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The Ceiling Principle Is Not Always Conservative in Assigning Genotype Frequencies for Forensic DNA Testing

To the Editor:

In forensic DNA typing for individual identification, when a suspect's DNA pattern matches that from a crime scene specimen, a crucial step is the assignment of a probability that the specimen genotype would match that of a person randomly selected from the population of potential perpetrators. On the presumption that a suspect is innocent until proved guilty, a method of assigning a probability to a suspect's genotype, given the same genotype from a crime specimen, should be conservative in the sense that the assigned probability should be greater than or equal to the true

probability. A "ceiling principle" has been recommended as a conservative method of providing an upper bound on the true match probability, assuming no laboratory mix-ups (Lander 1991; National Research Council Committee on DNA Technology in Forensic Science [hereafter NRC] 1992, p. 3-13). It is shown here that the ceiling principle does give an upper bound if the product rule applies within and across loci in all genetically differentiated subpopulations. However, a counterexample with correlations across loci is given here in which the ceiling principle gives an estimated "upper bound" that in fact is strictly smaller than the true match probability for every observed genotype. In this case, the ceiling principle exaggerates the power of the evidence to inculpate the suspect and is not conservative. Apparently, examples in which the ceiling principle fails to be conservative have not been described previously. The ceiling principle may be inappropriate for general use in forensic DNA typing unless additional information is available that justifies the use of the product rule within each subpopulation of a genetically heterogeneous population. Alternative methods of estimating a match probability should be explored.

The ceiling principle is presented (NRC, pp. 3-10–3-11) as "a practical and sound approach for accounting for possible population substructure . . . applying the ceiling principle involves two steps: (1) For each allele at each locus, determine a *ceiling frequency* [emphasis in original] that is an upper bound for the allele frequency that is independent of the ethnic background of a subject; and (2) To calculate a genotype frequency, apply the multiplication rule, using the ceiling frequencies for the allele frequencies. [¶] How should ceiling frequencies be determined? . . . The [NRC] committee strongly recommends the following approach: Random samples of 100 persons should be drawn from each of 15–20 populations, each representing a group relatively homogeneous genetically; the largest frequency in any of these populations or 5%, whichever is larger should be taken as the ceiling frequency."

After giving a numerical example of the procedure, the NRC observes (NRC 1992, p. 3-11): "Because the calculation uses an upper bound for each allele frequency, it is believed to be conservative given the available data, even if there are correlations among alleles because of population substructure and even for persons of mixed or unknown ancestry." Further (NRC 1992, p. 3-13), "The calculation is fair to suspects, because the estimated probabilities are likely to be conservative in their incriminating power."

The ceiling principle is indeed conservative if a population contains subpopulations in each of which there is no linkage disequilibrium between loci and in each of which Hardy-Weinberg equilibrium holds within every locus. In this case, the product rule is valid within each subpopulation. Since a product of nonnegative numbers is a monotonically increasing function of each factor, the result given by the ceiling principle necessarily is an upper bound on the probability of any genotype in the population and is therefore conservative.

Without the assumption of independence between alleles, within and between loci, the ceiling principle need not be conservative. For example, consider a population with three subpopulations, S1, S2, and S3. For purposes of intuition, these subpopulations may be thought of as major ethnic groups, but the example is not intended to be realistic. Assume that each subpopulation is ⅓ of the whole population. Suppose a DNA test is performed at three loci—A, B, and C—each of which has exactly three alleles. The three alleles of the A locus are A1, A2, and A3; of the B locus, B1, B2, and B3; and, of the C locus, C1, C2, and C3. Suppose that the alleles at the three loci are so strongly associated that only three haplotypes are found in the population: A1B1C1, A2B2C2, and A3B3C3. These three haplotypes may be called "H1", "H2", and "H3", respectively. (A haplotype such as A1B2C1 is assumed not to occur at all.) Suppose the haplotype frequencies are as shown in table 1 and that any two haplotypes combine at random within each subpopulation to form a genotype. Thus each locus is assumed to be in Hardy-Weinberg equilibrium within each subpopulation. For example, since H1 and H2 occur with

Table 1

Frequencies of Haplotypes in a Hypothetical Population with Three Subpopulations, Three Loci, and Three Alleles at Each Locus

