Letters to the Editor

Discussion of "Recent Application of DNA Analysis to Issues of Paternity"

Dear Sir:

In a recent correspondence to the *Journal of Forensic Sciences* (Vol. 33, No. 5, Sept. 1988, pp. 1107–1108), several concerns were raised regarding the use of deoxyribonucleic acid (DNA) analysis for paternity determination. Drs. Kobilinsky and Levine suggested several ways in which DNA analysis could lead to false exclusion in paternity determination. To illustrate their points, they presented three scenarios which would result in a child appearing to inherit DNA sequences which could not be attributed to the biological father.

The first scenario is the occurrence of a meiotic cross-over "which takes place at the site where a restriction enzyme cleavage would normally take place." Having reviewed this hypothetical situation (the authors have provided no details other than meiotic recombination occurring at the site where a restriction enzyme should cleave), I can envision only one way in which such an event could result in false exclusion. If both father and mother were homozygous for the particular restriction site (for example, EcoRI GAATTC) (the mother being homozygous for either the presence or absence of the site and the father being homozygous for the absence of the site) and the father's two alleles were marked by complementary mutations (for example, GTATTC and GAATCC), recombination could occur between the mutated bases in the two paternal alleles and generate two "new" alleles, one of which is marked by a normal EcoRI site (GAATTC), the other by a mutant site (GTATCC). If the allele that contains the normal EcoRI site is inherited by the child and the presence or absence of that particular EcoRI is measured by the DNA analysis, false exclusion would result. Given the requirements for (1) a recombination event to occur within a particular restriction site and (2) recombination occurring between alleles having complementary mutations of that site, this scenario should be considered unlikely.

The second scenario involves a mutational event in generation of the single spermatozoa (that which is responsible for the conceptus in the paternity dispute) which alters the restriction site being analyzed. The mutation rates of human coding and noncoding sequences are generally not expressed in mutations per generation or mutations per meioses because of the fact that such rates are extremely low and, thus, are more accurately expressed in terms of evolutionary timeframes (for example, percent mutation per million years) [*1*]. Again, given the low frequency of this event, it seems unlikely that germline mutation of restriction endonuclease sites will pose a serious problem for paternity determination.

The last scenario deals with yet another rare meiotic event, the case of meiotic nondisjunction resulting in uniparental disomy (that is, the child carries two copies of a maternal chromosome and lacks a paternally derived chromosome). Although the estimated frequency of such occurrences is low (/30 000 conceptions), there is a documented case of uniparental disomy of chromosome 7 [*2*].

There are two points I wish to stress in response to the above concerns. First, each of the scenarios rely on the occurrence of rare genetic events (meiotic recombination within a restriction site and between complementing mutations thereof, germline mutation of restriction site, and meiotic nondisjunction resulting uniparental disomy of a particular chromosome). Although it is recognized that such events could occur, their likelihoods of occurrence makes them improbable causes a false exclusion.

A second, and more important point, is that each of these scenarios could also lead to false exclusion by conventional serological tests. Alterations of virtually any genetic determinant could result from the same recombination, mutation, and nondisjunction events. Thus, if these are to be considered serious sources of error for DNA analysis, they must also be considered points of controversy for the more conventional protein and enzyme typing systems used in paternity analysis.

Although the incidence of the above events is rare at best, there are genetic events that can result in the de novo generation of new alleles during spermatogenesis. The most informative DNA markers currently being used in forensic science and paternity labs are the so-called VNTRs [*3*], loci defined by variable numbers of tandem repeat units. Alleles are operationally defined by the length of fragments generated by restriction enzyme sites which flank the tandem array. Meiotic recombination between arrays can result in the generation of "new" fragment lengths, the inheritance of which could lead to false exclusion. As many of these loci have measurable rates of mutation as a result of recombination (rates are defined by examining many large pedigrees where paternity is not an issue), this is indeed a concern of paternity analysis. In fact, this has been recognized for several years [*4–7*], and guidelines have been proposed which require that mutation rates of individual loci be established and that loci exhibiting high rates of mutation not be employed for paternity analysis.

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Authors' Response

Dear Sir:

We welcome the foregoing comments on our recent correspondence to this *Journal.* Dr. Waye's letter is an indication of the timeliness and importance of the issue we have raised and serves to flesh-out the "scenarios" we have proposed. However, some of the points raised by him call for additional discussion.

