CORRESPONDENCE

DNA Database Size

Sir:

The Technical Working Group on DNA Analysis Methods (TWGDAM), in its Guidelines for a Quality Assurance Program for DNA Analysis (1), requires population databases to be established in different racial and ethnic groups as part of the validation process of each DNA loci, but there is no guidance given on how big a database should be.

The size of a database is, however, of real interest to laboratory managers introducing new DNA typing systems into casework. In these situations a balance must be achieved between development costs, the desire to begin reporting new systems as soon as possible, and the need to know that reliable estimates of allele frequencies are being achieved. Obviously, the larger the number of samples the more definitive the population data, but large numbers are costly in terms of both time and reagents.

There is no clear guidance in the literature on the question of how large a database should be, possibly because there is no simple answer (2). Published populations for STR loci are generally of the order of either 100 or 200, but sometimes smaller numbers are reported for sub-populations. The International Society of Forensic Haemogenetics has recommended that 100 persons are sufficient (3), but no basis for this size is given and the number may well be a carry-over from what was considered adequate (4) for polymorphic protein systems. The Committee on DNA Technology in Forensic Science, when originally reporting the ceiling principle, also suggested databases of 100 (5). More recently, though, in describing databases generally the Committee suggests at least a few (or several) hundred persons (6). Several authors (Lander (7) and Devlin et al. (8,9) for example) have, however, argued that a sample of 100 individuals is too small. Lander, moreover, has suggested that even a database of 500 is too small although he has now given qualified support (10) for a database of 100 individuals. Weir (11) also has implied that samples of 100 individuals are too small because tests for independence of allele frequencies will have low power. Nevertheless, some practical support for a sample size of 100 was shown by Pacek et al. (12) in a study which compared allele frequencies obtained from individuals to allele frequencies of pooled blood samples.

We propose here a simple and graphical approach which we believe will help demonstrate when a database has reached a suitable operational size. The basis of the approach is to plot the frequency of each allele at a locus against the number of people in the database as the database is developed.

To demonstrate the procedure, DNA samples were obtained from blood samples collected from unrelated persons in South Australia and amplified at four STR loci. The amplified samples were analyzed by electrophoresis on a Model 373A DNA Sequencer using Genescan® 672 analysis software version 1.2 (Applied Biosystems, Foster City, CA, USA). Allele frequencies were calculated for each allele sequentially and progressively in groups of 10; that is, the frequency was calculated for each allele for the first 10 individuals tested, then for the first 20 individuals, then the first 30, and so on, and plotted against the number of individuals. The plots for HUMVWFA31 and HUMFES/FPS are shown to illustrate the process (Fig. 1).

It can be seen from the figures that the plotted frequencies show considerable variation for low population numbers, with the more common alleles generally showing the largest changes; some alleles show a general increase in frequency, whilst others show a decrease. As the database grows in size, however, the plots settle down to more or less horizontal lines indicating a population size above which the allele frequencies will not change greatly as more samples are added to the database. This then represents the population size which appears to be big enough to begin offering reliable estimates of allele frequencies. Inspection of the graphs presented shows that the minimum number of samples required to reach this point varies according to the system. Thus about 100 people are sufficient to form the database for HUMFES/FPS with the population studied, whereas for HUMVWFA31 the minimum number of samples would be approximately 170 to 180 (that is, about 200 would be appropriate).

It is important to note that this method can not be used to predict the number of samples required and neither can it be used to say that all possible alleles have been detected. Rather, it can only be used to assess frequencies already determined. Note also that the process has to be applied to each typing system in each population under study. This method should not be used to supplant sophisticated statistical methods such as those of Chakraborty (13) for determining sample size nor the recommendations of the 1996 NRC report (6) for testing population structure, but we believe it does provide a useful preliminary tool that can be used in the operational laboratory as an adjunct to these more rigorous procedures. Furthermore, the simplicity of the approach and the very visual nature of the results makes this a valuable method that can also be used for demonstrating to juries or to legal counsel that a database used in reporting is indeed large enough to provide reliable estimates of allele frequencies and that the addition of further samples will not change the frequencies greatly.

We acknowledge Andrew Dinan for his assistance with the production of the graphs and the (Australian) National Institute of Forensic Science for supporting Dr Swanson during the work. We especially thank Dr Bruce Weir for his encouragement and critical comment.

