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The Efficiency of Multilocus DNA Fingerprint Probes for Individualization and Establishment of Family Relationships, Determined from Extensive Casework

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Summary

The properties of human DNA fingerprints detected by multilocus minisatellite probes 33.6 and 33.15 have been investigated in 36 large sibships and in 1,702 Caucasian paternity cases involving the analysis of over 180,000 DNA fingerprint bands. The degree of overlap of minisatellite loci detected by these two probes is shown to be negligible (~1%), and the resulting DNA fingerprints are therefore derived from independent sets of hypervariable loci. The level of allelism and linkage between different hypervariable DNA fragments scored with these probes is also low, implying substantial statistical independence of DNA fragments. Variation between the DNA fingerprints of different individuals indicates that the probability of chance identity is very low ($<<10^{-7}$ per probe). Empirical observations and theoretical considerations both indicate that genetic heterogeneity between subpopulations is unlikely to affect substantially the statistical evaluation of DNA fingerprints, at least among Caucasians. In paternity analysis, the proportion of nonmaternal DNA fragments in a child which cannot be attributed to the alleged father is shown to be an efficient statistic for distinguishing fathers from nonfathers, even in the presence of minisatellite mutation. Band-sharing estimates between a claimed parent and a child can also distinguish paternity from nonpaternity, though with less efficiency than comparison of a trio of mother, child, and alleged father.

Introduction

DNA typing provides a powerful tool for the establishment of associations or exclusions between forensic specimens and criminal suspects and for the determination of family relationships in, for example, paternity cases and immigration disputes. Numerous DNA-based test systems directly or potentially applicable to such investigations have now been developed, including multilocus minisatellite probes (Jeffreys et al. 1985*b*, 1985*c*; Ali et al. 1986; Vassart et al. 1987; Fowler et al. 1988; Georges et al. 1988) and single-locus minisatellite or VNTR probes (Nakamura et al. 1987; Wong et al. 1987). Amplification of DNA by PCR (Mullis et al. 1986; Saiki et al. 1988) has been applied to informative marker systems such as minisatellites (Jeffreys et al. 1988*b*, 1990*b*; Boerwinkle et al. 1989; Horn et al. 1989), simple dinucleotide repeats, or "microsatellites" (Litt and Luty 1989; Tautz 1989; Weber and May 1989), HLA loci (Saiki et al. 1986; Gyllensten and Erlich 1988), and mtDNA (Wrischnik et al. 1987; Higuchi et al. 1988), and in principle raises the possibility of individualization at the level of a single cell (Jeffreys et al. 1988*b*; Li et al. 1988).

Of all of these marker systems, the greatest individual specificity per analysis is provided by multilocus DNA fingerprint probes (Jeffreys et al. 1985*b*, 1985*c*). Two probes termed 33.6 and 33.15 have been developed which have been extensively applied to civil and criminal casework (Gill and Werrett 1987; Helminen et al. 1988) and to the resolution of immigration disputes (Jeffreys et al. 1985*a*; Home Office 1988). These probes each consist of a naturally occurring human

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minisatellite located on chromosomes 1cen-q24 and 7q31.3-qter, respectively (Jeffreys et al. 1985*b*, 1990*a*). These probes hybridize to multiple variable minisatellite fragments in human DNA to produce a highly individual-specific DNA fingerprint (Jeffreys et al. 1985*b*, 1985*c*). Analysis of two large sibships has shown that there is little if any overlap in the hypervariable loci detected in these families by probes 33.6 and 33.15, and that most of the resolved DNA fingerprint fragments detected by each probe assort independently in offspring (Jeffreys et al. 1986). Similar conclusions were obtained from another multigeneration pedigree analysis in Mormons (Smith et al. 1990). Substantial genomic dispersal of the loci detected has been confirmed by analysis of individual hypervariable minisatellite loci cloned from human DNA fingerprints (Wong et al. 1987; Royle et al. 1988; Armour et al. 1989*b*, 1990).

The limited data published to date, while indicating the extreme statistical resolving power of DNA fingerprints, do not address a number of points of concern. These include the degree of independence of the DNA fingerprints detected by probes 33.6 and 33.15, the validity of the assumption of band independence in DNA fingerprints, the potential problems of individual variation in band number and of relatively invariant human subpopulations, and the effect of minisatellite mutation (Jeffreys et al. 1985*b*, 1988*a*) on parentage testing. We now present a review of 1,702 cases of disputed paternity which have been analyzed using these probes, and show that such large-sample analyses can provide novel information about the relevance of these concerns.

Material and Methods

The data set consists of all the Caucasian paternity cases received at the U.K. laboratory of Cellmark Diagnostics between September 1988 and September 1989. The only preselection of data for this study was that of ethnicity, which was determined on the basis of photographic evidence. The paternity cases came from all areas of the United Kingdom and abroad. In addition, 36 families of Asian (Indian subcontinent) origin with at least five bona fide children established by DNA fingerprinting were used to investigate the issues of band linkage and allelism. Blood samples from the mother, child(ren), and alleged father were taken principally by hospital hematologists and forwarded to Cellmark.

For each case, DNA was extracted, digested to completion with *Hin*fI, and duplicate 2–4 μg samples electrophoresed in the order mother–child(ren)–alleged father in a 0.7% agarose gel, until a 2.3-kb marker DNA fragment had migrated 20 cm from the gel origin on control tracks in each gel. DNA was transferred by blotting to a Hybond-N membrane (Amersham), and duplicate Southern blots hybridized with ^{32}P-labeled probes 33.6 and 33.15. Full details of procedures used are given elsewhere (Smith et al. 1990), except that 80 ng of probe were used per labeling reaction.

The autoradiograph for each family group was scored by eye. The total numbers of bands in the mother, alleged father, and each child were scored for DNA fragments larger than 3.5 kb. Bands which clearly did not align between two individuals were also noted, and pairs of bands which failed this criterion were scored as "shared" bands, even if minor misalignment and/or difference in hybridization intensity indicated that the shared bands were not identical. Repeat measurements on pairs of bands scored as "mismatches" showed band centers more than 0.5 mm apart. Shared bands therefore correspond to bands with centers less than 0.5 mm apart, irrespective of hybridization intensity. False mismatches due to electrophoretic band shift, which rarely occurred, were identified by visual comparison of the profile of the mother, alleged father, and children and, for most cases, by rehybridization of the Southern blots with a cocktail of single-locus minisatellite probes MS1 plus MS31, and MS8 plus MS43A plus pλg3 (Wong et al. 1987) (see fig. 1). Any case showing evidence of band shift was subjected to complete reisolation and reanalysis of DNA. From these analyses, the number of bands shared by the mother and alleged father, by the mother and child, and by the alleged father and child were also recorded. The autoradiographs and band-scoring data were checked by a second assessor. If any discrepancies arose, the data were fully reanalyzed by a third independent assessor and if necessary a full retest was performed. All data were recorded in an ASCII file and converted to Lotus 123 spreadsheets for analysis. For families with more than one child, the data from only one child selected at random were used in most analyses performed, giving a total of 1,702 different trios of mother, child, and alleged father.

Results

The Paternity Dispute Data Set

We analyzed 1,702 Caucasian paternity cases referred to Cellmark Diagnostics by DNA fingerprinting

