Letters to the Editor

Source Individuality Versus Expressed Individuality

Discussion of "Probability Analysis and the Evidential Value of Bolt Arrangements"

Dear Sir:

The case report by Even et al. in your September issue [*1*] is a critically constructed, well-reasoned analysis of the variation in the positioning of bolt heads in a commercial assembly-line product. The paper is an excellent example showing the analytical process necessary to evaluate individuality in a source. The article is exemplary in that the authors went beyond simple empirical comparison with alternative sources and attempted to generalize the variation that occurs in possible sources for these marks.

In my view the authors did an exceptional job at what the paper purports to do: to estimate the probability of finding a source with a bolt arrangement similar to the case source. The conclusion that is given is that, ". . . it is clear that there is only a negligible probability that another (source) with the same bolt arrangement is the one that (made the print)."

Although exemplary within the scope of its application, the authors' analysis fails to extend into a necessary and (usually) most difficult phase of the analysis: distinguishing between the individuality present in the source and the individuality that is expressed by the source.

Based on the authors' well-reasoned work we can accept that the individual bolt positions are statistically independent, that positions of 5° are distinguishable when looking at the bolt heads, and that from these two propositions their probability model follows. We cannot, however, apply this calculation (directly) to the case example. A critical step, critical both practically and philosophically, has been omitted. We need to evaluate how reliably bolt positions are represented *when looking at the print or impression resulting from the source*. Given the print (which in this case is associated with the criminal act) we need to evaluate the commonness of a compatible set of bolt orientations. This is a fundamentally different question from determining how common bolt arrangements are that would be indistinguishable from the suspect's source [*2*].

In the case discussed the prints were found on a coat, where wrinkling and contour will certainly have some effect on how well the bolt positions are represented on the coat. We must evaluate how reliably we can determine the bolt positions of the *actual* source from the less perfect information on the coat.

I am not implying that either the authors' work or their ultimate conclusion is faulty. In the case example the marks are clearly defined and there is sufficient related detail in the mark suggesting both that the distortions are not great and that any imprecision introduced during the "printing" process is not severe. Obviously the authors had to consider the quality of the mark to judge it reliable enough to compare against the source, and quite likely their estimate of distinguishable angles took these considerations (for their case) into account. There are grounds for concern, however, that the distinction between source individuality and expressed individuality has not been explicitly made. This distinction *must* be recognized for the evidential questions behind the statistical calculations to be properly formulated. The issue is *not* simply how well possible sources for the mark can be distinguished from one another. Rather, the issues are (1) how well can we tell the bolt positions in the actual source, given what we see on the coat, and

(2) how common are alternative sources that meet these, less precise, criteria. It is often easier to characterize variation among possible sources than it is to consider the ability of a less-perfect pattern to predict characteristics of the *actual* source. Sometimes we cannot objectively address this latter issue, yet the analytical process must consider it, so that proper questions can be formulated and fair predictions can be made regarding the frequency of *all* possible sources for the mark.

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Author's Response

Dear Sir:

Dr. Stoney's letter contained constructive and accurate remarks for which we would like to thank him. We share his view concerning the necessity of extending the analysis to distinguish between "the individuality present in the source and the individuality that is expressed by the source." The importance of this stage of the analysis is explained and well founded both in his letter to the editor as well as in his article which is cited in the References of his letter.

We would like to note that our main purpose in our article was not to "estimate the probability of finding a source with a bolt arrangement *similar to the car source*." Our main purpose was however to consider "the possibility of generalizing and concluding that any such arrangement of bolts is set in an accidental way since the orientation of each bolt is an independent one" and thus may be used as a means of identification of the object on which it is found (that is, the source).

The actual case described was intended to serve only as an example of when such an identification is needed. This can be seen by the fact that we did not go into details regarding the actual comparison between the marks found on the coat and the source arrangement.

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Discussion of "Repetitive Deoxyribonucleic Acid (DNA) and Human Genome Variation—A Concise Review Relevant to Forensic Biology"

Dear Sir:

A broad understanding of the various classes of DNA that comprise our genome is critical to the development and implementation of forensic deoxyribonucleic acid (DNA)

analysis programs. Recognizing this, Fowler and co-workers recently published a short review (with extensive bibliography) concerning human repetitive DNAs and how DNA polymorphisms may be useful for [forensic] analysis [*1*]. As this review is likely to be a primary reference source of many forensic scientists, it is essential that the information be accurate and comprehensive. This was indeed a formidable task given the wide scope and concise format of the review. Accordingly, the purpose of this correspondence is twofold: (1) to discuss some types of human repetitive DNA which were omitted from the review yet may be relevant to forensic science and (2) to offer further information regarding some of the human repetitive DNAs discussed.

