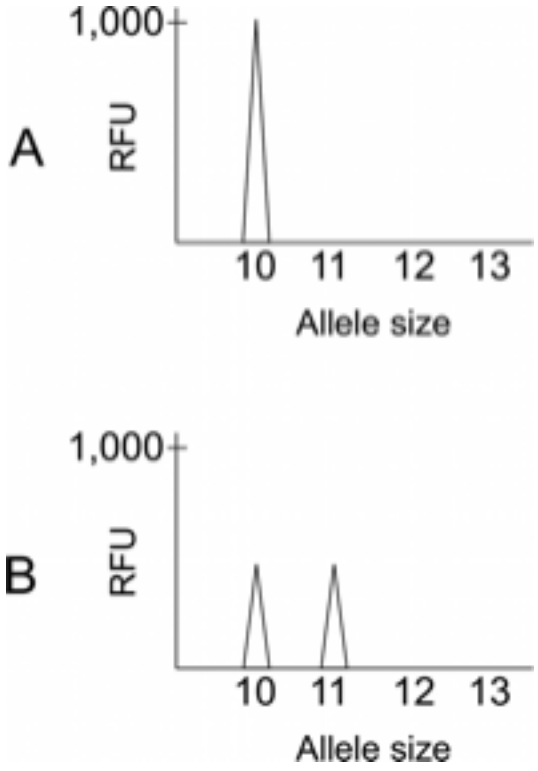

Figure 3. A DNA mixture combines two or more genotypes, and can produce data having more than two alleles. Here, an individual with genotype 10,11 has contributed half as much DNA as a second individual having genotype 12,13.

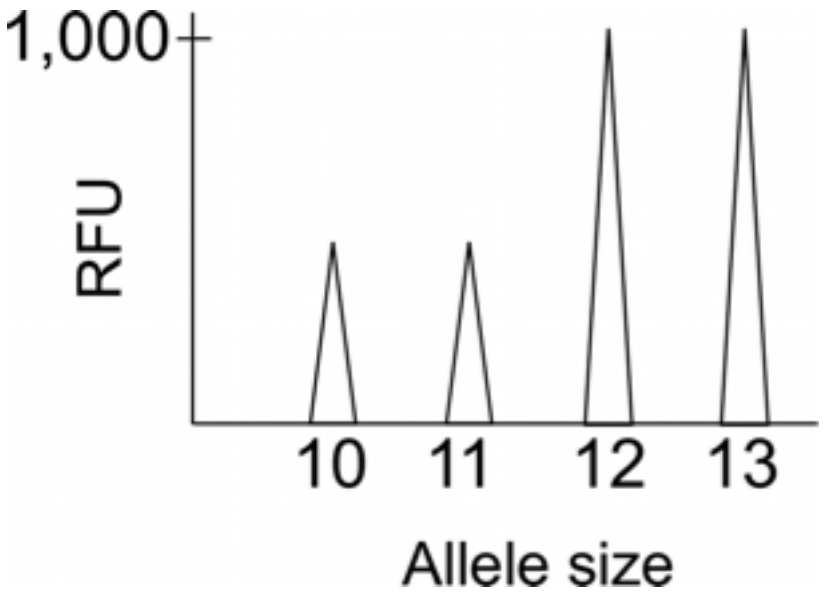

Figure 4. Allele 11 (tall peak) has a nonallelic PCR stutter peak at position 10 (very short peak).