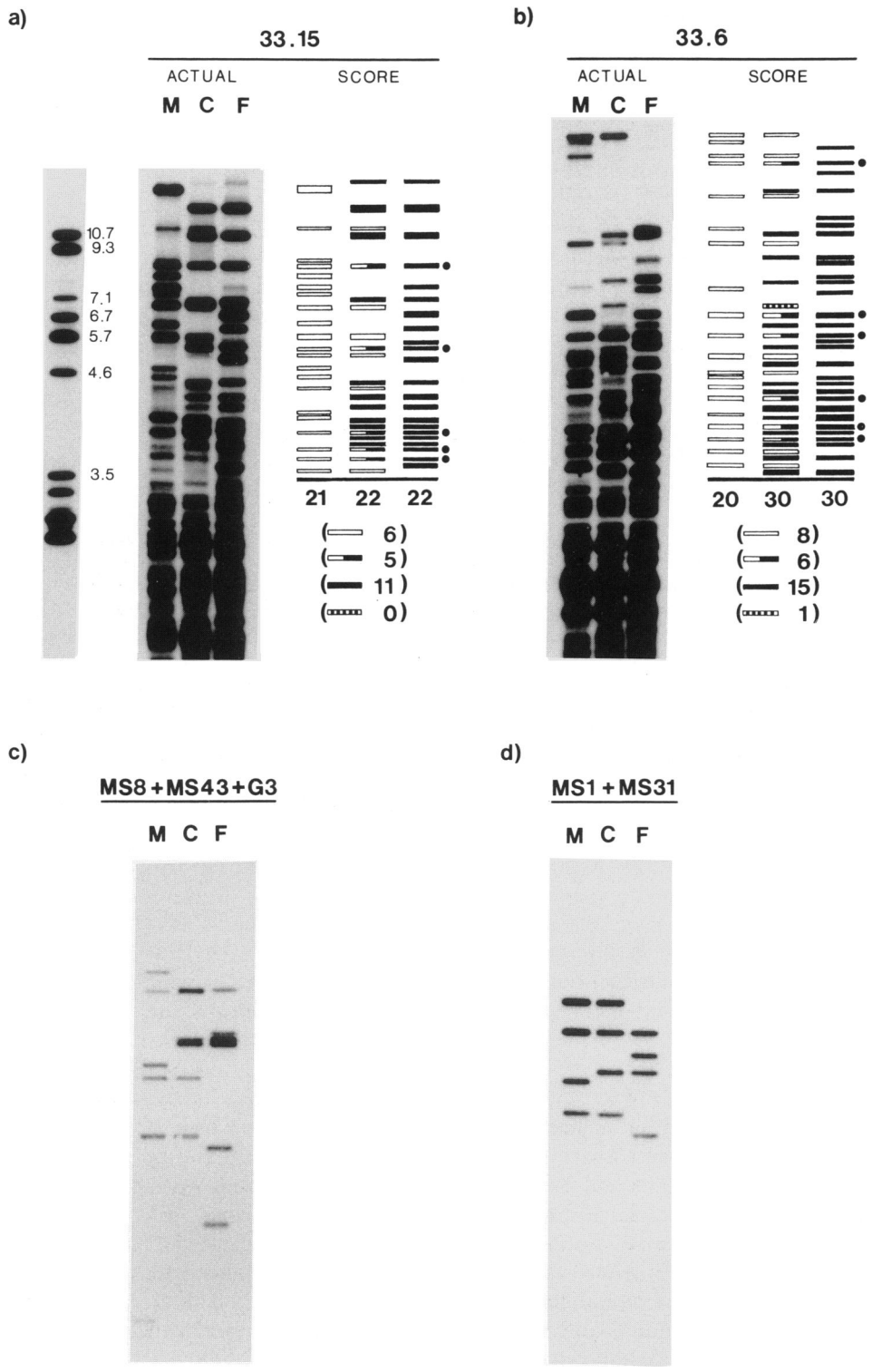

Figure 1 Example of a paternity case analysis. DNA fingerprints were produced from *Hin*fI-digested genomic DNA prepared from the mother (M), child (C), and alleged father (F) using multilocus probes 33.15 (*a*) or 33.6 (*b*), as described in Material and Methods. Molecular weight markers for 33.15 are restriction digests of single-locus minisatellite clones that are detected by the 33.15 probe. Sizes are in kilobases. A corresponding set of markers detected by probes 33.6 was also used but not shown. The scoring analysis of the DNA fingerprints produced by each probe is shown schematically. ▭, Maternal band; ▬, paternal band; ▭▬, child band from either or both parents; ●, bands "shared" between parents; ▪▪▪▪, unassignable offspring band. Band scoring is limited to band >3.5 kb. Some faintly hybridizing bands scored on the original autoradiographs are not visible in these reproductions. Total number of bands scored in each individual are given, together with details

Table 1

Summary of DNA Fingerprint Data from 1,702 Paternity Cases

	Probe 33.6	Probe 33.15
In mother, child, and alleged father:		
Mean no. of bands in alleged father, n_F (±SD)	17.91 ± 3.57	16.99 ± 3.41
Mean no. of bands in mother, n_M (±SD)	18.22 ± 3.59	17.15 ± 3.28
Mean no. of shared bands, s (±SD)	2.63 ± 1.71	2.37 ± 1.71
Mean band-sharing frequency between mother and alleged father, x (±SD)[a]	.144 ± .088	.137 ± .095
Mean band-sharing frequency between mother and child (±SD)	.573 ± .093	.567 ± .093
Expected band-sharing frequency between mother and child[b]	.555	.553
In child:		
Mean no. of paternal bands (±SD)	8.08 ± 2.47	7.58 ± 2.22
Mean no. of maternal bands (±SD)	8.11 ± 2.47	7.67 ± 2.29
Mean no. of bands common to father and mother (±SD)	2.48 ± 1.58	2.22 ± 1.57
Mean no. of unassignable mutant bands	.098	.195
Mutation frequency/offspring band[c]	.0052	.0110
(95% confidence limits)	(.0044–.0062)	(.0098–.0124)

NOTE.—Data on band numbers and band sharing are derived from the full set of 1,702 paternity cases, irrespective of whether paternity was correct. In total, 181,430 multilocus probe bands >3.5 kb were recorded. Data on offspring bands and mutation frequencies were taken from the set of 1,419 cases in which paternity was established; the remaining 283 cases in which nonpaternity was established were ignored in this analysis.

[a] Determined by $2s/(n_F + n_M)$.

[b] Estimated from the assumption that at steady state the mean number of DNA fingerprint bands does not change from generation to generation, and that each parental band has a uniform probability, t, of being transmitted to a child. A child will therefore inherit $t(n_F - s)$ bands specifically from the father, $t(n_M - s)$ bands from the mother, and $s(2t - t^2)$ shared bands from the mother and/or father. At steady state, the number of bands in mother, father, and child will all be equal to the mean number n of bands per individual, and thus $2t(n - s) + s(2t - t^2) = n$. If x is the mean level of band sharing between "unrelated" individuals (e.g., father compared with mother), then $s = nx$, whence it can be shown that $t = [1 - (1 - x)^{1/2}]/x$. From this, the expected level of band sharing between mother and child can be shown to be $[2x - 1 + (1 - x)^{3/2}]/x$.

[c] Mutation frequency is given by the observed proportion μ_o of offspring bands which cannot be assigned to either parent, and will slightly underestimate the total proportion μ_t of mutant bands due to instances of new mutant bands comigrating with other offspring bands. Since the probability of comigration is x, μ_t can be estimated as $\mu_o/(1 - x) = 1.16\mu_o$.

with multilocus minisatellite probes 33.6 and 33.15 (as well as, in most cases, with single-locus minisatellite probes MS1, MS8, MS31, MS43A, and pλg3 (Wong et al. 1987). Typical results for a case in which true paternity was deduced are shown in fig. 1. Information on band numbers and band sharing was derived for DNA fragments larger than 3.5 kb, corresponding to the well-resolved region of the autoradiograph. The criterion for the identification of shared bands is described in Material and Methods. Below 3.5 kb, the density of DNA fragments becomes too high for the reliable scoring of bands (Jeffreys et al. 1985*c*; Gill et al. 1987), and data from this region were not included in the following analyses. Correct maternity was not assumed in casework evaluation, but was established in all 1,702 cases. Paternity was established in 1,419 cases (83.4%) and excluded in the remaining 283 cases (16.6%) (see below). The basic DNA fingerprint parameters (band numbers, band sharing, mutation frequencies) are summarized in table 1.

Individual Variation in DNA Fingerprint Band Numbers

To investigate in more detail variation between individuals in the numbers of bands scored with probes 33.6 and 33.15, the frequency distributions of band

of the band composition of the child. *c*, Hybridization results using cocktail of single-locus minisatellite probes MS8, pλg3, and MS43A (Wong et al. 1987). Up to six hybridizing bands per individual are detected, as expected, with no evidence for paternal exclusion. The locus detected by MS43A does not contribute bands to a *Hinf*I DNA fingerprint (Royle et al. 1988). *d*, Corresponding results using single-locus probe cocktail of MS1 plus MS31 (Wong et al. 1987) showing four bands per individual as expected, with no evidence for paternal exclusion. The conclusion in this case is that paternity has been established, and that the child contains one mutant band not attributable to the loci detected by MS1, MS8, MS31, or pλg3.

