Perspective

The Utility of DNA Typing in Forensic Work

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AFTER THE DISCOVERY IN 1980 OF A HYPERVARIABLE DNA polymorphism in the human genome (*1*) and the subsequent demonstration that such hypervariability is widespread in humans (*2*), forensic scientists have recognized the potential of DNA typing for identifying a criminal from biological samples left at a crime scene. With the variable number of tandem repeat (VNTR) loci (*3*) currently used, the value of DNA typing as an investigative tool is enormous because an extremely large number of genotypes exists in the population, which yields a high probability of finding different patterns in different individuals. A high probability of different patterns in different individuals means a large chance of excluding a falsely accused individual and small chance of a coincidental match between a DNA profile of a suspect and that in an evidentiary sample. Courts in the United States and England have admitted DNA evidence in criminal and civil litigations (*4*). By 1990 >2000 U.S. court cases in 49 states and the District of Columbia had used DNA tests for such purposes (*4*). In England, civil litigations have used DNA evidence in immigration and paternity dispute cases.

Despite the acceptance of DNA evidence in court cases, there have been criticisms. In this issue of *Science*, Lewontin and Hartl (LH) (*5*) conclude that the use of DNA typing in courtroom applications of forensic genetics must wait for a thorough and extensive sampling of populations, because the "estimates of the probability of a matching DNA profile based on VNTR data, as currently calculated, are unjustified and generally unreliable" (*5*). We believe this claim is incorrect.

Our purpose is to provide a critical appraisal of the principles and basis of DNA typing in a legal setting. In the context of courtroom applications of DNA typing, it is necessary to draw the distinction between exact values and valid estimates. The issue under debate is whether, when a match occurs, a meaningful estimate can be obtained for the frequency of the DNA pattern. An estimate deliberately biased to favor the defendant is acceptable. The existing population data allow valid estimates to be calculated for the large U.S. racial groups. Existing data are sufficient to allow DNA typing of VNTR loci to be used in U.S. courts.

The legal settings of DNA typing. For forensic purposes, it is necessary to determine whether the evidentiary DNA profile matches that of the suspect and if it does, the significance of the match. DNA typing adds a powerful forensic tool, not only because of its high level of discrimination among individuals, but also because it can be done on many sources of materials that are otherwise unusable for traditional blood typings. We focus on the issues surrounding DNA typing in criminal investigations, although their use in civil litigations (for example, to establish or negate biological relatedness) may be supported by the same logic.

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Once DNA typing is done according to strictly established guidelines (*4, 6,* 7), three possible outcomes exist. First, DNA typing may result in inconclusive results, either because of insufficient DNA from the sample or because of technical problems in the test. Second, when the DNA profiles of the relevant samples do not match, the evidence may be declared exculpatory, as observed in the first criminal application of DNA typing (*8*). No population genetic issues arise in interpreting this outcome. In 60 to 65% of criminal cases, DNA typings result in one of these two outcomes. In the cases of exclusions obtained by DNA typing, a substantial fraction would have remained inconclusive without DNA data (*9*).

In the third alternative, when a match is found, the legal question for the defense of the suspect is the following: What is the likelihood that such a match occurs by chance and that the suspect is not linked to the sample? Sometimes, in the defense of a particular suspect, a more restrictive question could also be relevant: Is it likely that there are other individuals in a particular subpopulation who also have the same DNA profile? Although the reference populations to be used in answering these two questions could be different, in reality no precise genetic definition of either population emerges from any legal principle (*10*). We argue that this is the crux of the legal question to which population genetic methods should be applied: The significance of a DNA match should be evaluated in a legal setting (*11, 12*).