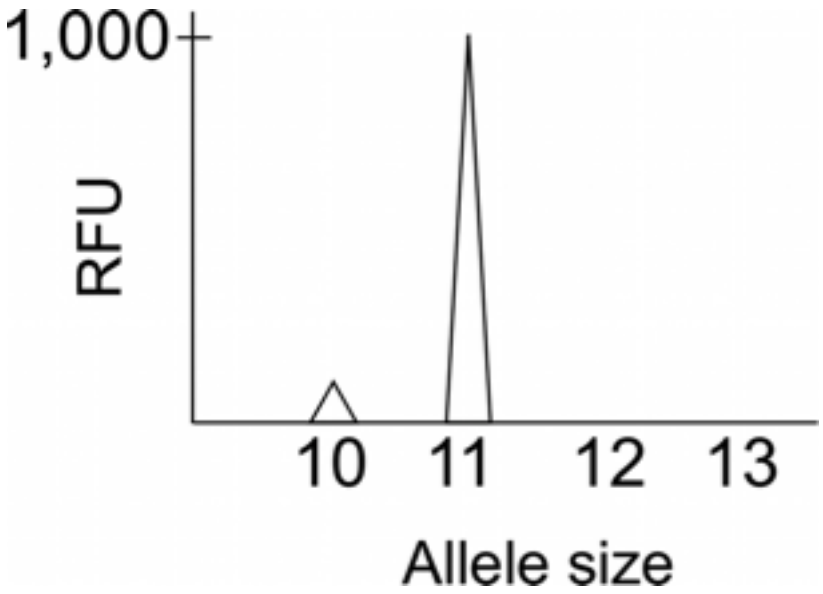

Figure 5. **(A)** STR mixture data (triangles) can support different genotype explanations. **(B)** One explanation of this 50:50 mixture combines genotypes 10,11 and 12,12. **(C)** In an alternative explanation, allele pairs 10,12 and 11,12 are combined.

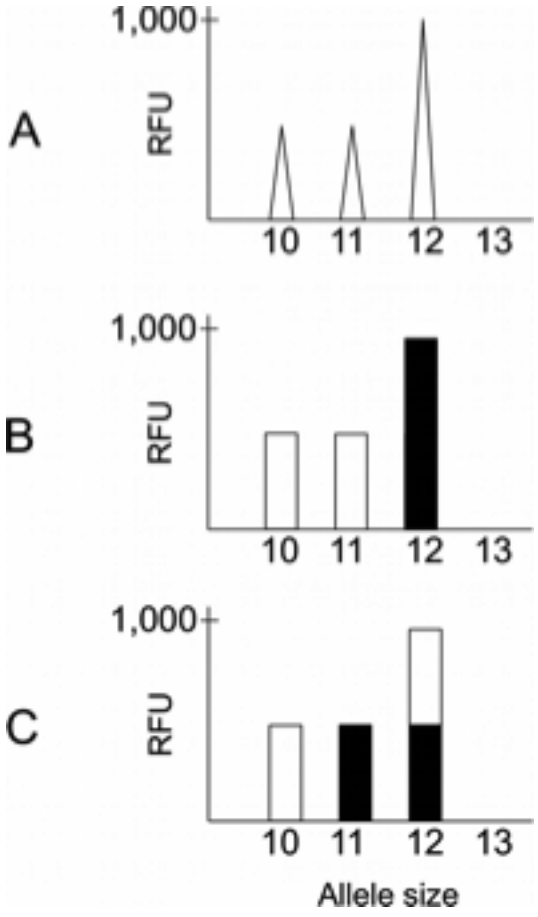

Figure 6. An evidence genotype is a probability distribution that can be represented as a bar chart. The x-axis lists the possible allele pairs, while the y-axis shows the probability of each allele pair.

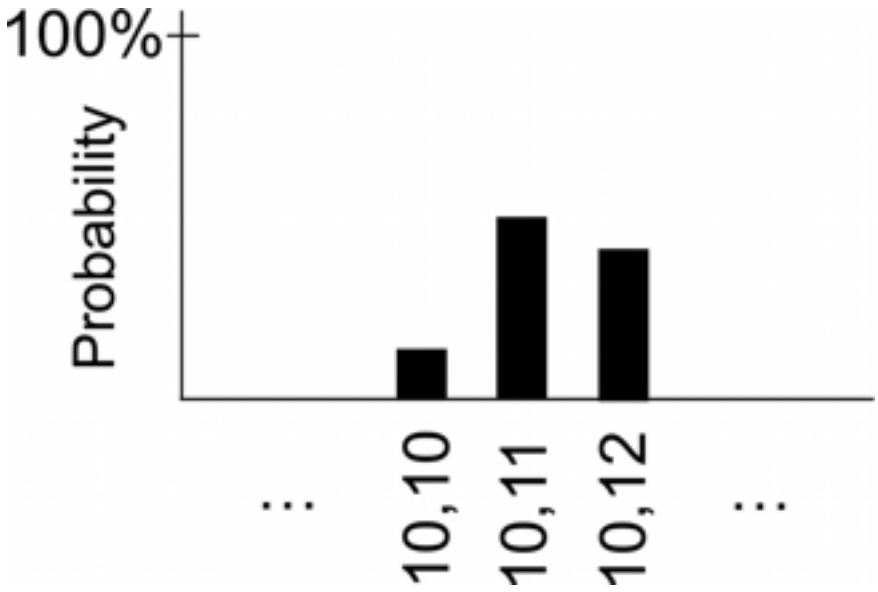

Figure 7. A population genotype is a probability distribution over allele pairs. The bar chart shows some of the hundreds of possible allele pairs, with each assigned a probability mass (bar height) proportional to its prevalence in the population.

