

## Increased band sharing in DNA fingerprints of an inbred human population

R. J. Bellamy<sup>1</sup>, C. F. Inglehearn<sup>1</sup>, I. K. Jalili<sup>2</sup>, A. J. Jeffreys<sup>1</sup>, and S. S. Bhattacharya<sup>1</sup>

<sup>1</sup>Department of Human Genetics, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne, UK

<sup>2</sup>St. John's Ophthalmic Hospital, Jerusalem, Israel

<sup>3</sup>Department of Genetics, Leicester University, Leicester, UK

Received July 17, 1990 / Revised December 18, 1990

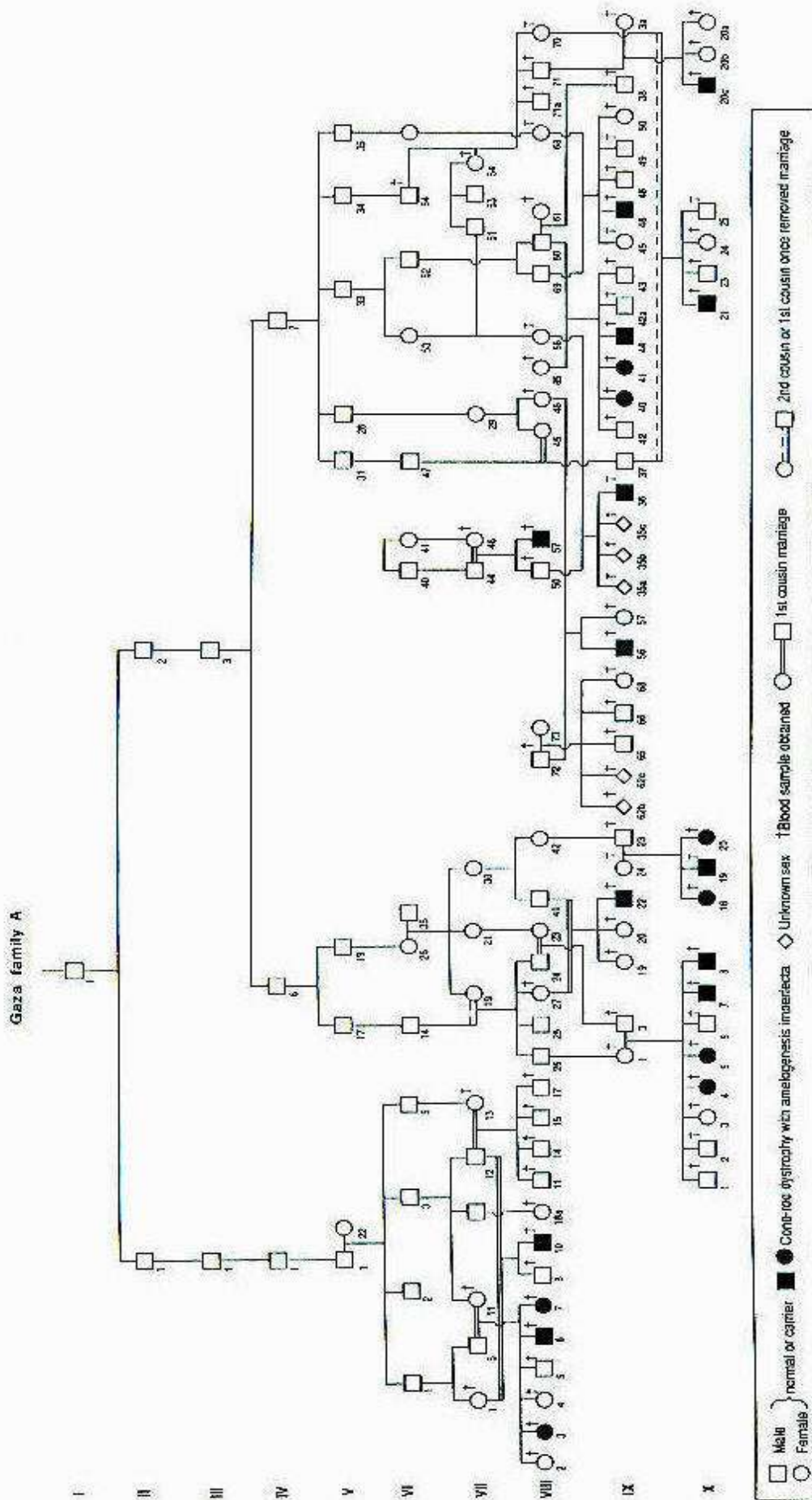
**Summary.** We have compared band sharing between the DNA fingerprints of members of an inbred human population with band sharing between members of an outbred population. It had not previously been determined whether the high rate of mutation at minisatellite loci is sufficient to prevent an increase in band sharing in moderately inbred populations. We have found that there is an increase in band sharing in the 2-kb to 9-kb size range, but not in the >9-kb size range, in the inbred population. The difference was consistently observed using four different multi-locus probes, viz. 33.6, 33.15, (CAC)<sub>n</sub> and M13. Thus, we have demonstrated that moderate but prolonged inbreeding can lead to increased similarity in human DNA fingerprints. This should be considered when analysing DNA fingerprints in forensic or paternity cases involving members of an inbred community.