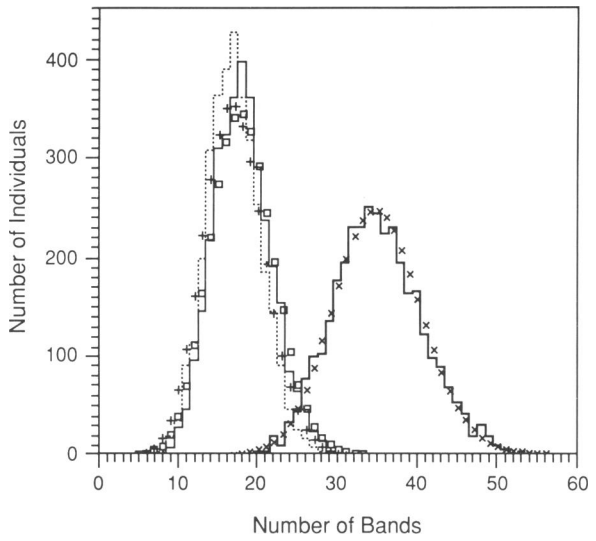

Figure 2 Individual variation in the number of bands scored with probes 33.6 and 33.15, determined from 3,404 mothers and alleged fathers in the 1,702 paternity cases. Distributions are shown for 33.6 (——), 33.15 (····), and both probes combined (▬). The predicted binomial distribution (indicated by □, +, and x, respectively) are derived from the mean number n of bands scored per individual and the mean band-sharing frequency x (table 1). Assuming total independence of bands and uniformity of x over all molecular weight intervals on the DNA fingerprint, the total number N of bands which can contribute to a DNA fingerprint is given by n/x, whence the probability that an individual will contain precisely i bands is given by $x^i(1-x)^{N-i}\cdot{}^NC_i$. Conversely, given the observed distributions, it is possible to estimate values of N and thus x which generate binomial distributions not significantly different from the observed distribution. The 95% confidence limits for x so determined are, for 33.6, x = .21–.33 (observed, .14); for 33.15, x = .27–.38 (observed .14); for both probes combined, x = .00–.16 (observed, .14). The observed distributions for probes 33.6 and 33.15 alone, but not for both probes combined, are therefore significantly narrower than the binomial distributions predicted from the observed band-sharing frequencies.

numbers scored in each of the 3,404 mothers and alleged fathers in the paternity casework data set were determined (fig. 2). The number of bands scored per individual ranged from eight to 33 for each probe alone, and from 20 to 57 for both probes combined. The combined probe distribution closely followed the binomial distribution predicted from the mean number of bands scored per individual and the mean band-sharing frequency (see fig. 2). For each probe alone, the predicted binomial distributions were slightly but significantly broader than the observed frequency distributions.

Probe Overlap, Allelism, and Linkage

To determine the extent to which probes 33.6 and 33.15 detect the same DNA fingerprint fragments, 36 families containing five to nine children per family were analyzed (table 2). A codetected DNA fingerprint fragment is defined by bands detected by 33.6 and 33.15 with equivalent electrophoretic mobilities and with identical parent-offspring segregation patterns. In a total of 1,077 parental bands scored with 33.6 and 970 bands detected by 33.15 there were only 12 instances of apparently codetected bands. Thus, only 1.2% of bands detected by one multilocus probe were also detected by the other probe. This is likely to be an over-estimate, since some instances of codetection may result from a 33.6-specific band and a 33.15-specific band in a given parent which have similar sizes and which by chance (or possibly by linkage in coupling) are transmitted to the same set of offspring. As expected, most instances of apparent codetection of bands occurred in the smaller sibships, where the chance of fortuitous cosegregation is greatest. We

Table 2

Co-detection of Variable DNA Fragments by Probes 33.6 and 33.15

No. of Offspring per Family	No. of Families Tested	No. of Bands Detected by[a]		No. of Common Bands[b]	% Common Bands[c]
		33.6	33.15		
5	10	305	240	8	2.9
6	13	413	381	3	.8
7	5	149	131	1	.7
8	6	162	170	0	0
9	2	48	48	0	0
Total	36	1,077	970	12	1.2

NOTE.—Thirty-six families each comprising a father, mother, and five to nine offspring were typed with probes 33.6 and 33.15.

[a] Bands present in one parent and not shared by the other parent were scored; the total numbers of bands scored in all parents are given.

[b] Apparently codetected bands were defined as bands in a given parent detected by 33.6 and 33.15 which differed in electrophoretic mobility by <3 mm and which showed identical parent-to-offspring segregation patterns.

[c] The mean proportion of bands detected by one probe which were also apparently detected by the other probe.

therefore conclude that the degree of overlap between loci scored in the 33.6 and 33.15 fingerprints is negligible.

The frequency of linkage or allelism between pairs of bands detected by a given probe was assessed in the same set of 36 large families by searching for pairs of parental DNA fingerprint fragments which cosegregated perfectly in the offspring (linkage) or showed allelic segregation (see Jeffreys et al. 1986) (table 3). As expected, the levels of apparent linkage and allelism decreased with increasing sibship size, indicating that many of these cases resulted from the chance dissegregation or cosegregation of DNA fragments from different loci in the relatively small sibships analyzed. Subtraction of this estimated "background" suggests that the true level of allelism and linkage is low, with <10% of bands having allelic or linked partners in a given parent. The 35 bands typically detected by probes 33.6 and 33.15 will therefore be derived from approximately 32 distinct and recombinationally separable hypervariable loci in a given individual.

Individual Variation in Human DNA Fingerprints

For probe 33.6 and 33.15, 14% of bands in one individual were on average "matched" by bands of a similar electrophoretic mobility in a second unrelated individual (table 1). To analyze DNA fingerprint variability in more detail, we have investigated the variation in the number of bands which discriminate between the DNA fingerprints of the mother and alleged father in each of the 1,702 paternity cases (fig. 3). Each mother–alleged father pair showed on average 30.9 discordant or discriminating bands with probe 33.6, 29.4 with probe 33.15, and 60.3 with both probes combined. The minimum numbers of discordant bands seen in any mother–alleged father comparison were 14, 12, and 31, respectively. The observed variation in the number of discordant bands among different mother–alleged father pairs closely followed the Poisson distribution predicted from the mean number of discordant bands, both for probe 33.6 and for probe 33.15.

In contrast, the corresponding distribution for data from both probes combined was slightly but symmetrically broader than the Poisson prediction, indicating that the frequency distributions for 33.6 and 33.15 are not completely independent. To determine whether this dependency could be caused by periodic minor variations in gel electrophoretic resolution and hybridization efficiency, which would tend to simultaneously increase or decrease the number of resolved bands detected by probes 33.6 and 33.15 in a given paternity case, the paternity cases were pooled into 17 sequential groups of 100. Comparisons between these groups showed a highly significant positive correlation between the mean number of discordant bands scored with 33.6 and 33.15 within each group (fig. 4). Similarly, there were significant positive correlations between the mean numbers of bands scored in the mother and in the father by a given probe, and in the mean numbers of bands scored by each probe in a given individual. In contrast, there was no significant correlation between the mean number of bands scored and the mean band-sharing frequency (data not shown).

Table 3

Incidence of Allelism and Linkage between DNA Fingerprint Bands Detected by Multilocus Probes

No. of Offspring per Family	No. of Families Tested	No. of Bands Scored	"Allelic" Bands (%)	"Linked" Bands (%)	Background (%)	% Bands with Allelic Partners	% Bands with Linked Partners
5	10	545	26.8	31.7	41.7	. . .	. . .
6	13	794	22.4	24.9	23.4	. . .	1.5
7	5	280	19.3	17.1	10.7	8.6	6.4
8	6	332	15.1	9.0	5.1	10.0	3.9
9	2	96	8.3	4.3	2.1	6.3	2.1

NOTE.—Families were typed with probes 33.6 and 33.15, and data pooled for both probes. For each probe and each parent, putative alleles were identified as pairs A,B of bands, present in the given parent and absent from the other parent, which segregated A – or B – into the offspring. Similarly, linked pairs were defined as pairs which cosegregated AB or – – into each offspring. The total numbers of bands which could be paired as apparent alleles, or as "linked" pairs, were pooled across all parents and both probes. The expected background incidence of spurious "allelic" bands, assuming that all bands in a given parent are derived from recombinationally separable loci, is given by $\Sigma\, n_i\,(n_i-1)/2^b$, where n_i is the number of bands scored in the ith parent by a given probe and b is the number of siblings analyzed. The expected incidence of spurious linked bands is identical. The final estimates of the proportions of true allelism and linkage are derived by subtraction of this background. These estimates, which are not significantly different for probes 33.6 and 33.15 (data not shown), do not include possible instances of allelism or linkage masked either by electrophoretic comigration of bands or by mutation.