Figure 8. A DNA match statistic is evaluated at the suspect's genotype 10,12, forming a ratio of the probability of the evidence genotype divided by that of the population genotype. This ratio of 40% to 4% describes a 10-fold increase in DNA match information at the locus.

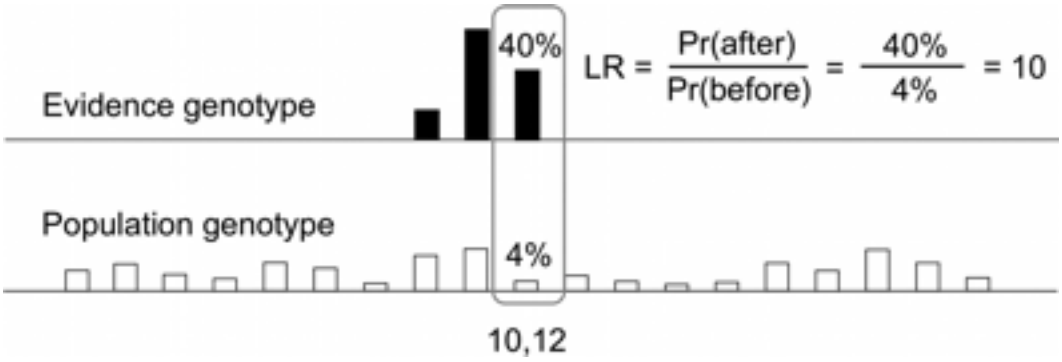

Figure 9. Different mixture combinations of same genotypes produce different allele peak data patterns. Combining genotypes 10,12 and 11,12 produces **(A)** an ascending pattern in a 25:75 mixture proportion, **(B)** two equal peaks adjacent to a higher peak when in a 50:50 combination, and **(C)** a "high-low-high" pattern for a 75:25 weighting.

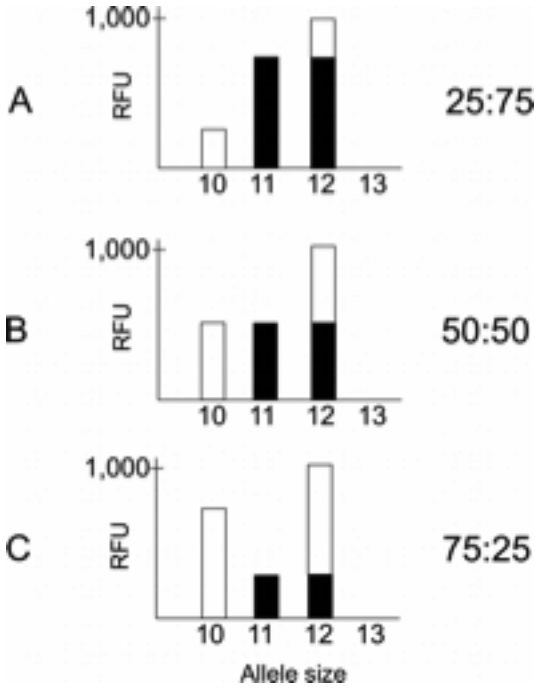

Figure 10. For **(A)** the two-person DNA mixture data shown (triangles), **(B)** a good explanation of the data pattern is a 50:50 combination of genotypes 10,12 and 11,12, while **(C)** a poor explanation would combine allele pairs 10,13 and 11,12 in the same proportion.