Harry WJ Harding, Ph.D.*
Principal Forensic Scientist
Rhett Swanson, Ph.D.*
Senior Forensic Scientist
Forensic Science
Adelaide, South Australia 5000
Department of Microbiology and Immunology
*Present address:
Department of Microbiology and Immunology
University of Adelaide
Adelaide, South Australia 5005

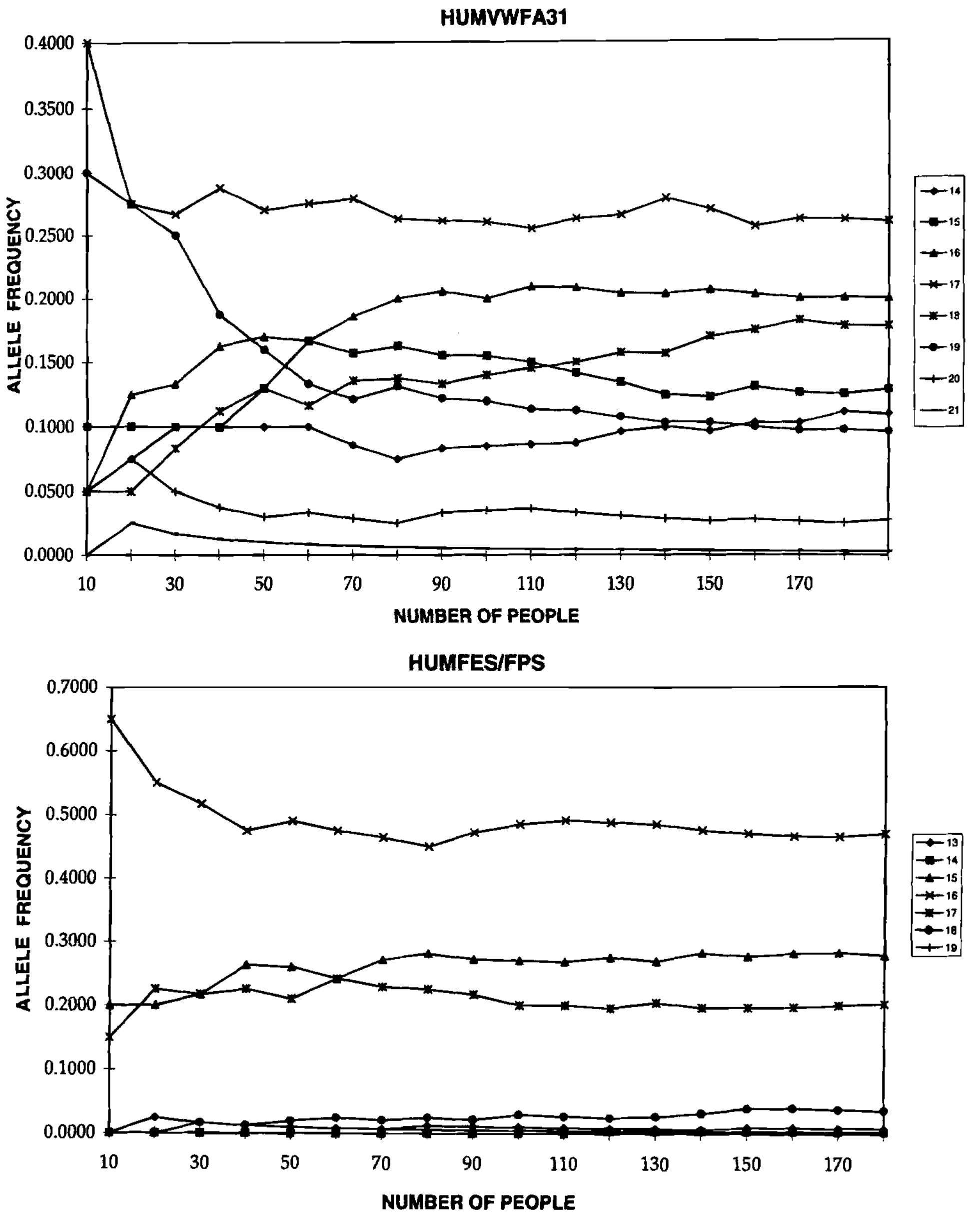

FIG. 1—*Allele frequency vs number of people plots for HUMVFWA31 and HUMFES/FPS.*