We thank Dr. Waye for providing an example of how our proposed crossing-over scenario could lead to a unique DNA restriction fragment in a child's genome. We would like to take this opportunity to emphasize further the importance of meiotic crossing-

over as a source of unique DNA fragments by pointing out that unequal crossing-over taking place either close to or at the site where restriction enzyme cleavage would normally occur can also result in banding patterns different from those expected based upon analysis of parental DNA. Unequal crossing-over is well established as the cause of a number of abnormal hemoglobins [*1*] and is considered a major factor in producing the different sized segments found in hypervariable loci in human DNA [*2*]. The high frequency of the latter event can be seen in surveys of families where parentage is not in dispute. In these studies, an average of 1 hypervariable offspring fragment in 300 cannot be detected in either parent [*2,3*], indicating a considerable possibility of incorrect exclusion where parentage is in dispute.

With reference to mutation, we were startled by the apparent brushing aside of the considerable body of information available on mutation rates. In evolutionary studies, it may be desirable and even necessary to express such rates as percent mutation per million years. However, in population studies mutation rates are expressed in terms of per million gametes per genetic locus. It is estimated that the human genome contains between 50 000 and 100 000 potentially identifiable genes, and a conservative estimate of mutation rate is one per million gametes per locus [*1*]. As a result of the above, 5 to 10% of all gametes should contain a newly mutated gene. In 1988, 143 million births occurred worldwide, representing the fusion of 286 million gametes [*4*]. We cannot estimate how many of the 14 to 28 million newly formed mutations affected restriction site bases, because we do not know how many such sites are present in the human genome. In view of the above, mutation is not a phenomenon to be dismissed.

In considering meiotic nondisjunction and its potential for resulting in uniparental disomy (1/30 000 births), one can use the information supplied in the above paragraph to conclude that more than 4700 such affected children were produced worldwide in 1988. As the world population continues to increase, the number of instances where a biological parent does not contribute a full complement of chromosomes to an offspring will also increase.

We do not agree that the issue of mutations applies equally well to protein and enzyme typing tests. Mutation of a polymorphic protein or isozyme usually results in an altered banding pattern which can generally be detected by comparison with the banding patterns of control isozymes run on the same gel. The analyst can recognize the variant by direct observation of the electrophoretic or isoelectric focused gel. On the other hand, since restriction fragment length polymorphism (RFLP) analysis does not incorporate the same sort of internal controls, the analyst may not be aware of the possibility that he/she may be detecting a variant allele. Because DNA fingerprinting is quickly becoming the test of choice in paternity analysis we decided to focus our discussion on DNA rather than protein analysis.

In his remarks about our scenarios, Dr. Waye states, "Although it is recognized that such events could occur, their likelihoods of occurrence makes them improbable causes of false exclusion." We would like to point out that it is not a matter that ". . .such events could occur. . .," but that in fact they do occur. It is also clear that although each event may have a small probability of occurrence, the probability of false paternal exclusion is the sum of the individual probabilities as any one of them can occur in a given case. Finally, for the family faced with the trauma of a paternity dispute, probabilities are meaningless if the individuals involved are unfortunate enough to be faced with an ambiguous situation.

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How Similar Is Substantially Similar?

> "When *I* use a word," Humpty Dumpty said, in rather a scornful tone, "it means just what I choose it to mean—neither more nor less."
> "The question is," said Alice, "whether you *can* make words mean so many different things."
> "The question is," said Humpty Dumpty, "which is to be master—that's all."
> *Through The Looking Glass*
> Lewis Carroll, Macmillan, 1871

Sir:

In October 1986, the 99th Congress passed, and President Reagan signed, legislation called the "Anti-Drug Abuse Act of 1986." One of the several sections of this Act is known as the "Controlled Substances Analogue Enforcement Act of 1986." Many states, including California, enacted legislation that essentially mimicked the wording of this Federal Law. What it does is to make the laws and regulations that are in place for controlling illegal acts with scheduled drug equally applicable to unscheduled drugs, if these latter can be viewed as analogues. And to the extent that an analogue is intended for human consumption, it shall be treated as a Schedule I drug.

What is an analogue?