There are, in my view, three types of human repetitive DNA that were not mentioned in the review but may be useful for forensic science analysis. First, there are the simplest of tandem DNAs (excluding homopolymers), consisting of short arrays of (TG) n [*2,3*]. These sequences are found throughout the human genome and the number of repeats found at a given locus can vary considerably. As such, one may consider these sequences to be VNTRs (variable number of tandem repeats) based on a dinucleotide repeat unit.

A second type of repetitive DNA not mentioned in the review is the telomeric sequences of human chromosomes [*4*]. Given their tandem organization and terminal location (telomeric regions of chromosomes appear to be enriched for hypervariable loci [*5,6*]), these sequences may also be polymorphic.

Lastly, the mitochondrial genome could be dealt with in the context of human repetitive DNA. Although these sequences are extrachromosomal, they do, nevertheless, constitute part of our total DNA complement. Regions of the mitochondrial genome are polymorphic [*7,8*] and, because the mitochondrial genome is inherited in a maternal fashion (and hence may be considered clonal for a given individual), these sequences may be well-suited for enzymatic amplification and direct nucleotide sequencing [*9*]

There were several points raised in the review that merit discussion. To begin with, the authors have used the acronyms STR and LTR (short and long tandem repeats, respectively) to distinguish between different classes of tandem DNA. The problem with this system is that the acronym LTR has been used for many years in molecular biology to describe the *Long Terminal Repeats* found in the genomes of many eukaryotic viruses. In fact, over 1100 citations have been made from 1983 through 1988 with reference to LTRs of viruses (643 to "long terminal repeat"and 503 to the acronym "LTR," *Index Medicus*). Thus, to use the LTR acronym to describe what has widely been termed *satellite DNA* can only lead to confusion. As an alternative to the STR/LTR designations for tandem DNA, it may be prudent to adhere to established classification schemes based on copy number and genomic distribution. For example, if a tandem DNA family is dispersed throughout the genome in a seemingly random fashion (for example, VNTRs), it should be designated as *interspersed*. This is not to undermine the fact that the sequence has a tandem organization, but rather to acknowledge that the arrays are not localized to any particular region of the genome. Conversely, if a tandem DNA is present in high copy number and is localized to a limited number of regions in the human genome, it should be designated as *satellite DNA*. Under the only universally accepted system of nomenclature for human DNA sequences [*10*], tandem DNAs are given designations that reflect both the chromosome assignment and copy number (example: chromosome 17 sequences are designated "D17"; single-copy loci, tandemly organized or not, are designated "S" [for example, D17S30], and highly repetitive satellite DNAs are designated "Z" [for example, D17Z1].

Several misconceptions from the literature have been restated in this review and perhaps merit clarification. First, alpha satellite and Sau3A DNA have been dealt with as separate repetitive DNA families (although it is noted that the Sau3A family is alphoid-related). Alpha satellite is defined as tandemly repeated DNA which is based on a ~171 basepair repeat unit which bears homology to the prototype primate or human alphoid DNAs [*11*]. The Sau3A DNA family defined by Kiyama et al. [*12*] corresponds to a subset of

alpha satellite which is specific for human chromosome 11 [*13,14*] and, as such, does not constitute a distinct class of repetitive DNA.

In reference to the preceding point, note that the authors have made reference to two distinct types of satellite DNA in their description of the Sau3A family. The first, described by Kiyama et al. [*12*], is chromosome 11 alpha satellite DNA which contains Sau3A sites at ~850-bp intervals [*13,14*]. The second, described by Menervi et al. [*15,16*], has Sau3A sites every ~66 bp and bears no sequence homology to the alpha satellite DNA family. This type of satellite DNA has recently been designated beta satellite in recognition of its unique tandem repeat unit [*17*].

A last point of discussion concerns the various types of DNA polymorphisms described in the review. The authors suggest that the polymorphisms of satellite DNA are due predominantly to the sporadic loss or gain of restriction sites in individual repeat units within a tandem array. While this most certainly is true of a number of reported polymorphic systems (for example, Ref *18*), there are also examples of amplified polymorphic fragment lengths which may be present in hundreds or thousands of copies per array. These polymorphisms may arise either from point mutations (loss or gain of a restriction site) or from cross-over events (that alter the distance between flanking restriction sites) which are subsequently spread throughout an array and fixed within the population [*19,20*]. Because the actual polymorphism is present in multiple copies per haploid genome, these RFLPs are by nature amplified and may be detected from smaller amounts of DNA than conventional single-copy RFLPs.