Genetic systems used in DNA typing. Any of the more than 2000 defined and catalogued DNA polymorphisms (*13*) could be used in a forensic application, just as blood groups and classical markers have been used for years. The VNTR loci (D1S7, D2S44, D4S139, D10S28, and D17S79, among others) used by the U.S. crime laboratories were chosen for their power of discriminating between genotypes observed in different individuals. However, because some VNTR alleles are similar enough in size that they cannot be distinguished on gels, a "binning" approach (*14*) is used. Although this pooling has been questioned (*5*), the same procedure is used for blood groups and proteins. All A alleles at the ABO locus are not identical at the molecular level; the division of A alleles into subclasses (A_1 and A_2) has long been recognized, and more recent molecular characterization of the ABO polymorphism shows that molecular heterogeneity exists even within these subtyped alleles (*15*). Similarly, studies of amino acid and DNA sequencing have suggested that DNA sequence variation exists among HB*A alleles at the hemoglobin locus and among the isoalleles at the ADH locus (*16, 17*). These show that the definition of allele as a distinguishable type has always been recognized as a technology-defined pool that might be subdivided by other technologies. The theories of population genetics apply, provided a consistent definition is used at a particular level of resolution. Occasionally critics have compared VNTR alleles with phenotypes such as height, weight, eye color, or hair color (*5, 18*). This is misleading because VNTR alleles are discrete entities at every single locus, even if measured with error (*19*).

Most forensic methods use a binning protocol to estimate grouped allele frequencies (*14*). Bins are size classes (in base pairs) of DNA fragments whose class limits are determined by measurement errors and the size standards used in the test. For some VNTR probes, it is likely that numerous alleles of similar sizes will be grouped within the same bin. The binning method was suggested (*14*) as an operational means to obtain conservative (*20*) estimates of the probabilities of DNA profiles. Population genetic methods have been developed to take into account these features of VNTR data gathered by Southern blot (DNA) restriction fragment length polymorphism (RFLP) analysis (*21*). These methods, when applied to VNTR data on population samples of presumably mixed origin, revealed no significant deviation from random combinations of VNTR alleles within individuals. These results and other informa-

tion (*22*) suggest that the computation of match probability by the binning approach generally yields valid, but conservative estimates.

Population genetics of VNTR loci. Extensive studies have demonstrated that VNTR alleles segregate according to Mendelian principles. The only significant difference between VNTR loci and traditional markers is the high mutation rate documented for some VNTR loci (*23*), which could be a problem for paternity cases but not for individual identification (*24*). While the current knowledge of intra- and interpopulation genetic variation for the traditional blood group and protein loci (*25, 26*) is much greater than that of the VNTR loci, simply because the VNTR technology is more recent, the literature on VNTR loci is growing fast (*27, 28*). The features of intra- and interpopulation variation at VNTR loci appear to be congruent with those at traditional genetic markers (*29, 30*). There are only two distinctive features of VNTRs: There are more alleles than at the functional loci previously used in forensics (*23*), and the current technical limits result in a quasicontinuous series of alleles which means that distinguishing among similarly sized alleles can be a problem.

Statistical evaluations of the significance of a match. The evaluation of coincidental match probability must take into account a reference population from which the crime sample was derived. An eyewitness account might indicate the racial background of the criminal but certainly not his or her precise ethnicity. A logical choice of an appropriate reference population should be the one that contains potential perpetrators, determined only by the place and time of occurrence of the crime (*10*). Thus, the reference population is inherently a theoretical construct, probably containing individuals of mixed race or ethnicity (or both) and defined largely by geographic criteria. The question could be asked: Can one compute a valid estimate of the coincidental match probability, and can such an estimate be justified on the basis of population genetic principles? We argue that they can be both computed and justified, and that the current practice provides conservative (*20*) approximations of the relevant frequencies.

The current practice of computing the probability of a DNA profile. Allele frequencies for various single locus probes that are used in criminal investigations have been gathered for populations representing various race, ethnic, and geographic groups (*7, 27, 28*). These data are not collected from homogeneous endogamous groups (*31*); there are subgroups, but these databases are congruent with the operational definitions of reference populations. Frequencies of binned alleles are determined from such databases by simply counting, parallel to the traditional population genetic gene count method (*32*). No assumptions regarding "random mating" or "population substructure" are needed in such computations. In fact, classic population genetic principles show that even if the reference population was a mixed one, these "binned allele frequencies" are unbiased estimates, of the averages of all underlying ethnic or endogamous subgroups contained within the reference population.