### Introduction

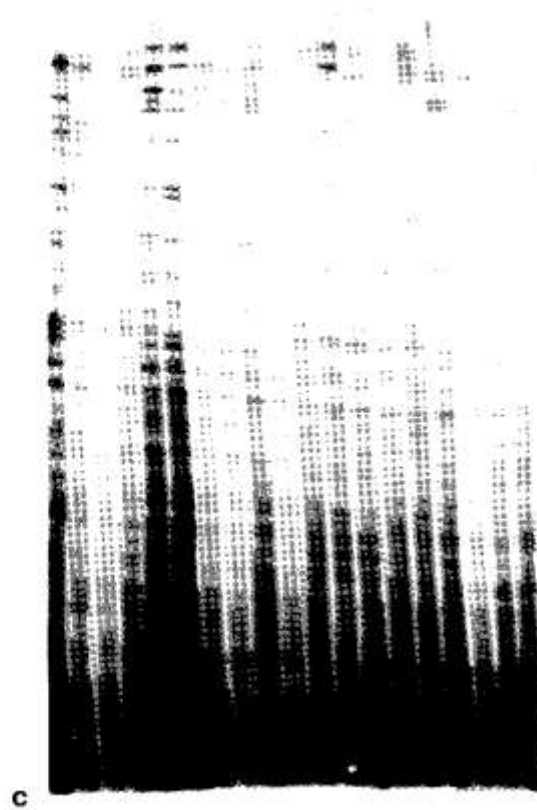
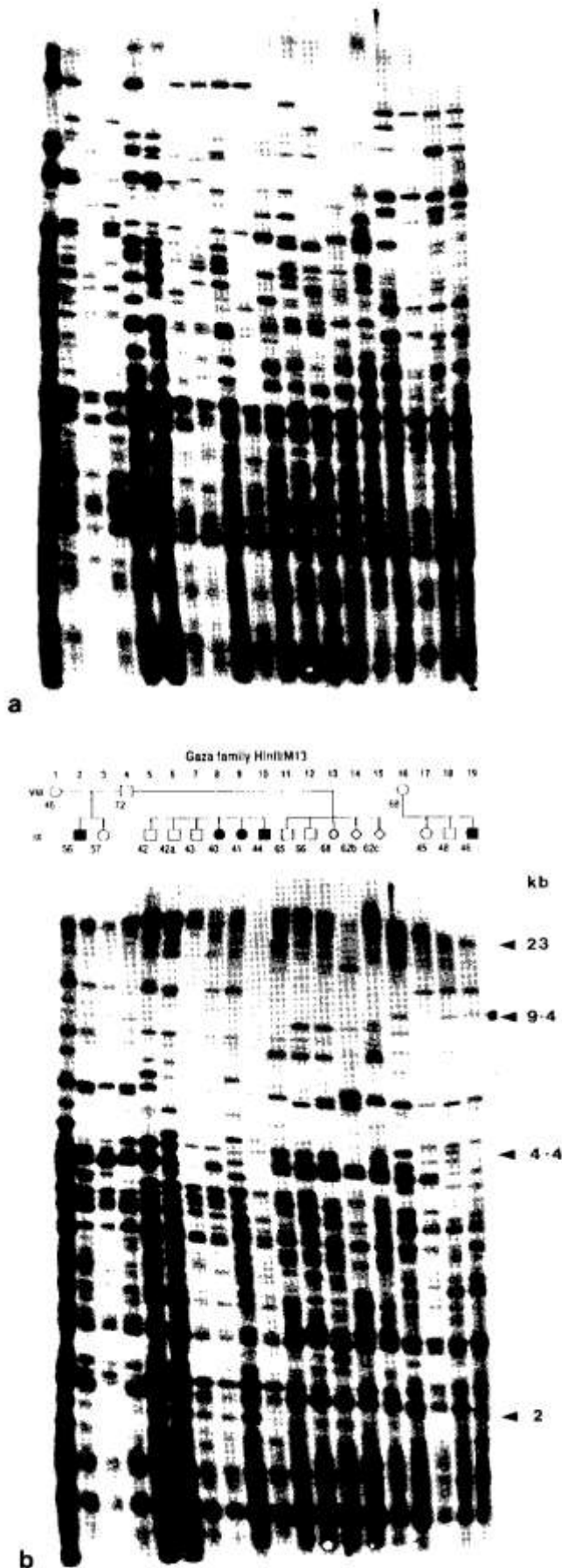
DNA fingerprints consist of a complex pattern of bands derived from many highly polymorphic VNTR (variable number tandem repeats) loci (Jeffreys et al. 1985a). These hypervariable regions contain multiple repeats of varying complexity, from simple repeat motifs of as little as 2 bp, to "minisatellites" of up to 80 bp in length (Bell et al. 1982; Capon et al. 1983; Nakamura et al. 1987; Litt and Luty 1989). VNTR length variation is caused by variation in the number of repeats within the hypervariable region. Probes with homology to the core sequence can simultaneously detect many of these hypervariable regions, thereby producing a totally individual-specific DNA profile, analogous to a "genetic barcode" (Jeffreys et al. 1985b). The DNA fingerprinting technique has found widespread applications in forensic medicine (Gill et al. 1985), paternity testing (Helminen et al. 1988),