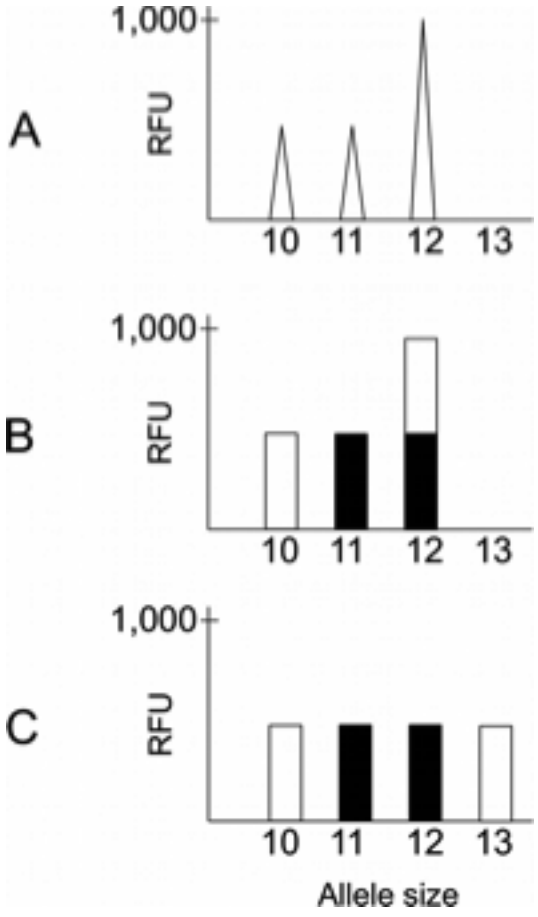

Figure 11. An inferred DNA mixture weight is a probability distribution computed from STR locus data. The mixture weight shown here is a bell-shaped curve centered at 33%, with a standard deviation spread of 8%.

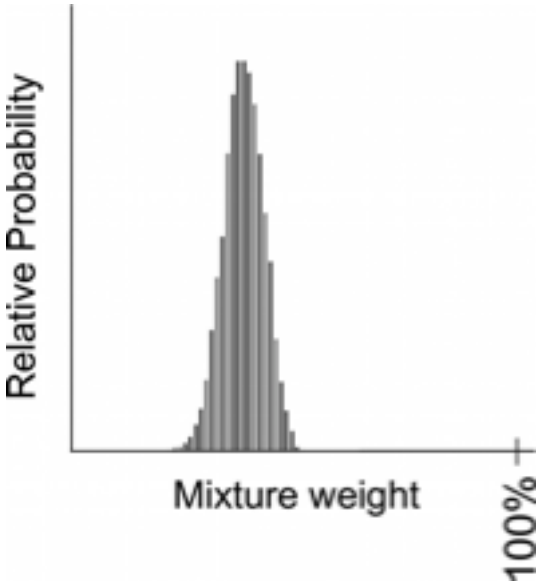

Figure 12. Human review applies thresholds that truncate DNA evidence data. **(A)** The threshold of 200 RFU shown keeps some STR peaks, and discards others. **(B)** After applying this threshold, quantitative information is lost and peaks 10, 11 and 12 are classified as alleles present in the data, while peak 13 is thought to not be an allele.

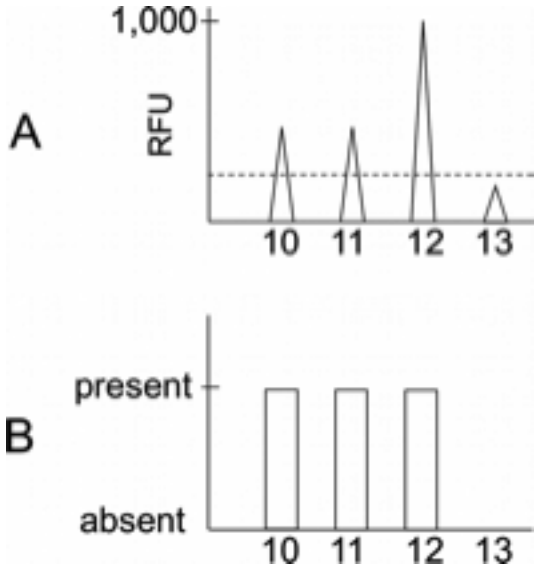

Figure 13. **(A)** A computer can infer a genotype that places probability where the data indicate, while on the same DNA mixture data **(B)** using a threshold diffuses evidence genotype probability off to infeasible allele pairs. This diffusion moves probability away (horizontal arrow) from the correct 10,12 genotype, artificially reducing evidence probability (vertical arrow) in the match statistic numerator.