The next step is to compute the single-locus profile probability (the probability of finding a given combination of two "binned" alleles in an individual) by means of these estimated binned allele frequencies. Here the Hardy-Weinberg expectation (HWE) principle is used (*32*). Then the single-locus profile probabilities are multiplied to obtain the multiloci profile probability for the entire battery of loci used in the investigation. This last step assumes linkage equilibrium (LE) (such that there is no preferential combination of the alleles). Hence, HWE and LE are assumed even though the reference population is a mixed one. When the technical limitations of generating such data are taken into account, analyses of the data support the use of these assumptions (*21, 22, 27*). This is not equivalent to saying that there is no substructuring within the reference population. It only suggests that even if the subgroups contained in the reference database have significantly different allele frequencies, their effect on deviation from HWE and LE is so small that the effect cannot be detected in practice.

Response to LH's criticism of the probability calculations. Critics have claimed that assuming subpopulations are absent has yielded "flawed" calculations (*5, 18*). Demographic and genetic evidence has been provided to support these arguments. Actually no such assumption is ever made. The data on blood groups provided by LH (*5*) can be used to show that the current practice is valid. Suppose that a crime is committed in an area where inhabitants are of either Polish or Italian descent, but the ethnicity of the perpetrator is unknown. To consider the worst scenario, assume equal numbers of Poles and Italians and that we have a sample of this mixed population to use as a reference population. LH depict the Italians and Poles as having disparate allele frequencies at the ABO, Rh, and, Kell blood group loci [although the frequency differences are actually much smaller (*33*) (Table 1)]. Because ethnicity-specific allele frequency data exist for blood groups, the multiloci genotypic frequencies would be computed in each subgroup (as done by LH, yielding 7.4×10^{-5} for Poles, and 3.0×10^{-7} for Italians). The weighted average of these estimates with equal relative sizes of these subgroups in the reference population yields an estimate of 3.69×10^{-5}, which would be the most adequate estimate of the coincidental multiloci genotypic probability.

Now assume that the ethnicity-specific allele frequencies are not available; only the allele frequencies for the pooled population are available (Table 1). Using them yields a probability, the "admixed" estimate of 1.19×10^{-5}, or only 3.1-fold smaller than the best estimate. Considering the absolute values of these two probabilities (of the order of 12 versus 37 in 1 million), no one would argue that these estimates are substantially different. With some intergroup marriages each generation, increasing proportions of the population will be of ethnically mixed origin. For such individuals, the estimate based on average allele frequencies is even better. In this Italian-Polish example, the frequency of the multiloci genotype (Table 1) for an individual born of an Italian-Polish marriage is 7.58×10^{-6}. This is only 1.6-fold smaller than the "admixed" estimate. Thus, the

Table 1. Effects of allele frequency differences at blood group loci among subpopulations on the estimation of multiloci genotypic frequencies in the pooled population. Notes: The first three columns use the data reported in LH (*5*). The revised data for the Rh locus is from table 4.25, p. 487 of (*25*) for the Poles and from simple averages of 14 listings of Italian samples is taken from table 4.19, p. 438 of (*25*). Revised Kell blood group data is from Table 6.3 (*25*), and the same for the ABO locus are from simple averages of 31 listings on the Poles (pp. 167–168) and 99 listings on Italians (pp. 163–167), both of (*25*). The three-locus heterozygotes genotype probabilities are computed assuming HWE and LE within each population. The mixed population (with an equal mixture of Poles and Italians) would have multiloci genotypic probabilities shown in the "mixed" column; in the parentheses are the estimates calculated from the "mixed" allele frequencies assuming HWE and LE.

Locus allele	Frequencies used by LH (*5*) in			Revised frequencies (*30*) in		
	Poles	Italians	Mixed	Poles	Italians	Mixed
Rh: cDe	0.047	0.0065	0.0268	0.0423	0.0333	0.0378
Cde	0.044	0.015	0.0295	0.0112	0.0196	0.0154
Kell: K	0.058	0.015	0.0365	0.0430	0.0489	0.0460
k	0.942	0.985	0.9635	0.9570	0.9511	0.9540
ABO: A	0.37	0.37	0.37	0.2590	0.2393	0.2492
B	0.22	0.07	0.145	0.1422	0.0814	0.1118
MGP	7.36×10^{-5}	2.98×10^{-7}	3.69×10^{-5}	5.74×10^{-6}	4.73×10^{-6}	5.24×10^{-6}
			(1.19×10^{-5})			(5.69×10^{-6})