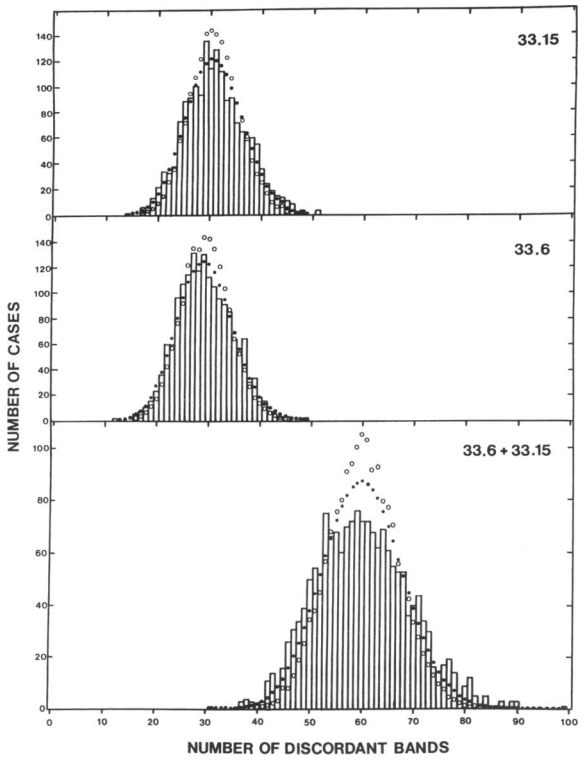

Figure 3 Variation in number of discordant bands between DNA fingerprints of mother and alleged father in each of the 1,702 paternity cases. The number d_i of discriminating bands in the ith case is related to the total number of bands in the mother, n_{Mi}, and in the alleged father, n_{Fi}, and the number s_i of bands shared by the two individuals by $d_i = n_{Mi} + n_{Fi} - 2s_i$. Frequency distributions are shown for probes 33.6 and 33.15 separately, and for data for both probes combined. The mean number of discordances are 30.9, 29.4, and 60.3 bands, for 33.6, 33.15, and both probes combined, respectively, and the Poisson distributions with these means are indicated (●). Corresponding frequency distributions for the mean of 10 computer-simulated sets of 1,702 mother-father pairs are also given (○) (see fig. 7).

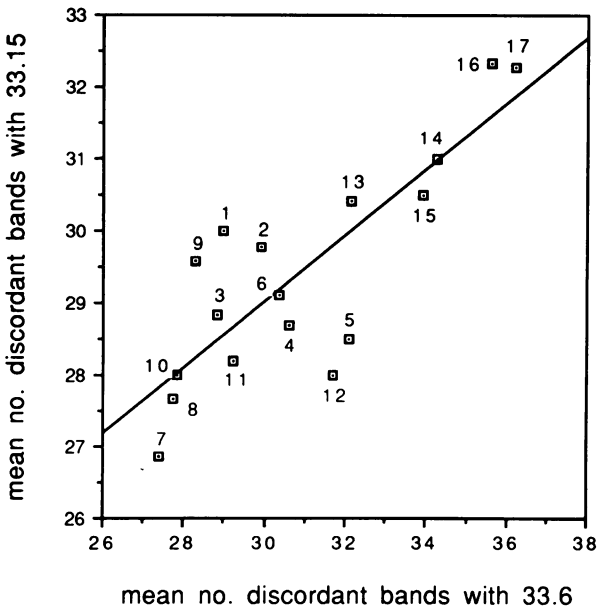

Figure 4 Periodic fluctuations in mean number of discordant bands scored by probes 33.6 and 33.15. The set of 1,702 paternity cases was divided into 17 sequential groups of 100 cases, each group corresponding on average to 3 wk casework. The mean number of discordancies within each group is shown, together with the sequential number of each group (1 = earliest group tested; 17 = most recent group analyzed). Note that the most recent cases (groups 13–17) show relatively high numbers of discordant bands with both probes, presumably reflecting steady improvements in band resolution/detection. The mean numbers of discordant bands scored with 33.6 and 33.15 show a highly significant positive correlation (r = .823, $P <$ .001).

Superimposition of 17 different Poisson distributions, with mean numbers of discordant bands determined from each pool of 100 paternity cases, produced a broader joint distribution more similar to that observed in figure 3 (not shown), and resulted in the elimination of 70% of the observed probability mass excess lying outside the original Poisson distribution. We conclude that minor periodic variations in resolution/detection efficiency account for most, if not all, of the minor statistical dependency observed between probes 33.6 and 33.15.

Discrimination of Fathers and Nonfathers by DNA Fingerprinting

Paternity disputes are most simply investigated by counting the number p of bands in a child that cannot be assigned to the mother and determining how many of these p bands can be detected in the alleged father. In the absence of minisatellite mutation, there will be no unassignable bands. In contrast, a nonfather would be expected to contain only xp of the offspring's p nonmaternal bands, where x is the mean band sharing frequency, and thus an expected value of $(1 - x)p$ nonmaternal bands in the child would not be attributable to the falsely accused man.

Figure 5 shows the frequency distribution of the number of nonmaternal offspring bands which cannot be assigned to the claimed father across the full set of 1,702 paternity cases. As expected, the frequency distribution is strongly bimodal, with modes at 0 and 11 unassigned bands with data pooled for probes 33.6 and 33.15. The first distribution is highly skewed toward zero unassignable bands and must represent true fathers. The shoulder on the first distribution, corresponding to one or two unassignable bands, is distinct from the second distribution and must be due to a

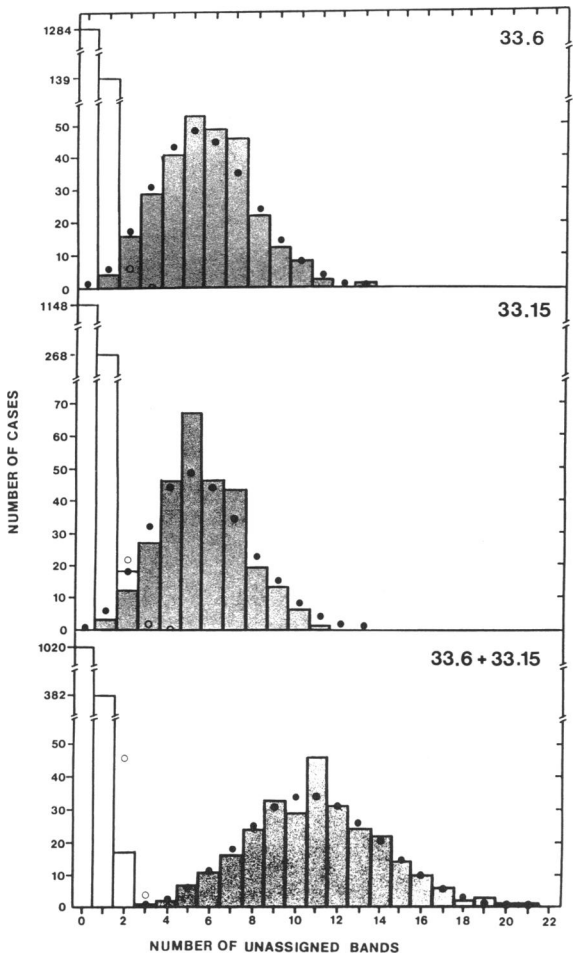

Figure 5 Variation in number of nonmaternal bands in a child which cannot be assigned to alleged father in a paternity case. The data are derived from 1,702 paternity cases. Open bars, correct paternity (1,419 cases); shaded bars, nonpaternity (283 cases). The paternity of marginal cases with one to five unassigned bands with probes 33.6 plus 33.15 was further determined by sequential Southern blot hybridization with cocktails of single-locus minisatellite probes pλg3 plus MS8 plus MS43A and MS1 plus MS31, which reveal additional information including small alleles not scored in the DNA fingerprints (MS43A does not contribute to the DNA fingerprint [Wong et al. 1987; Royle et al. 1988]). Cases of nonpaternity show a mean of 5.54, 5.46, and 10.98 nonmaternal bands in the offspring which are not present in the falsely accused man, with probe 33.6, probe 33.15, and both probes, respectively. Poisson distributions with these means are indicated (●). Poisson distributions for ≥2 mutant bands per offspring are also shown (○), using the mean frequencies of mutant offspring bands given in table 1.