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Non-Amplification of a vWA Allele

Sir:

While typing 600 population samples as part of Phase 3 of the FBI's STR Standardization Project, our laboratories (NIST and VA DFS) encountered an African American sample that typed differently at the vWA locus depending on the primers used. The two STR kits initially used were Promega Corp. Powerplex™ (lot #72792) and Perkin-Elmer-Applied Biosystems Inc. (PE-ABI) AmpF1STR Blue™ (lot #A7F005). Both multiplex systems amplify the vWA locus. Using the vWA locus to verify the typing data, this sample typed 16, 19 using Powerplex™ and 16, 16 with AmpF1STR Blue™. Both laboratories repeated the typing procedures using new bloodstain cuttings from the original sample. Results of the test were the same. Subsequently this sample was amplified with monoplex vWA primers from both Promega and PE-ABI. Both vWA monoplex kits typed the sample as 16, 19. The PE-ABI monoplex vWA primers were designed to amplify a shorter product than the vWA primers of the AmpF1STR Blue™ multiplex system. Therefore, these primers do not bind to the same location on the genome. PE-ABI is aware of this problem and they are actively pursuing an explanation for this allelic dropout by sequencing the sample.

We would like to know if other laboratories have seen a similar phenomenon with other samples so that we can establish the frequency of this occurrence. We realize that probably few laboratories perform duplicate typing at the same locus using different primer sets. Laboratories should be aware of the existence of this potential problem when exchanging samples and/or data for this locus. One way to circumvent the problem would be to re-test samples that show apparent homozygosity by using alternative primers.

Margaret C. Kline
Research Biologist
DNA Technologies Group
National Institute of Standards and Technology
Gaithersburg, MD 20899
Brad Jenkins
Forensic Scientist Senior
Stephen Rodgers
Analytical Chemist
Department of Criminal Justice Services
Virginia Division of Forensic Science
1 N. 14th Street
Richmond, VA 23219

Commentary on Dawid AP and Evett IW. Using a graphical method to assist the evaluation of complicated patterns of evidence. J Forensic Sci 1997 Mar; 42(2):226–31.

Sir:

Probabilistic Expert Systems (PES) are popular applications for modern computers' facility for massaging great quantities of data with relative speed and accuracy. The ease with which complex statistical analyses are computed has led to a misconception that data manipulation can somehow compensate for substandard forensic field work, inadequate data quality controls or illogical analyses.

Probabilistic analyses assess the likelihood of something happening, most often inferring future occurrence from known historical data or conjecture. On the other hand, forensic science most often concerns itself with retrospective analyses of ***that which has already happened.*** The challenge to the forensic scientist is not to find Dawid and Evett's "... all-important likelihood ratio between the defense and prosecution propositions ...", but to find out ***what happened.*** Whereas attorneys and theoreticians may engage juries in statistical disputes (at their peril), forensic field work is accomplished in a binary world: events which have happened have a probability of 1.0, and the myriad possibilities which did not occur have a probability of ***zero.***

The unsuitability of attempting to apply predictive methodology retrospectively has been chronicled in various rebuttals of conventional investigatorial wisdom (1–5 *inter alia*). A common thread throughout these expositions of failed predictions and botched investigations is the uncritical acceptance of unverified false assumptions, leading to the adoption of "retrospective fallacies" to which subjective investigations fall prey (5).

A glaring deficiency of most attempts to apply PES retrospectively is the absence of valid methodology for testing data quality and assuring analytical logic. *E.g.,* In Dawid and Evett's fictional exemplar, they "... regard *G2* as truthful evidence of the facts it reports, and uninformative about anything else...." (p. 227), apparently assuming that similar credence can be granted in real life. This offhand treatment of the necessity for data verity can easily lead an eager client-oriented forensic investigator or attorney to believe that statistical legerdemain may prove appropriate remedy for factual insufficiency.

