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 recom-
mended as a conservative method of providing an up-
per 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 incriminate the suspect and is not
conservative. Apparently, examples in which the ceil-
ing principle fails to be conservative have not been
described previously. The ceiling principle may be
in-
appropriate 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 account-
ing for possible population substructure . . . applying
the ceiling principle involves two steps: (1) For each
allele at each locus, determine a ceiling frequency [em-
phasis in original] that is an upper bound for the allele
frequency that is independent of the ethnic back-
ground of a subject; and (2) To calculate a genotype
frequency, apply the multiplication rule, using the ceil-
ing frequencies for the allele frequencies. [Q] 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 repre-
senting 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 sus-
pects, because the estimated probabilities are likely to
be conservative in their incriminating power."

The ceiling principle is indeed conservative if a pop-
ulation 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 non-
negative numbers is a monotonically increasing func-
tion of each factor, the result given by the ceiling prin-
ciple 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 sub-
population is ½ 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
two 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 subpopu-
lation to form a genotype. Thus each locus is assumed
to be in Hardy-Weinberg equilibrium within each sub-
population. For example, since H1 and H2 occur with

### Table 1

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency for Subpopulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1B1C1</td>
<td>0.5</td>
</tr>
<tr>
<td>A2B2C2</td>
<td>0.5</td>
</tr>
<tr>
<td>A3B3C3</td>
<td>0</td>
</tr>
<tr>
<td>All others</td>
<td>0</td>
</tr>
</tbody>
</table>

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frequency .5 in $S_1$, the frequency of the triple heterozygote $H_1H_2 = A_1A_2B_1B_2C_1C_2$ genotype in $S_1$ is $2 \times .5 \times .5 = .5$. The factor of 2 allows for the fact that the $H_1$ haplotype may come from the father or the mother. Likewise the frequency of the triple homozygote $H_1H_1 = A_1A_1B_1B_1C_1C_1$ genotype in $S_1$ 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 $H_1H_2$, $H_1H_3$, and $H_2H_3$ is $(2 \times .5 \times .5)^3 = .125$. The predicted frequency, by the ceiling principle, for each triple homozygote $H_1H_1$, $H_2H_2$, and $H_3H_3$ 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 $H_1H_2$, $H_1H_3$, and $H_2H_3$ is $(\frac{1}{3}) \times .5 = \frac{1}{6}$, because $H_1H_2$ can occur only in $S_1$, $H_1H_3$ only in $S_2$, and $H_2H_3$ only in $S_3$; the frequency of each triple heterozygote is .5 in the subpopulation in which it occurs, and each subpopulation is $\frac{1}{3}$ of the whole population. The actual frequency for each triple homozygote $H_1H_1$, $H_2H_2$, and $H_3H_3$ is $(\frac{1}{3}) \times (.5 \times .5) \times 2 = \frac{1}{6}$, because each triple homozygote occurs (with frequency $\frac{1}{3} \times .5$) in two subpopulations, each of which is $\frac{1}{3}$ 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, $.125 < \frac{1}{6}$. For triple homozygotes, $.015625 < \frac{1}{6}$. 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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Joel E. Cohen
Rockefeller University
New York
References


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