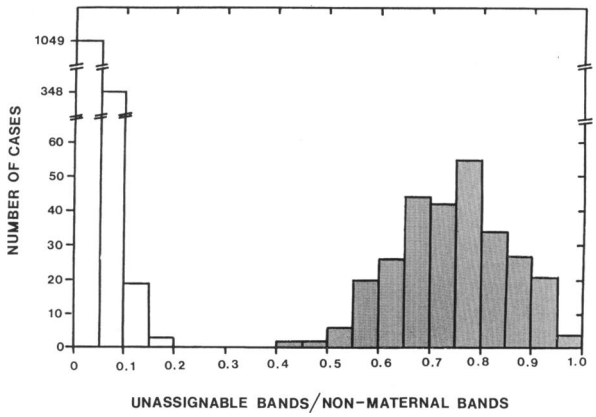

Figure 6 Variation in proportion of nonmaternal offspring bands which cannot be detected in alleged father. The frequency distribution for probes 33.6 and 33.15 combined is given for 1,702 paternity cases (1,419 cases of correct paternity [open bars] and 283 cases of incorrect paternity [shaded bars]), with data binned in 0.05 intervals. The shaded distribution approximates a normal distribution (mean = 0.744, SD = 0.110) and the nonshaded to the exponential distribution $N_c = 7{,}300e^{-45.5u}$ ($u > .05$), where N_c is the number of cases and u is the ratio of unassignable to nonmaternal bands.

low frequency of mutant bands in the offspring. The second distribution (mode 11) corresponds to nonfathers with multiple excluding bands and follows a Poisson distribution. The two distributions overlap marginally for each probe alone, but are just separate for both probes combined. From the Poisson distribution, the probability that all of the nonmaternal bands in a child's DNA fingerprint would be present by chance in a nonfather can be estimated at 1.7×10^{-5}, that is, once in 60,000 cases. Almost all such hypothetical cases would be due to a very low number of nonmaternal bands in the child, and thus failure to exclude in such circumstances would not be associated with a large number of inculpatory bands. Almost all such rare cases would therefore be inconclusive rather than lead to the erroneous conclusion of paternity for a nonfather, and would in any event be subjected to further analysis using single-locus probes.

Since the discrimination of fathers and nonfathers depends not only on the number of unassignable bands but also on the total number of nonmaternal bands in a child, it is more appropriate to analyze the variation in the *proportion* of these offspring bands which cannot be assigned to the alleged father. For true fathers, even with mutation, this proportion should be low. For nonfathers, the proportion should be high. In practice, the frequency distribution of this proportion among the 1,702 paternity cases defines two completely separate classes of men corresponding to fathers and nonfathers, respectively (fig. 6). For fathers, the proportion lies in the range 0–.18, and for nonfathers, .43–1. If the range for declaring paternity is arbitrarily set at 0–.2, and for declaring nonpaternity .4–1, then it is possible to estimate the probabilities of a false call in a paternity case. For nonfathers, the

frequency distribution of the proportion is approximately normal (fig. 6), and the probability of obtaining a proportion of $\leq .2$ for a nonfather under this distribution is 3×10^{-7}. Similarly, the frequency distribution for fathers is approximately exponential (fig. 6), and, under this distribution, the probability of a proportion of $\geq .4$ for a true father is 2×10^{-8}. While further data are needed to characterize these distributions, particularly in the range .2–.4, it is already clear that the ratio of unassignable bands to the total number of nonmaternal bands in a child is an efficient single statistic for distinguishing fathers from nonfathers, even in the presence of mutation.

Frequencies of Mutant Bands in DNA Fingerprints

There were 399 cases of genuine paternity (out of 1,419 cases in total defined in fig. 6) in which the child's DNA fingerprints showed at least one unassignable, presumably mutant, band (fig. 1; table 4A). Of these cases, 390 showed all bands assignable with one probe and one or two mutant bands with the other probe (fig. 6). In the one case with three unassigned bands and the two cases with four unassigned bands there were relatively few nonmaternal bands overall, and these cases could be clearly assigned to the nonpaternity group (see fig. 6). In each of these cases there were also multiple exclusions of paternity by single-locus probe analyses. The mutation rate per offspring band (table 1) is twice as high for 33.15 (.011 per band) as for 33.6 (.0052 per band). The numbers of mutant bands in offspring are distributed with a lower variance than predicted from the Poisson distribution (table 4A; fig. 6), which again suggests that not all bands are equally prone to mutation.

In an attempt to identify the loci contributing mutant bands, 388 of the families with mutant offspring were reprobed with the locus-specific minisatellite probes MS8 plus pλg3 and MS1 plus MS31 (Wong et al. 1987) (fig. 1; table 4B). Allele length change mutation rates for these loci, established in the CEPH panel of families where parentage is beyond dispute, have previously been reported (<.003, .003, .007, .052 per gamete for MS8, pλg3, MS31, and MS1 respectively [Jeffreys et al. 1988*a*]), and all four loci can contribute bands to DNA fingerprints. Of 405 mutant bands, 88 (22%) could be detected with one or the

Table 4

Incidence of Mutant Bands in the DNA Fingerprints of Offspring

A. Frequency of Mutant Bands

No. of Mutant Bands in Offspring	No. of Cases	% of Cases	No. Expected[a]
0	1,020	71.9	1,058
1	382	26.9	311
2	17	1.2	45.7
3	0	.3[b]	4.5
4	0	.02[b]	.33

B. Origin of Mutant Bands

	Parental Origin of Mutant Band			
	Maternal	Paternal	Not Determinable[c]	Total
Total no. of mutant bands	. . .	. . .	. . .	405
No. detected by MS8 plus pλg3	2	6	3	11
No. detected by MS1 plus MS31	26	44	7	77

NOTE.—In part A, data for probes 33.6 and 33.15 are combined. Part B describes the identification of mutant loci in the DNA fingerprints of 388 families showing one or more mutant offspring bands, by rehybridization of Southern blots with cocktails of single-locus minisatellite probes MS8 plus pλg3 and MS1 plus MS31 (Wong et al. 1986, 1987; Royle et al. 1988). Of 405 mutant bands, 88 (22%) were detected by these cocktails.

[a] Predicted from the Poisson distribution with a mean of 0.293 mutant bands per child (Table 1).

[b] Maximum estimate based on these Poisson frequencies.

[c] Mother and father shared an indistinguishable allele which was transmitted to the child.

other probe cocktail. Of these mutant bands, most (88%) were attributable to the most unstable loci detected by MS31 and MS1; alleles from these loci are detected by 33.15 but not by 33.6 (Wong et al. 1987). Of the identified mutant bands, 64% were paternal in origin, indicating a small but significant excess of paternally derived mutants ($\chi^2 = 5.7$; Yates's correction; 1 df; $.02 > P > .01$).

The Efficiency of DNA Fingerprint Band Sharing for Distinguishing Parents from Nonparents

In some parentage cases (though not in the cases reviewed in this paper), one or the other parent may be unavailable for DNA testing. In such cases, multilocus DNA fingerprinting can only give information on the degree of band sharing between the child and an alleged parent. On average, 14% of bands will be shared by two unrelated individuals, and 57% by a parent and offspring (table 1). To estimate the efficiency of this band-sharing statistic in discriminating fathers from nonfathers, variation in band sharing with probes 33.6 and 33.15 was analyzed across all 1,702 paternity cases (fig. 7).

The variation in mother–alleged father band sharing (mean 14%, range 0%–51%) is markedly different from mother-child sharing (mean 57%, range 32%–76%), as expected, since maternity is not in dispute in any of these cases and the degree of mother-child band sharing should therefore represent that for true parent-offspring comparisons. The observed frequency distribution of child–alleged father band sharing is, in contrast, strongly bimodal, indicating that fathers and nonfathers can be substantially distinguished by their degree of band sharing with the claimed offspring. The efficiency of determining paternity and nonpaternity can be assessed empirically from these distributions. Let the limits for calling nonpaternity and paternity be arbitrarily set at 0%–35% and 45%–100% band sharing, respectively. Of nonfathers, 97.2% would be correctly diagnosed as such. Of nonfathers, 2.4% would fall into the range 36%–44% in which no call would be made, and only 0.4% would be incorrectly classified as fathers. Similarly, the correct-call, no-call, and false-call percentages for true fathers are 96.8%, 3.2%, and <0.2%, respectively. Although band-sharing analysis does not provide definitive exclusionary bands for nonfathers, DNA fingerprint comparisons can nevertheless discriminate the substantial majority of fathers and nonfathers.