In the broadest usage, in the letters and arts, something is an analogue of something else if it is similar to it in function but different in structure or origin. The parent stem, analogy, was a Greek word that quite simply signified an agreement or correspondence between things that were in other respects different. And analogy can imply that if two things are alike in one way, they may be in another. In zoology, the wing of a bird and a butterfly wing are analogues. In linguistics, a potato and an apple are probably analogues in German (Kartoffel) and French (pomme de terre) but not in English or Italian. In mathematics, analogy was originally the basis of comparing ratios, but in current usage, analogue is contrasted to digital as a representation of continuous function.

In the area of chemistry and chemical structure, the use of the term has ranged from the most narrow sense to the most broad. The primary question that is being asked is, how can we compare the structures of two molecules? Are they both long and spindly, or short and squat, or planar or lumpy, or big and heavy, or small and light? Do they both have rings and/or chains and/or bumps or valleys or do they share similar weird atoms? The comparison of molecules very much depends upon which particular lens you are using for viewing.

In the narrowest of all interpretations, two compounds are analogues only if they differ by the replacement of one atom with another above or below it in the periodic table. The sulfur analogues of ethers. The silicon analogues of hydrocarbons. The next level of broadening comes from a horizontal license with the periodic table. The replacement of a carbon with an oxygen or a nitrogen, for example.

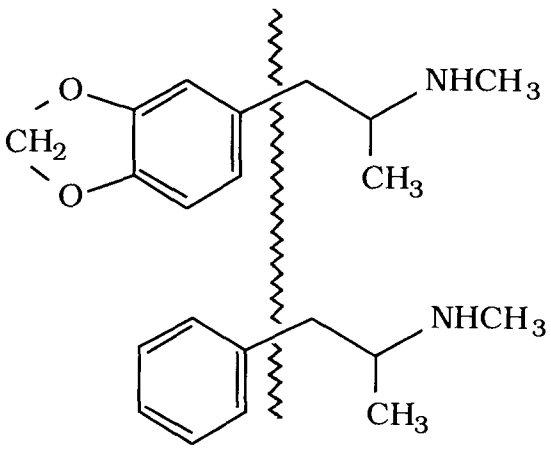
Yet looser rules can apply when an entire unit of a chain of units can be replaced with another. The analogues of a polypeptide may have a different amino acids at the valine position. Polysaccharide analogues might have new sugars at the terminal galactose position. And there are nucleic acids and synthetic polymers. Sometimes one group can be replaced with another to create an analogue, such as halo groups for alkyl groups.

And in the broadest examples, even the lengthening of a chain (theoretically, a homologue) or the rearrangement of atoms (theoretically, an isomer) have been structural changes referred to as analogues. Pick up a random copy of the *Journal of Medicinal Chemistry*. A sizable percentage of all titles incorporate the word "analogue" and all of the above usages are represented.

What applies to chemical structure applies equally to pharmacological function. Two drugs may be compared in an unlimited number of ways, comparing activity from the gross (they were both lethal) to the subtle (they showed similar receptor site kinetics). If the observer chooses one particular response and two drugs show it, he may well say that they are pharmacologically analogous. They become analogues. Another observer, looking for something else, may find them different from one another, and to him they are not analogues.

But all this is legally moot, since the term "analogue" has been explicitly defined in the 1986 law. A chemical is an analogue if its structure is "substantially similar" to that of a Schedule I or II drug. Or it is an analogue if it has a stimulant, depressant, or hallucinogenic activity that is "substantially similar" to that of a Schedule I or II drug. In short, the definition that is to be used in the enforcement of law has built into it a carefully worded vagueness. Nowhere are the terms "substantially" or "similar" defined. In everyday usage, the term "similar" means having something in common, that there is a close resemblance. The word "substantial" implies having substance, rather than being imaginary. Or being major (or strong, or heavy, or serious) rather than being minor (or weak, or light, or trivial). It is linguistically understandable to say that two structures are similar or that they are substantially identical. Either term means that they "kinda look alike." This calls for some subjective input by the speaker, but if his way of looking at two chemical structures (or two pharmacological responses) shows them to be somewhat alike, he can certainly call them "similar."

But the term "substantially similar" is hopelessly vague. I believe that it was crafted with this very goal in mind. By designing the net which has a completely variable mesh size, one can catch whatever fish one wishes to and let escape another fish that is not wanted. There is no objective standard to the term "similar" and certainly none for the phrase "substantially similar."