Likewise, Dawid and Evett shortcut a substantial complication by "... simplify[ing] things to assume that the guard's evidence with regard to the number of offenders ... is completely reliable." (p. 228) This is quite handy, yet ignores both the reality of eyewitness inaccuracy and the statistical inconvenience that for each increase in the number of independent variables, the possible permutations of dependency increase factorially; *i.e.,* by N!. Although our friendly computer nerds can easily include the additional computations in their programming, each broadening of the base introduces new opportunities for data error unless robust quality controls are invoked.

The inherent pitfall of Dawid and Evett's proposed methodology is summed up in their comment: "Note that this probability (like all others considered) is implicitly conditional on all the remaining evidence in the case, which will affect [the constant of proportionality]." (p. 230) In other words, one significant piece of bad data, or invalid assumption, will poison the well. Yet the authors address neither verification nor validation of the data, but appear to accept their purity uncritically. Neither do they acknowledge a possibility that data might be missing, or apply any tests to substantiate the logic applied to the data analysis.

Starting with Wigmore (1937), most of Dawid and Evett's references relate to evaluating evidence jurisprudentially, within the context of litigation, rather than scientifically, within the context of truth (6). In acknowledging that forensic science supports litigation, it is even more important to remember that "forensic" is an adjective; "science" is the noun. Nordby addressed the issue succinctly in his 1992 paper on expert disagreement (7). What the seeker finds too often depends on that which is sought.

One must not diminish the rigor of scientific inquiry to meet the whims of adversarial jurisprudential combat. Successful forensic investigations result when investigation customers demand to know ***what happened,*** and investigators persevere to prove the unique confluence of events which produced the outcome under study.

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Ira J. Rimson, P.E.
Forensic Engineer
P.O. Box 11008
Albuquerque, NM 87192

Authors' Response

Sir:

Ira Rimson's comments on our paper display some fundamental misunderstandings, combined with misguided distortions of our purpose. Nowhere do we, nor would we, suggest that our methods can in any way compensate for substandard work. Although our focus is not specifically on the issue of data quality, that is no justification to imply that we must therefore consider it to be unimportant or irrelevant. On the contrary, the better the evidence, the better will be any inference drawn. Likewise, the fact that we choose a particular example, reasonably complex to indicate the scope of our methods but, for illustrative purposes, not over-complex, and that we then make further simplifying assumptions to streamline the exposition, cannot be taken as serious criticisms of our approach. But in any case, we are not proposing that graphical analyses such as ours be presented in evidence in court; rather, like Wigmore's chart method, they are intended to guide the forensic scientist in understanding and clarifying his own view of the relationships between different items of evidence, and in assessing their combined effect.

Rimson's immature interpretation of probability leads him to conjure up problems where none exist. Probability is the quantitative measure of uncertainty. We can be uncertain about past events just as much as about future ones, and consequently there is no difficulty in assigning them probabilities different from 0 and 1. But our uncertainty, in turn, must depend on our evidence, as well as on our individual prior uncertainty, before that evidence was taken into account. This understanding of Probability, as simultaneously evidence-based and subjective, forms the basis of the modern Bayesian approach to inference from data—which is just the problem we face in the forensic context. For further background on applications of probability to evidence interpretation, see the Appendix to the book "Analysis of Evidence," by T. Anderson and W. Twining (1991); Little, Brown and Co: Boston, Toronto, London.

In an ideal world the evidence would lead to logically certain and uncontestable conclusions. But in general this is not possible, and no amount of wishful thinking can then make it possible: Rimson may wish and believe it were otherwise, but the world of forensic fieldwork is very far from being binary. Then the residual uncertainty in the light of the evidence must be recognized and assessed. Moral, or practical, or legal certainty are appropriate only when this uncertainty is judged to be suitably tiny. Our work is aimed at simplifying the complex and subtle task of assessing the appropriate (necessarily subjective, though by no means arbitrary) uncertainty. We are frankly amazed that this vital task should engender such a reactionary and unhelpful response.

In conclusion, we can do no better than quote the final sentence of the letter of John I. Thornton (The DNA Statistical Paradigm vs. Everything Else) in the July 1997 issue of the Journal:

"To master statistical models to explain much of our evidence may be a slow, reluctant march through enemy territory, but we must begin to plan for that campaign."

We agree entirely with Dr. Thornton.

A. Philip Dawid
Professor
Department of Statistical Science
University College
London, UK
Ian W. Evett
Head of Interpretation Research
Forensic Science Service
London, UK.