Curiously, the mean level of band sharing between nonfathers and children (21%) is significantly higher

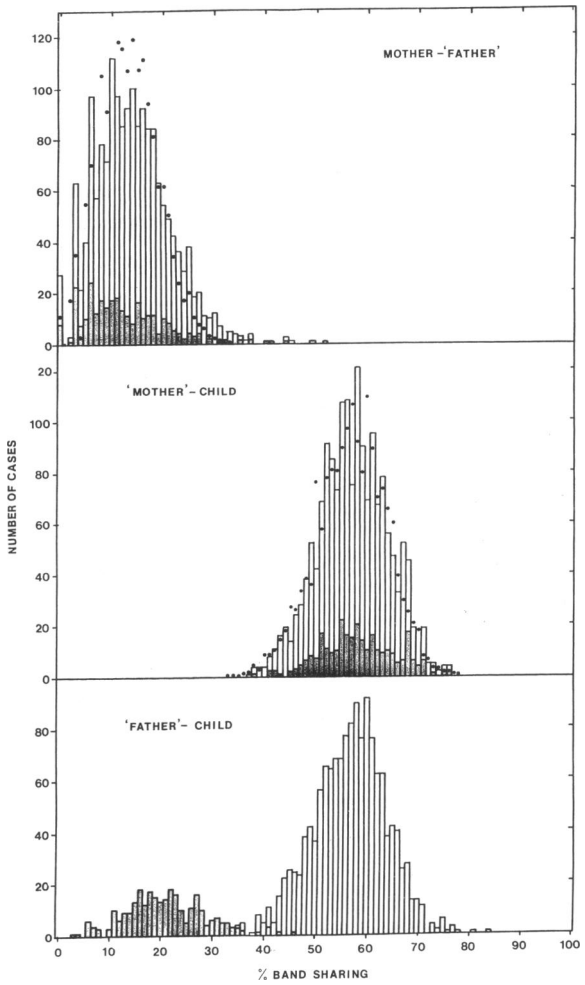

Figure 7 Variation in band-sharing frequency between alleged parents and between alleged parent-offspring pairs in 1,702 paternity disputes. For each paternity case, the percentage of bands shared by each pair of individuals is determined by $100 \cdot 2s/(n_A + n_B)$, where n_A and n_B are the total number of DNA fingerprint bands scored for probes 33.5 and 33.15 combined in individuals A and B, respectively, and s is the number of bands shared by A and B. The band-sharing frequencies are binned in 1% intervals. Open bars, true fathers; filled bars, nonfathers. The corresponding frequency distributions for a computer-simulated set of mother-father-child trios are also given. For each parent, the DNA fingerprint was generated using an array of 125 independent bins per probe, with each bin occupied or not occupied by a band according to the band-sharing frequency x. The child's DNA fingerprint was generated by independently transmitting each parental band to the offspring according to the transmission frequencies given in table 1; each band was mutated according to the mutation frequencies in table 1, with each mutant band, when generated, being placed at random in one of the 124 nonprogenitor bins. Band sharing was estimated from the proportion of array elements in two individuals sharing a band. Each point (●) represents the mean of 10 simulations of 1,702 mother-father-child trios (17,020 "families" in total).

than between mothers and alleged fathers (14%) (fig. 7). Since, for nonpaternity, nonfathers and children should not be genetically more similar than other pairs of unrelated individuals sampled from these populations (such as mothers and alleged fathers), the levels of nonfather-child and mother–alleged father band sharing should be identical. The reason for this discrepancy is unknown, though one intriguing possibility is that there may be a tendency for a falsely accused man to be weakly related to the true but unknown biological father. Alternatively, and more plausibly, there might be a small bias in band scoring against identifying the complete set of paternal exclusion bands in a child, in cases of nonpaternity. This bias would correspond on average to the incorrect classification of one nonmatching offspring band per probe as a band matching the falsely accused father, and might cause minor distortions in comparisons restricted to a child plus alleged father in the absence of the mother. Thus, estimates of the discriminating power of DNA fingerprinting in single-parent analysis given above should be regarded as provisional.

Discussion

Independence of DNA Fingerprint Bands

Pedigree analysis of DNA fingerprints in 72 different parents has demonstrated that, under the hybridization conditions used, probes 33.6 and 33.15 detect essentially nonoverlapping and therefore independent sets of loci. Similarly, within a given individual, the level of allelism or linkage between bands is low and comparable to levels previously reported (Jeffreys et al. 1986). Analysis of minisatellites cloned from DNA fingerprints has shown that the individual loci which contribute to DNA fingerprints are highly variable, with heterozygosities in some cases exceeding 99% and with a wide range of allele sizes. Frequently, the majority of *Hin*fI alleles are smaller than 3.5 kb, the lower limit of band scoring in DNA fingerprints. The relative lack of allelic pairs of bands in a DNA fingerprint therefore occurs because the majority of alleles at most loci are small and therefore not scored. Analysis of the cloned hypervariable loci has also shown that they are widely scattered in the human genome, although preferentially localized towards the ends of chromosomes (Royle et al. 1988; Armour et al. 1989*b*, 1990), which is consistent with the relative lack of linkage observed between DNA fingerprint bands. Another possible source of linked bands could arise from minisatellite alleles containing an occasional internal variant repeat unit cleavable by *Hin*fI to generate a haplotype of linked minisatellite DNA fragments from one allele. Such haplotypic fragments would be very tightly linked genetically, raising the possibility of linkage disequilibrium between different haplotypic fragments. An extensive set of linked bands has indeed been detected in one dog (Jeffreys and Morton 1987) and another set in mice (Jeffreys et al. 1987), with evidence in the latter for linkage disequilibrium. In contrast, such haplotypes have not been detected in any human family yet analyzed, even though DNA fingerprinting should be capable of detecting them. Furthermore, there is no evidence as yet that the relatively infrequent linked pairs of bands in human DNA fingerprints show significant linkage disequilibrium in human populations. Even if linkage disequilibrium between linked bands were to exist, the number of these nonindependent bands could be estimated from table 3 and subtracted from the DNA fingerprint data prior to statistical estimation. This procedure would result in the elimination of at most only one or two bands from a typical DNA fingerprint. With the proviso concerning population structuring (see below), we therefore conclude that the different bands resolved in a DNA fingerprint show a high degree of statistical independence.

Estimation of the Individual Specificity of Human DNA Fingerprints

The procedure generally adopted for estimating individual specificity is to determine the mean number n of resolved bands per individual and the mean probability x that a band in individual A is matched by a band of similar electrophoretic mobility in a second unrelated individual B (see Material and Methods for the "match" criterion). Assuming complete statistical independence of bands and uniformity of x over all bands, the probability that all of A's n bands are included within the DNA fingerprint of B is given by x^n. Heterogeneity in x, which exists (Jeffreys et al. 1985*c*; Smith et al. 1990), will reduce this probability estimate. Table 5 gives these probabilities for DNA fingerprint fragments >3.5 kb for the conservative value of x (.25) determined previously (Jeffreys et al. 1985*c*) and used in casework, and the lower values of x (.14) determined in the present study by comparison of the DNA fingerprints of the mother and claimed father in each of the 1,702 paternity cases (table 1). The probabilities are all very low ($<10^{-10}$ per probe).

Table 5

Estimation of Individual Specificity of Human DNA Fingerprints

	Probe 33.6	Probe 33.15
Probability that all bands in A are present in B:[a]		
Typical:[b]		
x = .25[c]	1.3×10^{-11}	5.3×10^{-11}
x as determined in this study[d]	6.2×10^{-16}	1.8×10^{-15}
Population mean[e]		
x = .25[c]	3.1×10^{-8}	5.8×10^{-8}
x as determined in this study[d]	2.1×10^{-10}	2.7×10^{-10}
Mean probability that no bands are unique to A or B:[f]		
x = .25[c]	1.8×10^{-15}	1.2×10^{-14}
x as determined in this study[d]	3.8×10^{-16}	2.5×10^{-15}
Poisson probability of no discordances[g]	3.8×10^{-14}	1.7×10^{-13}

[a] Determined from the mean number n of bands in an individual and the mean band-sharing frequency x by x^n.

[b] Using the mean number of bands per individual (table 1).

[c] Jeffreys et al. 1985*c*.

[d] Table 1.

[e] Using the mean determined from all mothers and alleged fathers analyzed, given by $1/T \cdot \Sigma x^{n_i}$, where T is the number of individuals typed (3,404) and n_i is the number of bands resolved in the ith individual.