MDMA

Methamphetamine

Let me offer one specific example. I was asked a question by a lawyer a few weeks ago, in regard to the invocation of the California Analogue Bill. The charge was directed at the possession of MDMA, and it was based on the assumption that it had a structure "substantially similar" to that of methamphetamine. Methamphetamine, in California, is a Schedule II drug, and MDMA is not Scheduled. Above are shown the two structures being compared. The question that I was asked, "Do I think that these structures are "substantially similar?"

I have drawn a wiggly line to separate the aliphatic from the aromatic portion of these structures.

To the right of this line, there is found the same carbon chain, the same hetero-atom, the same number of atoms connected to one another in the same way, the same chiral center, and on and on. These halves are more than "substantially similar"; they are out-and-out identical.

To the left of the line, one finds two rings rather than one, two new hetero-atoms (the two oxygens), an additional carbon atom, three rather than five substitution positions on the benzene ring, and on and on. These halves are by no stretch of the imagination "substantially similar"; they are totally different.

In the eyes of a chemist who is attracted to aliphatic chains, these molecules might be analogues. And in the eyes of another chemist, one who thinks first in terms of rings and substitution patterns, these molecules are not analogues. The same ambiguity is obtained in the search for some "substantially similarity" between them in regard to their pharmacology. If one looks with an eye to heart rate and loss of appetite, they can be thought of as being similar. But with an eye to subjective effects and abuse patterns, they are totally dissimilar.

There is no "right" answer. There can never be one. And yet, charges are being brought and convictions are being obtained based on the "scientific" opinions presented in criminal cases. Eventually, a challenge to this preposterous wording will be made that will result in its removal from the law statutes. But until then, I fear that it will be imposed by the law enforcement groups when desirable, and ignored at other times. Any law that allows selective enforcement is bad law.

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"Observations and Statistics Relating to Suicide Weapons": An Update

Dear Sir:

In a previous publication [*1*] data were presented for 202 weapons used in suicidal death. The experimental procedure has been continued and being presented herewith are data for a total of 650 cases.

As before, each of these cases has been ruled as a suicidal death by the Office of the Medical Examiner. Note also that each of the 650 cases is either a contact or loose contact wound.

These data are for weapons received in suicide cases from June 1985 to October 1988. Of the 650 cases, the distribution of weapon type is 54% revolvers, 20% pistols, 15% shotguns, and 11% rifles. In Table 1 the detection of blood inside and on the muzzle end of the various weapon types is given. Note that weapons submitted which had obviously been laying in a pool of blood were excluded from the study.

In Tables 2 and 3 the results of testing as broken down by caliber and weapon type for revolvers and pistols are given. In Table 4 the location of entrance wound sites by sex of decedent is shown.

TABLE 1—*Results of testing suicide weapons for blood: June 1985 to October 1988.*

Weapon Type	Positive Blood Inside Barrel	Positive Blood on Barrel	Weapons Total
Revolver	166 (47%)	251 (72%)	351
Pistol	63 (48%)	93 (70%)	132
Rifle	36 (52%)	54 (78%)	69
Shotgun	73 (74%)	83 (85%)	98
Total			650

TABLE 2—*Blood detected inside and on the muzzle end of revolvers.*

Weapon Caliber	Positive Blood Inside Barrel	Positive Blood on Barrel	Weapons Total
22 short, long, long rifle	23 (31%)	39 (53%)	74
22 rim Magnum	6 (60%)	7 (70%)	10
32 short, 32 long	10 (36%)	19 (68%)	28
357 Magnum	49 (65%)	63 (84%)	75
38 short Colt, 38 S&W	3 (50%)	5 (83%)	6
38 Special	68 (47%)	107 (73%)	146
41 Magnum, 44 Magnum	5 (56%)	8 (89%)	9
45 long Colt, 45 S&W	2 (67%)	3 (100%)	3

TABLE 3—*Blood detected inside and on the muzzle end of pistols.*

Weapon Caliber	Positive Blood Inside Barrel	Positive Blood on Barrel	Weapons Total
22 LR	12 (43%)	18 (64%)	28
22 rim Magnum	1 (100%)	1 (100%)	1
25 Auto	27 (55%)	35 (71%)	49
30 Carbine	0 (0%)	1 (100%)	1
32 Auto	3 (43%)	4 (57%)	7
380 Auto	9 (50%)	17 (94%)	18
38 Special (Derringer)			5
9MM Parabellum	5 (42%)	10 (83%)	12
45 Auto	7 (64%)	9 (82%)	11