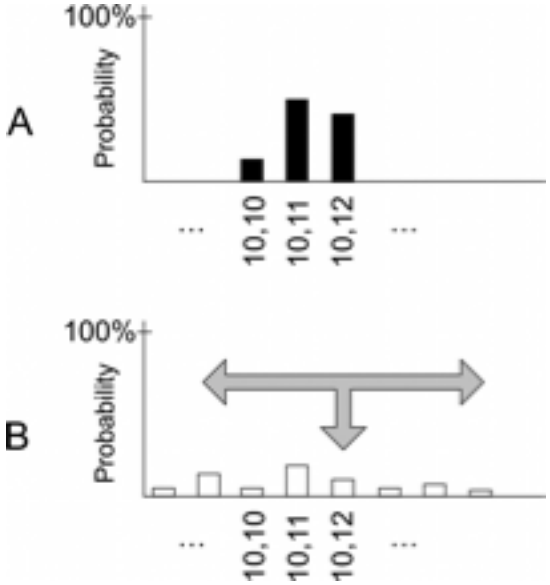

Figure 14. The genotype of a child (shaded square) can be inferred from known parental genotypes. The inferred child genotype is a probability distribution.

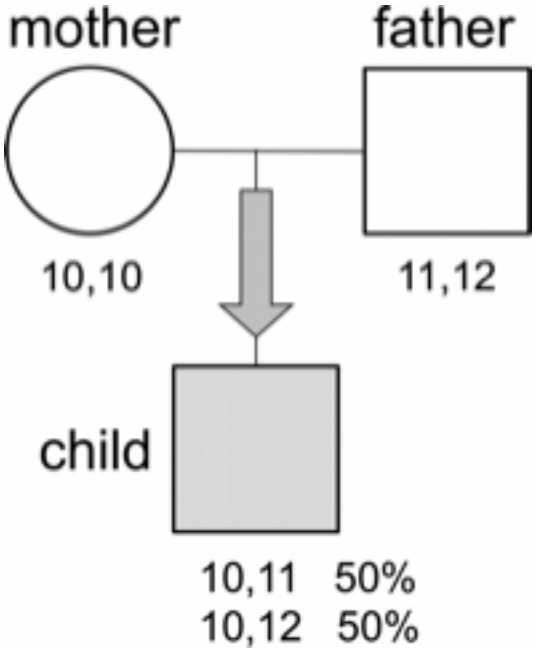

Figure 15. The genotype of a biological father (shaded square) can be inferred from the known genotypes of his child (white square) and the mother (white circle).

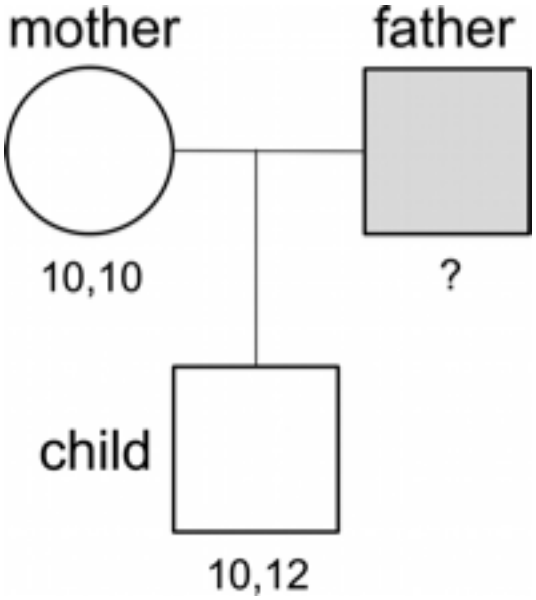

Figure 16. The father's genotype must contain allele 12, based on the child and mother genotypes. The other paternal allele comes from the population, and so the inferred father genotype is a probability distribution over all allele pairs that contain allele 12.

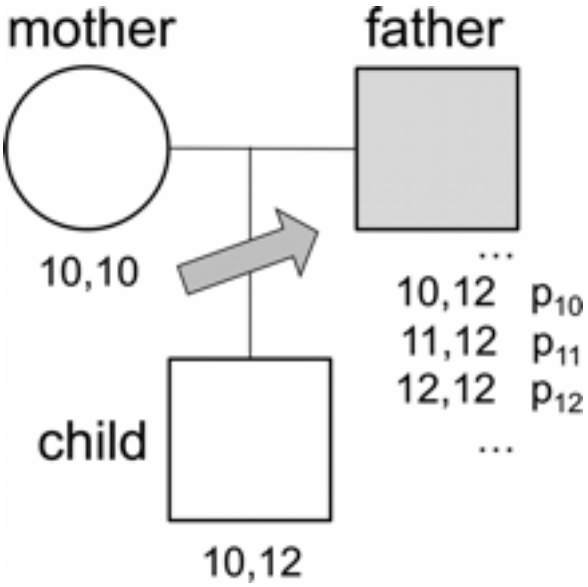

Figure 17. A missing person's genotype (shaded circle, mother) can be reconstructed from the known genotypes of their relatives. More kinship information generally produces a more definite genotype probability distribution, and a larger DNA match statistic.