[f] Number of bands (or more correctly gel intervals which may or may not be occupied by a band) can be estimated as n/x, where n is the mean number of bands per individual (table 1) (Jeffreys and Morton 1987). The probability that a given band is either present in both individuals A and B or absent from both individuals is given by $x^2 + (1-x)^2 = 1 - 2x + 2x^2$. The overall probability that the DNA fingerprint of A and B will be concordant for the presence/absence of all bands >3.5 kb is therefore given by $(1 - 2x + 2x^2)^{n/x}$.

[g] Determined from the Poisson distributions in fig. 3, whence the probability of no discordant bands is given by e^{-m}, where m is the mean number of discordant bands between a mother and alleged father.

These probabilities are for a typical person with n bands, and do not represent the mean probability in a population where the number of bands in A is variable (table 1). As expected, the population mean probabilities over all "parents" in the 1,702 paternity cases are higher ($<10^{-7}$ per probe; table 5) since rare individuals with small numbers of bands will contribute disproportionately to the mean probability estimate.

The above probabilities refer to the chance that all of A's bands are matched by similar bands in an unrelated individual B and do not take into account the possibility of additional nonmatching bands in B not present in A. To estimate the probability that the DNA fingerprints of A and B are concordant for all bands, it is necessary to estimate the total number N of bands which can contribute to a DNA fingerprint (Jeffreys and Morton 1987). Assuming uniformity of x, N is given by n/x (table 5). N is 125 both for probe 33.6 and for probe 33.15. Since the criterion for distinguishing different bands yields an electrophoretic mobility difference of approximately 0.5 mm or more, and the total length of the autoradiographic track scored for DNA fingerprint bands is approximately 90 mm, the number of resolvable intervals in the DNA fingerprint is given by approximately $90/(2 \times .5) = 90$, in good agreement with the values of N estimated for 33.6 and 33.15. Thus N and the band-sharing frequency x are largely determined by gel electrophoretic resolution. The chance that a given interval is occupied by a band in a given individual is x, and the overall probability that randomly selected unrelated individuals A and B are concordant for the presence/absence of bands in all intervals is given by $(1-2x + 2x^2)^{n/x}$ (table 5). This probability estimate for full concordance of A and B is a population mean, and allows for variation in band numbers in A and B. The probabilities are again very low ($<10^{-13}$ per probe; table 5). Note that these probability estimates do not refer to DNA fingerprint identity, since for full identity, the DNA fingerprints of A and B must also match in the nonscored region below 3.5 kb. Furthermore, for a true match, the two DNA fingerprints should correspond not only in band numbers but also in precise relative band positions and relative band intensities, in contrast to the much less stringent "match" criteria used to estimate the band-sharing frequency.

These estimates of the chance that the DNA fingerprints of A and B would be indistinguishable are therefore still highly conservative.

The "125-Bin" Model of DNA Fingerprints

As noted above, the simplest model for DNA fingerprints is that the autoradiographic track is composed of 125 intervals or bins per probe, with a uniform probability x that a given bin is occupied by a band in a given individual. To determine whether this "125-bin" model gives frequency distributions similar to those shown in figures 3 and 7, DNA fingerprints were simulated by computer. Mother and father DNA fingerprints were generated by an array of 125 independent bins, each of which was occupied or not occupied by a band according to the band-sharing frequency x. The child's DNA fingerprint was subsequently generated according to the transmission frequencies and mutation rates given in table 1 (see figs. 3, 7). The simulated discordancy frequency distributions for probes 33.6 and 33.15 fit the observed distributions fairly well, though with a slightly lower variance (fig. 3). Similarly, the simulated band-sharing distribution for husband-wife and mother-child comparisons are similar to the observed distribution (fig. 7), though in the former case there is a small but significant excess of pairs (approximately 5% of the total cases) who showed a higher-than-expected level of band sharing. The 125-bin model, which is based on complete statistical independence of bands, no variation in x between bins, and no variation in gel electrophoretic resolution or band detection between runs, does appear to provide a reasonable description of the properties of DNA fingerprints, at least with respect to the central tendencies of the observed frequency distributions.

Model-independent Estimates of Individual Specificity

All of the above estimates of individual specificity make assumptions, first about the molecular genetic properties of DNA fingerprints (genomic dispersal of detected loci, lack of allelism and linkage, lack of linkage disequilibrium between linked bands, uniformity of the band-sharing frequency over all molecular weight intervals, lack of interrun variation in band resolution/detection) and second about the complete absence of possible influences of population structuring. To avoid the first set of assumptions, we can make use of the band discordancy frequency distributions shown in figure 3, which for probe 33.6 and for 33.15 closely follow the Poisson distribution. Under the single assumption that the fit with the Poisson distribution continues to the extreme tail of the distribution, the chance of no discordant bands can be estimated by e^{-m}, where m is the mean number of discordant bands per mother–alleged father comparison. These probabilities correspond to the Poisson probability that a mother–alleged father pair, selected from the Caucasian population groups represented in the panel of 1,702 paternity cases, would show no discordant bands in the >3.5-kb size range. These probabilities are again very low ($<10^{-12}$ per probe; table 5) and comparable to estimates based on band-sharing frequencies.

Population Structuring

The above estimates of individual specificity make the further assumption about the absence of relatively invariant Caucasian subpopulations in which the probability of DNA fingerprint identity is substantially higher than estimates based on the population as a whole. We have optimized the chance of detecting such subpopulations by avoiding comparisons of randomly selected individuals and instead restricting our analyses to mother–father pairs who, by definition, would include representative pairs from any significant inbred subpopulations within our Caucasian population sample. The band discordancy frequency distributions shown in figure 3 show no evidence for a leftward skew which might signal the existence of such inbred subpopulations.

While the band discordancy frequency distributions for 33.6 and 33.15 follow the Poisson distribution, the distributions for data for both probes combined is slightly but symmetrically broader than the Poisson prediction (fig. 3), owing primarily to periodic minor variations in gel electrophoretic resolution and hybridization efficiency, which tend to simultaneously increase or decrease the number of resolved bands detected with probes 33.6 and 33.15 in a given paternity case. However, it remains formally possible that some of this distribution broadening might instead reflect population substructuring, with separate subpopulations showing relatively high and relatively low mean husband-wife band sharing. However, the broadening is modest, and the joint distribution in figure 3 shows a good fit with two superimposed Poisson distributions, with mean numbers of discordant bands of 55 and 64 corresponding to mean band-sharing frequencies of .22 and .07, respectively (data not shown). We therefore conclude that, even if subpopulation heterogeneity in band sharing were solely responsible for the distribution broadening in this Caucasian population

sample, the heterogeneity is modest and would be unlikely to affect significantly the statistical evaluation of casework data using the conservative band-sharing frequency of .25 used in casework evaluation. However, minor variations in band-sharing frequency between subpopulations, or the existence of rare and as yet undetected heavily inbred Caucasian subpopulations, could distort some of the statistical extrapolations given in table 5.

The only other possible indicator of subpopulation effects is the 5% excess of mother–alleged father pairs who show a minor increase in band sharing over that predicted from the 125-bin model (fig. 7). However, this model does not accurately describe the distribution tails (fig. 3) and thus this 5% excess may simply reflect inaccuracies in the model rather than the existence of subpopulations with a small increase in band sharing. Alternatively, our data set may include instances of consanguineous matings, and indeed we suspect that a very small proportion of the cases are in fact incest cases with the mother and alleged father thus being close relatives.

Criticism of DNA Fingerprint Evaluation

Cohen (1990) has recently raised a number of criticisms concerning estimations of the degree of individual specificity achievable with probes 33.6 and 33.15. His criticisms may be briefly summarized as follows:

1. Variation in DNA fingerprint band numbers between individuals is apparently lower than predicted from the binomial distribution; this is interpreted as evidence for statistical associations between bands.
2. Estimation of the probability that a typical individual (with the population mean of n bands) would show all bands present in a second unrelated individual, using the geometric mean x^n, substantially underestimates the population mean probability ($1/T \cdot \Sigma x^{n_i}$; table 5).
3. Statistical evaluation may be seriously distorted by population structuring.