Commentary on Huston, Germaniuk HB, Sidler AK. Three cases of fatal firearm use following external hinge removal from locked gun cabinets. J Forensic Sci 1997 Sep;42(5):956–7.

Sir:

Drs. Huston, Germaniuk, and Sidler make an excellent case for more secure gun cabinets, if more restricted access to the firearms is the main purpose of the cabinet. Obviously, for instance, if the firearms are in the cabinet for display purposes, eliminating the glass or decorative hinges would be counter to the purpose of the owner.

Although any case of death may be considered tragic, this article's statement that "firearms in the home are much more likely to cause the death of a family member, an acquaintance, or the firearm owner themselves rather than an intruder" is in error, as shown by numerous studies that have revealed the flawed methodology of the references quoted.

To be fair, in reality, firearm usage involves a benefit risk analysis on the part of the user.

The National Safety Council estimates that the fatal firearm accident rate fell to an all-time low in 1995. The new rate of 0.5 per 100,000 represents an 85% decrease from the high water mark registered in 1904 and is well below the incidence of fatal motor vehicle accidents (16.7), falls (4.8), poisoning (4.0), drowning (1.7), fire (1.6), or accidental choking (1.1).

The annual number of fatal firearm accidents has fallen to an all-time low even as the population has doubled and the number of firearms owned has quadrupled since the reference year of 1930.

The BATF now reports that some 65,000,000 U.S. citizens lawfully own firearms, that 11% of these owners report using a firearm successfully for self defense, and that actual criminal gun use involves less than 0.2% of all firearms.

Obviously, the benefit of responsible firearm ownership far outweighs the risk.

The 1990 Harvard Medical Practice Study of inpatient deaths from physician negligence in New York State, extrapolated to the country as a whole, reveals approximately 180,000 people die each year partly as a result of iatrogenic injury, nearly five times the number of Americans killed with guns. One might fairly conclude from such an analysis that doctors are a deadly public menace.

Why do we not reach that conclusion? Because, in balance, doctors save many more lives than they take, and so it is with firearms.

Daniel L. Orr II, D.D.S., Ph.D., J.D.
Clinical Professor of Surgery
University of NV Medical School
Forensic Odontologist
Clark County, NV, Coroner
DLOII/NP
Medical Education Bldg.
2040 W. Charleston Blvd.
Las Vegas, NV 89102

Authors' Response

Sir:

Dr. Orr's comments are appreciated. We hope that the major point of our article was not lost. Our paper points out a flaw in the current construction of gun cabinets and that firearm owners who think their weapons are securely locked may be wrong. They may not even have considered the possibility that someone would think of removing external hinges to obtain a weapon to commit a crime in their home. Also, it is not obvious that displaying firearms and a well constructed gun cabinet are mutually exclusive. Shatter-proof glass and internal hinges can be used in all gun cabinets regardless of the primary purpose of the cabinet. A cabinet can be both decorative and safe.

The remainder of Dr. Orr's comments concern firearms and accidental deaths. None of the cases presented in our paper were accidental, two cases were suicides and one case was a double homicide. Statistics concerning firearms fatalities are controversial. The statistics that Dr. Orr presents may be misleading. Accidental deaths may have decreased for a variety of reasons including better medical management of gunshot victims and increased use of proper gun storage methods since 1904. However, homicides and suicides continue to increase each year. And the number of non-lifethreatening injuries has also increased causing billions of dollars in health care expenses each year.

There are many reports stating the risk of firearms in the home, especially in homes with children (1–11). Therefore, we advocate that firearms be kept in combination locked, tamper proof handgun vaults or cabinets with internal door hinges. Children should not be allowed access to the combination. Any death that can be potentially prevented, whether it be homicide, suicide or accident should be avoided. A single preventable death is one death too many. Why should we be content with the current number of fatalities (no matter what statistics are chosen) when we can continually try to decrease violent deaths in this country?

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Butch M. Huston, M.D.[1]
Humphrey D. Germaniuk, M.D.[2]
Angela K. Sidler, M.D.[3]

[1]Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC.
[2]Chief Medical Examiner, Office of the Chief Medical Examiner, Washington, DC.
[3]Clinical Assistant Professor, Department of Pediatrics, University of North Carolina, Chapel Hill, NC.