current protocol is robust, even when of the allele frequencies are chosen to indicate that the subgroups are genetically well differentiated. The 247-fold difference between the subgroups' multiloci profile frequencies is of no consequence. We disagree with LH that this method is a major drawback of the current practices; we also disagree with their representation of the method (*34*). When more appropriate blood group data are used, the result is even more convincing (Table 1); for instance, the ratio of best to "admixed" estimate, calculated correctly, is 0.92, close to 1. The estimate for a child of a Pole and an Italian is 5.81×10^{-6}, or extremely close to the estimate of 5.69×10^{-6} calculated from average allele frequencies assuming HWE and LE. Clearly, substructuring does not invalidate the estimates.

We also challenge the data on Table 2 of LH (*5*). In most current VNTR allele frequency databases, bins are redefined so that no single bin contains fewer than five observations (7). Several of the bins of LH's Table 2 should have been merged. In addition, there is inaccurate allele sizing in their data (*35*). The correctly sized allele frequency distributions in the French and Israeli populations, after rebinning of alleles, differ from each other by at most a factor of 6.1 [not zero to infinity, as reported in LH (*5*)]. Consequently, the variances of allele frequencies in these two populations are much smaller than the ones obtained from the data of LH, which would make the approximate (mixed) estimate of any D2S44 profile in the mixed population accurate.

Other empirical support of the current protocol of estimating the multiloci genotypic probabilities is provided by Caskey (*36*) who has shown that the multiloci genotypic probability estimates, based on databases exhibiting substantial allele frequency differences, are robust.

The effect of nonrandom mating. We urge the reader not to be misled regarding nonrandom mating and the genetic consequences of the substructure in human populations. In relation to the "narrow sense" definition of random mating (*5*), LH agree that people do mate "at random" with regard to VNTR and blood group types (*5, 18*). However, LH claim that in the broad sense human populations form "endogamous" groups. To support their claim, they cite demographic studies (*37*), which suggest that different U.S. ethnic groups are "largely endogamous" and "the Americans tend to marry the girl or boy next door." The qualifications "tend to" and "largely," however, have significant implications in genotypic probability calculations, because population genetic theory shows that even a small amount of gene migration across ethnic and religious boundaries will quickly homogenize populations (*38*). Both the proportion of marriages of mixed ethnicity (20%) and that of marriages outside the 10-mile radius (67.6%) per generation (*37*) are high. Continued over even two or three generations, these rates must yield substantial homogenization, as shown in the example given earlier of the child of a Pole and an Italian.

Lewontin's (*39*) work is often cited (*5, 18*) as showing that substantial genetic variation exists within the major racial groups. However, when Lewontin's approach is applied to smaller levels of population structure, the majority of genetic differences are still found to be between individuals within villages or parishes rather than among villages or parishes (*40*). These results demonstrate the truism of biological diversity of individuals, even in extremely subdivided groups. The reality of human evolution (*41*) shows that even though marital preference is nonrandom at every level at which one can define populations (*42*), its effect on deviation from HWE of genotypic frequencies or linkage equilibrium is minimal. No new population genetic principles are needed to apply this thesis to forensic DNA typing (*22, 43*).

Generalizations about American marriage practice based on studies done before the "baby boom" are questionable, because such studies do not reflect the extensive mobility and mixing of groups in the general U.S. population that occurred during and following World War II. The present generation of Americans, the group most likely to commit violent crimes, are offspring of this postwar era, which is also an era in which multiple marriages are more frequent.

In summary, American demography for descendants of Caucasian immigrants is closer to a "melting pot" than to a rigid subdivision. Asians, blacks and whites do come closer to clear boundaries between groups. If the demographic and social issues raised by LH were indeed correct, why, then, are blood groups and protein polymorphisms justifiable in forensic work, but not VNTR polymorphisms, as they assert? In fact, the vast literature on blood groups and protein markers has demonstrated that the existing subdivisions within populations do not produce any appreciable departures of single or multiloci genotypic frequencies from the ones predicted with the Hardy-Weinberg and multiplication rules, and all present data on VNTR polymorphisms (*21, 27, 28, 29*) suggest that this also applies to DNA typing.