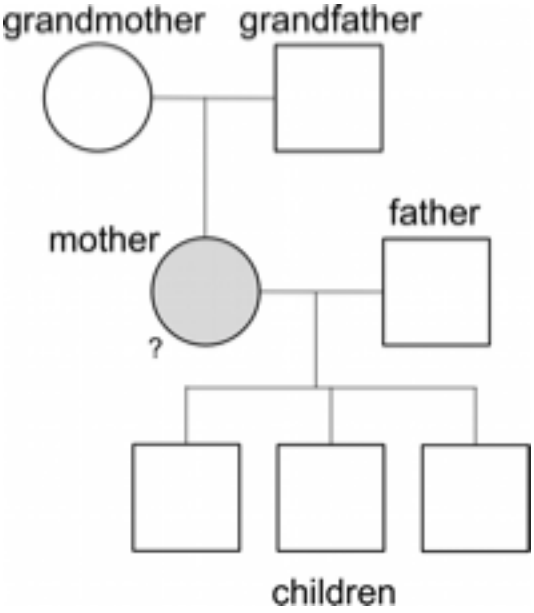

Figure 18. Two person DNA mixture comparisons from an investigative DNA database that uses probabilistic genotypes; information is shown along the x-axis as the logarithm of the match statistic. **(A)** Sensitivity was assessed on 80 known genotype matches, detecting all positive matches (right side, black) with an average positive exponent match statistic of $10^{14.5}$ (around a quadrillion). **(B)** Specificity was assessed on 80,000 random genotype comparisons, rejecting all these non-matches (left side, gray) with an average negative exponent statistic of $10^{-21.4}$ (around one in sextillion).

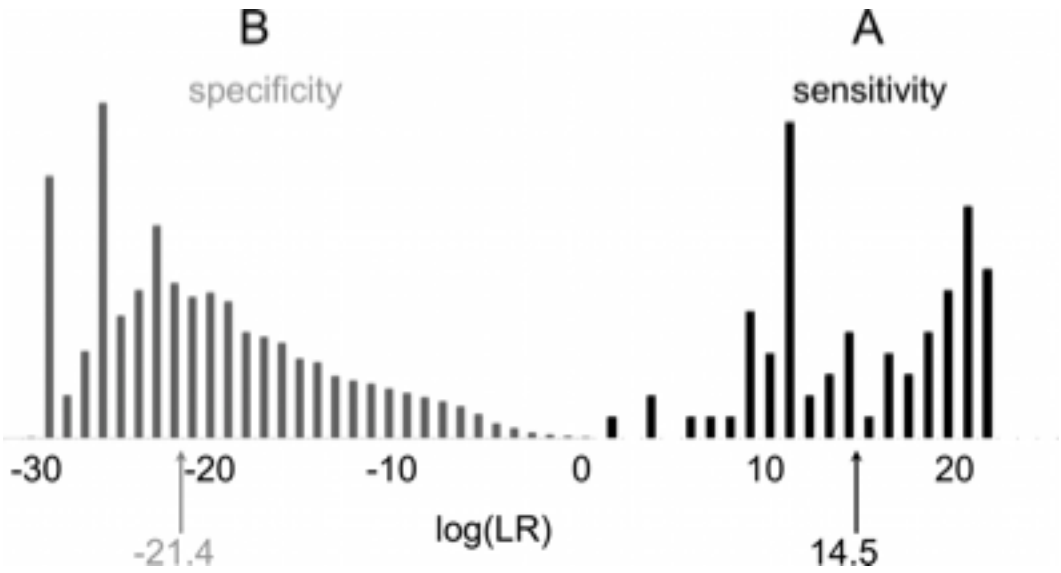

Figure 19. Match sensitivity and specificity of probabilistic genotypes on a set of 20 typical two and three person DNA mixture items from criminal cases. **(A)** The true positive DNA matches (right side, black) of inferred mixture genotypes to reference samples all show positive log(LR) match information. **(B)** Cross-case comparison of mixture genotypes to unrelated references in other cases (left side, gray) accurately reject these 1,664 false matches, as indicated by the negative log(LR) distribution.