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Author's Reply

Dear Sir:

The purpose of the published review [*1*] to which Dr. Waye refers was to provide operational forensic scientists with a concise overview of human genome organization in general, and repetitive DNA sequences in particular. The bibliography allows the flesh and fabric of detail to be assembled on this skeleton. The comments of Waye are valuable in expanding upon this process both with regard to suggested reconstruction of the skeletal outline and to providing additional detail.

The most constructive issues raised are those of nomenclature, as exemplified by the potential confusion in the use of the acronym LTR (meaning either long tandem repeat or long terminal repeat). Waye suggests adherence to established classification schemes. However, as the examples cited in the review show, interspersed tandem repeats (to use the designation suggested by Waye) have been variously called VNTRs, HVRs, "minisatellites,"and VLPs [*1*]. This indicates there has as yet been little adherence to an established nomenclature.

Under the classification scheme suggested by Waye, the features by which to distinguish satellite DNA and interspersed (tandem) DNA—the latter not to be confused with interspersed **non-tandem** DNA repeats such as LINES and SINES [*1*]—are copy number and localization. This is quite acceptable. However, it is as well to realize that such concepts are relative, the meaning of terms such as interspersed tending to change depending upon the perspective from which the genome is viewed. For example, at the base sequence level, individual "minisatellites"/VNTRs are interspersed within other sequences, but at a cytogenetic level many of these same sequences are apparently more localized in near terminal regions (see the text and Refs *4* and *5* in Waye above). Waye uses the term "enriched" to describe this while Ref *5* cited by him uses the term "clustering."

Conversely, the high copy number alphoid tandem repeats are localized to centromeres and would rightly be considered satellite DNA. However, at the base sequence level

there is evidence, obtained from cosmid clones of such regions, that sequences other than alphoid are occasionally interspersed within alphoid repeats [*2*]. Of twelve such clones, six contained 20 to 25% non-alphoid DNA interspersed within alphoid DNA.

These examples do not seriously undermine the scheme proposed by Waye but clearly demonstrate its limitations. Indeed the term interspersed is even somewhat inappropriate when referring to LINES and SINES [*1*]. Cytogenetic evidence [*3*] suggests L1 (Kpn) and Alu sequences are separately localized to G and R banding, respectively, in metaphase preparations (chromosome banding refers to alternate light and dark staining along the length of the chromosome induced by different staining techniques).

With regard to issues other than nomenclature, firstly the view that Sau3A sequences are members of the alphoid family rather than a distinct class of repetitive sequences is reasonable (see in particular Ref *10* cited in Waye above). Secondly, the review stated, but did not explain why, restriction fragments associated with alphoid and Satellite III DNA polymorphisms may occur in multiple copy number per haploid genome. This is an appropriate extension in detail (see Ref *4* below for a fuller discussion regarding nomenclature and models by which this might occur).

The citation by Waye of a number of important publications, some of which postdate the review (for example, Refs *3*, *5*, and *16* in Waye above), underscores the activity of research into human genome structure and organization. Among the most active is the advancement of polymerase chain reaction (PCR) technology [*5–10*] and the evaluation of the fidelity of the amplification process itself [*10,11*]. The PCR has been applied predominantly to chromosomal genic sequences [*5–9*]. However, the amplification and analysis of mitochondrial sequences [*12*] (and see Ref *8* in Waye above) as well as tandemly repeating sequences [*13*]—under suitable controlled conditions—are also possible. This makes more feasible the analysis of the small quantities of disintegrated DNA which might typically be found in forensic science specimens.

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Diagnostic/Orientation Errors Using #2 Size Dental Duplication Film

Sir:

In January 1988 (Vol. 33, No. 1) you published my letter about diagnostic errors with the #2 dental periapical size Kodak duplicating film. Reversal of either the original or the duplicate film in the duplicating process would cause the raised dot to be in an incorrect direction causing misorientation as to left or right.

Initially, Eastman Kodak insisted that they received no customer complaints concerning the potential misorientation other than from myself. The letter generated multiple additional complaints. As a result, Eastman Kodak has now implemented modifications of the edge print, the dot, and the packaging insert. The edge print now reads DUP. The dot has been changed to a pin dot which is a smaller embossed dot that actually penetrates the film. A caution has now been added to the packaging insert stating **"THE REVERSAL OF EITHER THE ORIGINAL OR THE DUPLICATE FILM IN THE DUPLICATING PROCESS MAY LEAD TO SUBSEQUENT DIAGNOSTIC ERRORS."**

The DUP edge print and the pin dot modifications will aid diagnosticians in identifying the film as a duplicate and will be a signal to check that the dot is facing the correct way.

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