Consequently, interpretations of the statement that the "statistical tests for Hardy-Weinberg expectation (HWE) are virtually useless as indicators of population substructure" (*5*) are opposite to what LH suggest; it implies that the HWE approximation is appropriate for genotypic probability calculations from allele frequencies. The same logic extends to LE, because the arithmetic and underlying principles are identical; HWE is simply the multiplication rule applied to

Table 2. Effect of population substructure on genotypic probability (single and multiloci) calculations in a mixed population. Hae III–digested DNA fragments were by the fixed bin method (*14*): bins 7, 8, 9, 19, 21, 26, and 31 represent alleles of base pair sizes of 1,197 to 1,352, 1,353 to 1,507, 1,508 to 1,637, 3,330 to 3,674, 3,980 to 4,323, 6,369 to 7,241, and equal or greater than 12,830, respectively. Binned frequencies are from samples of 215 and 213 unrelated Orange County Caucasians and blacks for the D2S44 locus and 217 and 210 Caucasians and blacks for the D2S44 locus and 217 and 210 Caucasians and blacks for the D4S139 locus; (*51*). The values in the "mixed" column assume an equal mixture of Caucasians and blacks. The values are simple averages of the individual racial groups; the numbers in parentheses are the ones computed from the allele frequencies in the mixed sample under the assumption of HWE and LE.

Locus	Binned alleles	Frequencies in		
		Caucasians	Blacks	Mixed
		Allele frequencies		
D2S44	7	0.035	0.106	0.071
	8	0.042	0.075	0.059
	9	0.140	0.070	0.105
	19	0.063	0.026	0.045
D4S139	21	0.025	0.069	0.047
	26	0.189	0.138	0.164
	31	0.104	0.031	0.068
		Single-locus genotypic frequencies		
D2S44	7/8	0.0029	0.0159	0.0094 (0.0084)
D2S44	9/19	0.0176	0.0036	0.0106 (0.0095)
D4S139	21/31	0.0052	0.0043	0.0048 (0.0064)
D4S139	26/31	0.0393	0.0086	0.0240 (0.0223)
		Two-loci genotypic frequencies		
Genotypes		*Caucasians*	*Blacks*	*Mixed*
7/8 and 21/31		1.51×10^{-5}	6.84×10^{-5}	4.17×10^{-5} (5.38×10^{-5})
7/8 and 26/31		1.14×10^{-4}	1.37×10^{-4}	1.25×10^{-4} (1.87×10^{-4})
9/19 and 21/31		9.15×10^{-5}	1.55×10^{-5}	5.35×10^{-5} (6.08×10^{-5})
9/19 and 26/31		6.92×10^{-4}	3.10×10^{-5}	3.61×10^{-4} (2.12×10^{-4})

a single locus. This is not equivalent to saying no substructuring exists within human populations. Substructuring exists everywhere, no matter how the population as a mating unit is defined. However, as the component subpopulations are genetically similar, because of gene exchange almost since the beginning of their evolution (*44*), the net effect of substructuring is trivial.

For the specific case of VNTRs, common VNTR alleles exist throughout diverse populations, and differences are less conspicuous among ethnic populations within racial groups than among racial groups (*27*). We conclude that LH's concern about the inappropriateness of the HWE and multiplication rules has no basis. Although this is evident from the data of Table 1, examples from existing VNTR data may be more convincing. In Table 2, we illustrate a worst-case scenario with data on two VNTR loci (D2S44 and D4S139) applied on the Orange County Sheriff's Department (OCSD), California database (*44a*). Genotypes were selected for binned alleles that depict the largest variation between the Orange County Caucasians and blacks; most bins at these and the other loci showed smaller differences between the two populations. The numerical evaluations (Table 2) show that for the mixed population the actual and estimated genotype frequencies differ, as expected. However, even in this worst case, the estimates, based on averaged bin frequencies and assuming HWE and LE, are excellent approximations. The deviations depend on the magnitude and direction of allele frequency differences in the subgroups, with the greatest effect occurring when both alleles are more common in one subgroup than the other for both loci [for example, those found for the genotype D2S44 9/19 and D4S139 26/31 (Table 2)]. Even in this case, the two loci genotypic frequency predicted by the current protocol (2.12×10^{-4}) does not differ in any meaningful way from that found in the mixed population (3.61×10^{-4}).