	Frequency for Subpopulation		
Haplotype	S1	S2	S3
A1B1C1	.5	.5	0
A2B2C2	.5	0	.5
A3B3C3	0	.5	.5
All others	0	0	0

frequency .5 in S1, the frequency of the triple heterozygote H1H2 = A1A2B1B2C1C2 genotype in S1 is $2 \times .5 \times .5 = .5$. The factor of 2 allows for the fact that the H1 haplotype may come from the father or the mother. Likewise the frequency of the triple homozygote H1H1 = A1A1B1B1C1C1 genotype in S1 is $.5 \times .5 = .25$.

Now the genotype frequency obtained from the ceiling principle will be compared with the correct genotype frequency. The maximum frequency (over all three subpopulations) of each allele at each locus is .5. Hence the predicted frequency, by the ceiling principle, for each triple heterozygote H1H2, H1H3, and H2H3 is $(2 \times .5 \times .5)^3 = .125$. The predicted frequency, by the ceiling principle, for each triple homozygote H1H1, H2H2, and H3H3 is $(.5 \times .5)^3 = .015625$. These are the only genotypes that can possibly occur in a crime specimen from the hypothetical population constructed in table 1 and therefore are the only genotypes that need be considered.

The actual frequency for each triple heterozygote H1H2, H1H3, and H2H3 is $(1/3) \times .5 = 1/6$, because H1H2 can occur only in S1, H1H3 only in S2, and H2H3 only in S3; the frequency of each triple heterozygote is .5 in the subpopulation in which it occurs, and each subpopulation is 1/3 of the whole population. The actual frequency for each triple homozygote H1H1, H2H2, and H3H3 is $(1/3) \times (.5 \times .5) \times 2 = 1/6$, because each triple homozygote occurs (with frequency $.5 \times .5$) in two subpopulations, each of which is 1/3 of the whole population. Since there are three triple heterozygotes and three triple homozygotes, the actual frequencies of all possible genotypes add up to 1, as they must.

In this example, for every possible genotype that could be found in a crime specimen, the ceiling principle gives a frequency that is lower than the actual frequency. For triple heterozygotes, $.125 < 1/6$. For triple homozygotes, $.015625 < 1/6$. While the ceiling principle gives positive estimates for genotypes other than triple heterozygotes and triple homozygotes, these other genotypes would never be observed in a crime specimen from the hypothetical population and are therefore irrelevant to evaluating the method. In this example, the ceiling principle is uniformly more incriminating than the evidence justifies.

It is not yet clear whether, in practice, the ceiling principle is likely to be conservative or nonconservative. Some evidence presented by Risch and Devlin (1992) apparently favors the applicability of the product rule within very broad ethnic groups, but the population-sampling procedure by which the data base is constructed is not specified and may not correspond in a meaningful way to a random sample of the population of potential crime perpetrators. Further, by matching genotypes, the analysis of Risch and Devlin (1992) did not test the applicability of the product rule *within* each locus, whereas the ceiling principle applies a product rule within (as well as between) loci after taking ceiling frequencies. Moreover, in practice, the FBI used a binning procedure different than that used by Risch and Devlin, and the effect of the actual binning procedure on match probabilities was not studied. Other evidence, extensively reviewed elsewhere (e.g., NRC 1992, pp. 3-6–3-8, and references given there), argues against the applicability of the product rule, within very broad ethnic groups. This evidence on DNA and protein polymorphisms and genetic diseases, which is also controversial, suggests that, within broad ethnic categories, there may exist genetically differentiated subgroups with differing allele frequencies, resulting in an association of alleles at the level of the broad ethnic group or the whole population.

One alternative to the ceiling principle is the counting method—dividing the frequency of the observed genotype in a reference data base by the number of individuals in the data base. Risch and Devlin (1992, p. 720) object "that such an approach is unnecessarily conservative." A second possible approach, suggested by Miron L. Straf (personal communication), is to apply Bonferroni's inequalities (e.g., see Feller 1968, pp. 110 and 142), which make no assumptions of independence within or across loci. The practical application of Bonferroni's inequalities remains to be investigated, as do the general conditions under which the ceiling principle fails to be conservative.

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