Criticism 1 is based on the analysis of a small survey of 20 individuals in which sampling errors in the SD estimates of band numbers were likely to be large (Jeffreys et al. 1985*c*). As noted by Cohen, the binomially expected SD for the number of bands per individual is $[Nx(1-x)]^{1/2}$, where N is the total number of bands which can contribute to a DNA fingerprint (or bins each of which may or may not be occupied by a band) and x is the band-sharing (bin-occupancy) frequency. From table 1, probe 33.6 detects on average 18.1 bands per individual, with $N = 125$ and $x = .144$. The expected SD (3.9) is significantly greater than observed (fig. 2) but lies within 10% of the observed value of 3.6. Likewise, the expected and observed SDs for probe 33.15 (3.8 and 3.4, respectively) are similar. The minor decreases compared with the expected SDs can be explained by the occasional instance of hypervariable loci where both alleles are usually, or always, represented in the scored region of a DNA fingerprint, and by the tendency for fainter bands to be obscured by more intensely hybridizing bands; these fainter bands will be more readily detectable in individuals with relatively low band numbers, causing a further minor drop in variance. Neither of these phenomena would significantly affect the assumption of band independence in the estimation of probabilities in identification and parentage analyses.

Cohen's criticism 2 concerning population mean estimates of individual specificity is directly addressed in table 5 and shown to be of minor significance. Of course, his argument concerning individual variation in band number is irrelevant in a given forensic case, where band numbers are fixed by the forensic specimen against which suspects are being compared.

Criticism 3 concerning relatively homogeneous subpopulations has been discussed above, where we have noted that our Caucasian population data show, at best, only minor evidence of structuring, of insufficient magnitude to distort the conservative statistical evaluation of casework data. We are currently extending this analysis to other populations with as yet no evidence for significant shifts in the mean band-sharing frequency between different ethnic groups (P. Debenham and A. J. Jeffreys, unpublished data). The possibility of relatively invariant subpopulations within a given ethnic group is much more difficult to test experimentally and will require very large surveys of the type reported here. However, there are a number of theoretical arguments which suggest that genetic drift between subpopulations is unlikely to affect band-sharing frequencies significantly except in the case of major chronic inbreeding within a small reproductively isolated community:

1. The band-sharing frequency between unrelated individuals is largely dictated by gel electrophoretic resolution, not population variability. As a result, many instances of band sharing between unrelated individuals arise through the fortuitous electrophoretic comigration of alleles from differ-

ent loci. Thus multiple loci can, and indeed do, contribute alleles to a given bin (Wong et al. 1987), from which it follows that "band" and "allele" are not synonymous, as assumed by Cohen. For the frequency of occupation of a given bin (i.e., the presence of a band in a given gel interval) to differ between subpopulations, it would be necessary for alleles from many or all of the contributing loci to drift in frequency in the same direction. This is improbable.

2. DNA fingerprint individuality will still remain high, even in an extreme example of a local subpopulation, namely, a single sibship. For a typical family, there will be 60 bands in total in the mother and father (ignoring shared bands) (table 1). The transmission frequency of each band to a given child is .52 (table 1), and the probability that two children would be concordant for the presence/absence of a given parental band is .50. With statistical independence of inheritance of bands, the overall probability of full DNA fingerprint concordance between two siblings is $0.5^{60} = 9 \times 10^{-19}$ for probes 33.6 and 33.15 combined. For the mother-father pair with the lowest number of discriminating bands (31; fig. 3), the probability of sibling identity is still low (5×10^{-10}). Even if we remove the experimentally justified assumption of independence (nonallelism) and assume that in this family each parent contains just seven or eight unlinked pairs of allelic DNA fragments (cf, table 3), then the probability of sibling identity still remains very low (2×10^{-5}). Thus even in local (hypothetical) subpopulations in which much of the variability is lost, the large number of characters scored by the multilocus probes still ensures extreme levels of individual specificity.
3. Rigorous inbreeding of mice by strict brother-sister mating over many generations fails to produce completely uniform DNA fingerprints, owing to the generation of new bands by recurrent mutation (Jeffreys et al. 1987; Kelly et al. 1989).
4. The deliberate use of the conservative value of the band sharing frequency x (.25) in evaluating casework automatically compensates for substantial levels of inbreeding. The degree of band sharing between related individuals can be estimated from $2F(1-x)+x$, where F is the inbreeding coefficient. Since $x = .14$ for probes 33.6 and 33.15, the conservative value of x corresponds to an assumption that $F = .06$, equivalent to first-cousin relationship. Thus, estimates of the statistical significance of DNA fingerprint similarity relate not to the probability that a man picked at random from an outbred population would fortuitously match, but to the probability that a first cousin of the true assailant would by chance show a DNA fingerprint "indistinguishable" (under the match criteria used) from that of the forensic specimen.

In contrast, single-locus minisatellite or VNTR probes are more likely to show interpopulation variation in allele length frequency distributions (Balazs et al. 1989). Such variation will preferentially occur at loci with smaller numbers of alleles with significant population frequencies (Flint et al. 1989). In contrast, recurrent allelic length mutation will tend to counteract the genetic drift of preexisting alleles at ultravariable loci such as MS31 and MS1, reducing interpopulation divergence of alleles defined on the basis of length (Jeffreys et al. 1990*b*). Also, we note that statistical evaluation of single-locus probe profiles can place considerable statistical weight on a given allele, particularly when rare, and that substantial uncertainties in allele frequency can arise from sampling errors, population heterogeneity, and allele sizing errors. In contrast, DNA fingerprint evaluations place only modest statistical weight on each band, with individual specificity arising from the large number of bands scored. Issues of population heterogeneity do not therefore apply equally to the statistical evaluation of single-locus-probe and multilocus-probe evidence, as claimed by Cohen (1990).

The Efficiency of Parentage Testing

Empirical analysis of the 1,702 paternity cases shows that the combined use of probes 33.6 and 33.15 can efficiently distinguish fathers from nonfathers in virtually every case. The most effective single discriminatory parameter is the proportion of nonmaternal bands in a child which cannot be detected in the accused man. Minisatellite mutation, which has been extensively documented not only in multilocus DNA fingerprints but also for loci cloned from DNA fingerprints (Jeffreys et al. 1985*b*, 1988*a*, 1990*b*; Armour et al. 1989*a*; Smith et al. 1990), occurs at a significant rate but does not significantly impede the discrimination of fathers from nonfathers. However, the occurrence of mutation does affect the statistical evaluation of a given paternity case. Consider for example a typical paternity case in which 16 nonmaternal bands are detected in the child (table 1). Suppose that all of these bands were present in the

accused father. Assuming full statistical independence of bands, the (conservative) probability that a nonfather unrelated to the child would contain all of these bands is x^{16} (see Hill 1986; Brookfield 1989; Evett et al. 1989*a*, 1989*b*, for more formal Bayesian approaches). For the conservative value of x used in casework (.25), this probability is 2×10^{-10}. Note that while the band-sharing frequency x for nonfather-child comparisons is for some reason higher than for mother–alleged father comparisons (fig. 7), it is still lower than .25. Now suppose that 15 of the 16 nonmaternal bands are present in the alleged father, with one unassignable, presumed mutant band. The empirically observed probability of one mutant band in a child is .269 (table 4). The probability that a nonfather would contain by chance 15 out of 16 nonmaternal offspring bands is $16\ x^{15}(1-x) = 1.1 \times 10^{-8}$. The likelihood ratio of paternity to nonpaternity can therefore be estimated at $.269/1.1 \times 10^{-8} = 2.4 \times 10^{7}$. For two mutant bands, this likelihood ratio falls to 4.7×10^{4}. Thus the level of mutation observed in DNA fingerprints does not seriously compromise the ability to establish paternity beyond reasonable doubt, and such cases would in any event be further analyzed by single-locus minisatellite probes. Note that the above likelihood ratio estimates do not include the prior probability of paternity (.834) estimated from this sample of U.K. casework. This prior probability is likely to vary substantially from country to country and between population groups. The prior probability also appears to alter depending on whether the adults involved are "married" or not (that is, share the same surname, data not shown).

Even in the absence of the mother, estimates of band sharing between an alleged father and child are remarkably effective at discriminating fathers and nonfathers (fig. 7), even though no definitive exclusion characters are generated. However, the band-sharing statistic for a given comparison is less effective in defining precise relationships and in detecting more distant relationships (Lynch 1988).

Conclusion

The present study supports earlier claims for the effectiveness of multilocus DNA fingerprint probes in individual identification and paternity testing based on a more extensive analysis of the genetic properties of DNA fingerprints and on empirical model-independent analyses of population data. Theoretical criticisms which have been leveled at the use of these probes (Cohen 1990) are largely based on unrealistic models and appear to be without significant foundation.

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