The generality of this conclusion may be illustrated with the probabilities of VNTR DNA profiles of 2046 individuals in the Federal Bureau of Investigation (FBI) database (*7, 14*) plotted on a scatter diagram. Each data point in Fig. 1 represents a specific DNA profile and gives the probabilities of this profile (in terms of 1 in x individuals) in the black and Caucasian populations, which we calculated assuming HWE and LE and using the rebinned allele frequencies (*7*). Obviously, specific deviations from the diagonal line represent the effects of (rebinned) allele frequency differences between races, but the message is obvious. In general, the rules are robust, and even if different databases are used, representing statistically significant allele frequency differences, the inference about the rarity of occurrences of each multiloci DNA profile remains virtually unaltered. This, we argue, is the reality of population substructure on DNA profile frequencies.

In the discussion of the origin and genetic makeup of the Hispanics, LH refer to the allele frequency differences among the Karitiana and Surui of Brazil. It is true that Hispanic is a term used for people that have diverse origins. However, the data on Amazon Basin tribes (*28*) are not the only considerations. Indeed, the Karitiana represent an extreme example of a small isolated inbred population. No Hispanic group in North America is so small or inbred; whatever tribal structure did exist among their Amerindian ancestors has long since been broken down (*45*). Although the accumulation of gene diversity among the Amazon Basin tribes is significant (*46*), the entire Amazonian population appears to have sufficient gene flow among populations, so that an equilibrium has been reached, and these populations behave over the long-term as a unit (*47*). Even within the Karitiana sample, which contains many pairs of individuals more closely related than full siblings, there were no two individuals with identical VNTR profiles.

Furthermore, whereas Cubans, Puerto Ricans, and Mexican Americans of Texas or Arizona differ in their admixture composition

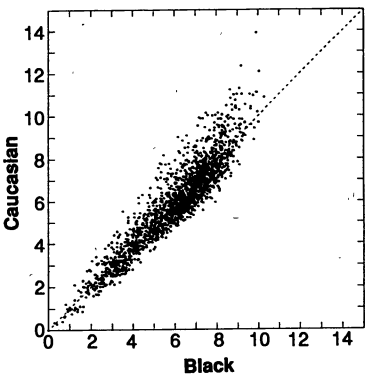

Fig. 1. Scatter plot of estimated probabilities (written as $1/x$, x in log scale) of DNA profiles based on up to four single-locus VNTR probes in 2046 individuals of the FBI database (*50*). Horizontal axis represents the estimated probabilities when rebinned allele frequencies are based on U.S. blacks, whereas the vertical axis represents the estimated probabilities of the same profile with rebinned Caucasian allele frequencies (*7*). The profile of every individual in the database is included in the graph. The points close to the origin represent those DNA profiles based on only one locus. This example was chosen because of all the pairwise database comparisons, this one showed the greatest spread from the diagonal.

and history of origin, these groups internally do not show any deviation from HWE, nor do they show any associations of alleles at different loci (*45*). Genetically admixed populations are not necessarily heterogeneous (*48a*), and the same conclusion is applicable to VNTR loci. Thus, we are not concerned with estimating the frequency of a DNA profile among individuals who have the same ethnic ancestry as a defendant, for example, one-eighth Irish, one-fourth Italian, one-eighth French, one-fourth Polish, and one-fourth Amerindian; no such database will ever exist, nor is it necessary (*48*). In this example, the U.S. Caucasian database and the U.S. Hispanic database from the Southwest will provide conservative estimates that indicate the degree of uncertainty that might exist.