TABLE 4—*Location of entrance wound sites by sex of decedent.*

Decedents	Head Area	Body Area
	HANDGUNS	
101 Females	70 (69%)	31 (31%)
382 Males	321 (84%)	61 (16%)
	LONG GUNS	
12 Females	8 (67%)	4 (33%)
155 Males	106 (68%)	51 (33%)

The additional data have produced only slightly different percentages with regard to men and women as to frequency of head versus body location of entrance wounds. Also, it appears clear from Table 2 that the incidence of blood detected inside the muzzle end of revolver gun barrels does increase as caliber increases. Although not shown in this paper, one sees this relationship between incidence of blood inside the barrel and caliber more clearly when only head wounds are considered. The intervening target of clothing significantly affects blood detected inside the barrel, but has relatively little effect on blood detected on the outer surface at the muzzle end of weapons.

Table 3 lists results of blood detected inside and on the barrel of pistols. The frequency of blood inside the muzzle varies from 43% detected in 22 long rifle caliber weapons to 64% "positive" in 45 caliber weapons. This spread of values is somewhat less than those obtained in revolver cases. Another way to evaluate the data might be to consider the relationship among caliber of bullet, muzzle velocity, and weapon type.

In the course of examining the suicide weapons for function, a question arose as to whether blood inside the barrel of the firearm might persist even after obtaining test rounds from the weapon for comparison purposes. Preliminary results regarding the first five handguns tested [2] revealed that blood was detected inside the muzzle end after test-firing in four of the five cases. Since then two additional handguns have been tested with both yielding positive tests for blood after discharging one or more test rounds. In one of the most recent cases, blood was detected inside the barrel of a 357 Magnum revolver after two rounds were discharged; no blood was detected after discharge of the third bullet.

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Metal Mesh Gloves for Autopsy Use

Dear Sir:

The emergence of AIDS has served to heighten concern regarding viral transmission during the performance of autopsies, especially by accidental cutting of the prosector's hands. Whether this is a significant risk remains to be proven, but it certainly is theoretically possible. Most of us who do autopsies can recall a time or two when the scalpel finally lurched through that calcific coronary artery and continued its path through our index finger or thumb. If the deceased is known to have had AIDS or hepatitis, it is more disconcerting than if he did not.

To help decrease the risk of accidental cuts, metal mesh gloves have been recently introduced, such as Armor-Touch™ gloves, available through Braintree Laboratories, Inc. in Braintree, Maine. The gloves cost $279.00 a pair, plus $3 to $5 for shipping charges, depending upon which side of the Mississippi River one resides. The gloves can be sterilized by autoclave or liquid soak and reused. We have tried these gloves and found them to be workable, but somewhat heavy and cumbersome.

At the suggestion of one of our laboratory technicians, Mr. John Lappin, we recently purchased the K-Steel™ Fillet Glove™, manufactured by Normark Corp. of Minneapolis, Minnesota. The gloves are widely available at sporting goods stores and cost about $13.00 a glove. By buying in bulk, the cost can be reduced to around $8.00 a glove, or $16.00 a pair. The glove will fit either hand. It is considerably lighter and far more flexible and comfortable than the Armor-Touch gloves. The glove is made of a combination of stainless steel and "high tech" synthetic material. It is machine washable and reusable. It withstands liquid disinfectant and autoclave treatments. It is much more comfortable to wear under a heavy rubber glove, such as that distributed by Ansell Industrial Products, Dothan, Alabama (Ansell FL 200's, Flocklined 20 mil, #254). We were unable to cut the glove by random slicing with a scalpel, both with and without our hands inside. However, we did cut the glove with a hand held razor blade by holding it taut and repeatedly cutting it twelve to thirteen times with maximal force. We could not cut the glove using an autopsy knife in the same fashion, however, We feel secure using the Normark glove, and because of their far cheaper price and better flexibility, highly recommend them for autopsy use. Note that neither the Armor-Touch nor the K-Steel glove is resistant to needle punctures.

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