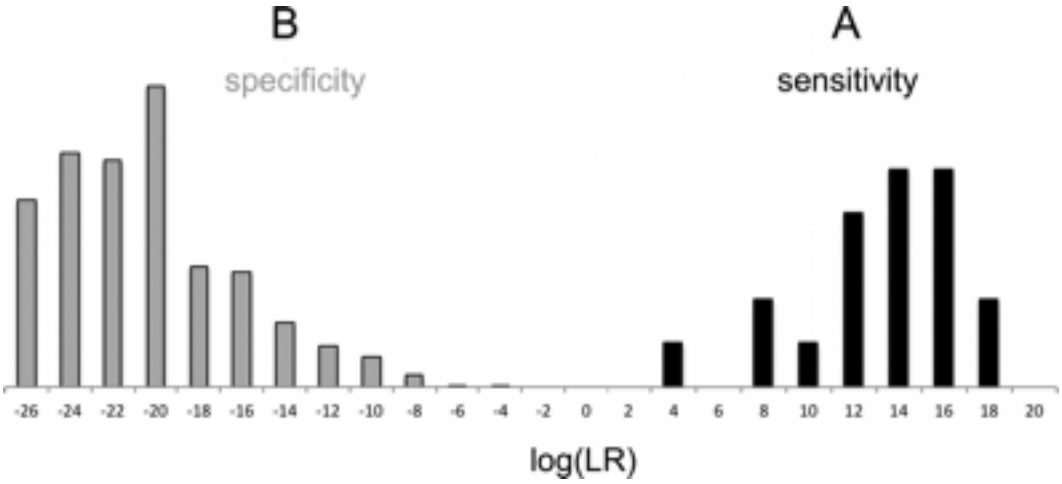

Figure 20. The reproducibility of DNA mixture interpretation is shown for a probabilistic genotyping method. The y-axis gives log(LR) match information in increments of 3 (i.e., thousand, million, billion, and so on). DNA match statistics from twenty genotype comparisons are shown in ascending order (black bars), along with replicated results from repeated computer runs performed on the same mixture data (white bars).

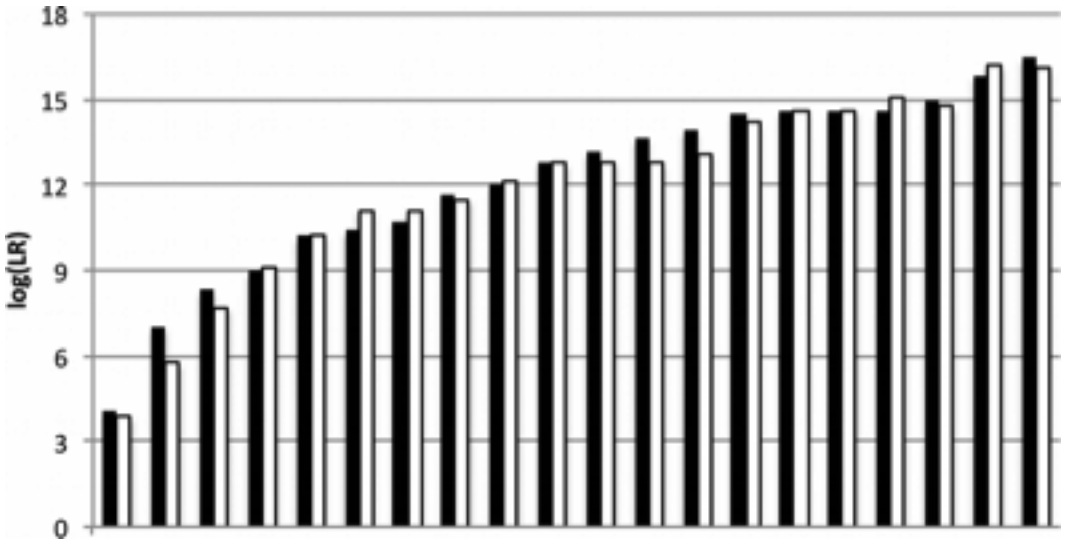

Figure 21. The non-contributor distribution for a genotype separated from DNA mixture evidence data in the Southampton rape case. The *x*-axis is the log(LR) match statistic expressed in logarithmic ban units; the *y*-axis represents probability. The distribution shows the relative occurrence of log(LR) values for people who did *not* contribute their DNA to the evidence. The average value of this match statistic distribution is one over 2,750 (–3.44 ban in log units), a number less than one that describes the genotype's exclusionary power.