The summary by LH discusses the uncertainty in estimates, a phenomenon common in statistics. We have shown that the uncertainty is far less than depicted by LH, because it is the general frequency in the total population that is desired, not the frequency in the subgroup to which the suspect or defendant belongs. The current practice produces good approximations of the frequency of a DNA profile in a mixed population. The conservative use of bin frequencies rather than allele frequencies and the practice of rebinning to pool the most poorly estimated frequencies into a larger, more reliable estimate assure that most of the frequencies used are overestimates. With multiple loci, the possible errors will more frequently "average out" than reinforce. In contrast, the overestimates used for each allele assure that the final multiloci estimate is greater than the best estimate for the specific genotype.

LH have advocated three solutions to the problems they see; we do not see the necessity of any of them. Their first option is "don't multiply," but use only the database available. Each new profile that has not been seen in the database would have a frequency less than $1/N$, where N is the number of individuals in the database. Although this would be an extremely conservative estimate, it ignores all Mendelian principles. Clearly there are more possible genotypes than can be realized in any moderately sized population and many more than in any sample. The fixed-bin approach (*14*) defines an average of 20 bins per locus, after rebinning, giving 20^4, or 1.94×10^9, possible four-locus profiles. The use of the "don't multiply" approach with a database of a few hundred samples fails to convey adequately the true significance of a match.

The use of ethnic ceilings to fix the current method, as advocated by LH, actually indicates that the current practice is valid, because HWE and LE are to be assumed to compute the relevant profile frequencies within groups. In essence, current procedures are based on a ceiling principle; the assumption is now made by some forensic laboratories that the source of the evidentiary sample is from a specific racial group (say, Caucasian, black, or Hispanics), and respective databases are used for estimating probabilities. The use of

the largest of the compared values provides an additional safeguard beyond those in place for each database alone. Further subdivision within each database is not necessary.

As we have shown both theoretically and with examples, the current procedure does not require "fixing" for it to be used in courts. Of course, it can and will be refined as more data become available. Real examples given above indicate that even if the defendant belongs to a small endogamous subgroup, no meaningful change in the interpretation of a DNA match occurs by using the current data.

Epilogue. Notwithstanding these comments, it must be reiterated that human population geneticists should not take an attitude of complacency with the current state of knowledge of VNTR polymorphisms, nor toward the method of detection of such polymorphisms. New technologies based on polymerase chain reaction (PCR)–based protocols are being suggested for VNTR analyses that are more incisive than simple binning of alleles (*49*). Collection of data in anthropologically well-defined populations is being vigorously pursued by several groups. The present practice of providing conservative estimates of a match probability demonstrates that, when the DNA typing is done with care, matches can provide overwhelming evidence that cannot be coincidental. If DNA evidence is excluded from courtroom applications, the prospect of convicting true criminals, as well as exonerating the falsely accused, will be substantially diminished.

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34. If $p_1, p_2, \ldots, p_k$ and $q_1, q_2, \ldots, q_k$ represent the frequencies of two specific alleles in k subgroups within a population, the frequency of individuals heterozygous for these two alleles in the total population is given by $2\bar{p}\bar{q} + 2\sigma_{pq}$, where $\bar{p} = \Sigma w_i p_i$ and $\bar{q} = \Sigma w_i q_i$ are the allele frequencies in the pooled population, and $\sigma_{pq} = \Sigma w_i(p_i - \bar{p})(q_i - \bar{q})$ is the covariance of allele frequencies across subgroups; w_i is the relative size of the subgroup at position i in the total population, and the summation is over all subgroups. Because $\sigma_{pq} = \Sigma\Sigma w_i w_j(p_i - p_j)(q_i - q_j)$, it is clear that the deviation from HWE due to population substructure is a function of products of allele frequency differences among subgroups; it is not determined by the ratio of allele frequencies across subgroups, as indicated by LH (*5*). The argument for homozygotes is exactly similar, when variances are considered instead of covariances. Therefore, the numerical effect of population substructure is generally negligible.
35. The D2S44 allele frequencies reported in LH (*5*) after correct sizing and re-binning would become 0.035, 0.035, 0.039, 0.086, and 0.039 for the French sample and 0.052, 0.052, 0.034, 0.014, and 0.039 for the Israeli sample for bins 1, 6, 8, 12, and 21, respectively (B. Budowle, personal communication).
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