immigration cases (Jeffreys et al. 1985c) and a variety of scientific and medical fields (Hill and Jeffreys 1985; Thein et al. 1986; Ponder et al. 1987; Wetton et al. 1987; Weitzel et al. 1988; Burke et al. 1989).

VNTR alleles are stably inherited in a Mendelian fashion in humans (Jeffreys et al. 1986) and in a range of other species (Ryskov et al. 1988). All bands present in an individual's DNA fingerprint can be traced to one or the other of the parents' fingerprints (unless a new mutation has occurred); approximately half of the offspring's bands will have been inherited from each parent. The expected band sharing between first degree relatives from an outbred population can be calculated from the number of coinherited band fragments (approximately 0.5) and the mean band sharing between unrelated individuals ( $x$ ).  $x$  is low in an outbred population (Jeffreys et al. 1985b). A single generation of consanguineous mating between first cousins should have little effect on band sharing between siblings produced by such mating, as the offspring are only one sixteenth more related to each other than the offspring of an outbred marriage. However, if a population were to become chronically inbred, either by many generations of consanguineous mating or because of prolonged population bottle necks, single VNTR alleles may become fixed at some loci. This could give rise to a population whose members have DNA fingerprints with more similarities than those from an outbred population. This has been demonstrated for highly inbred lines of laboratory mice (Jeffreys et al. 1987), domestic chicken strains (Kuhlein et al. 1990), and an almost genetically homogenous wild mammal population, the eusocial naked mole rat (Reeve et al. 1990). However, the rate of mutation to new length alleles at VNTR loci is high, and may be sufficient to prevent significant increase in DNA fingerprint similarity at intermediate levels of inbreeding. To date, it has not yet been demonstrated whether the levels of inbreeding found in culturally or geographically isolated human sub-populations is sufficient to increase band sharing in these populations. An increase in DNA fingerprint simi-



**Fig. 1.** Extended pedigree of the large inbred family from the Gaza strip. As records of each member's ancestry were incomplete, many marriages that are shown as outbred may contain a degree of consanguinity.



**Fig. 2a-c.** DNA samples from some of the family members shown in Fig. 1, digested with *Hinf*I, electrophoresed through a 40 cm, 0.7% agarose gel at 2V/cm for 52 h and probed sequentially with (a) 33.6 (b) M13 and (c) (CAC). The pedigree, which is numbered as in Fig. 1, applies to all three gels

ilarity in such populations would have important implications in legal medicine. Probability calculations of an alleged father being a child's true father or of a forensic sample coming from a suspect depend on the mean band sharing frequency ( $x$ ) between unrelated individuals. If  $x$  is increased in inbred populations then probability calculations based on estimates from outbred populations are invalid.

To investigate the effects of inbreeding on the complexity of human DNA fingerprints, we studied a large family from the Gaza strip (Valili and Smith 1988), where consanguineous marriages are a cultural norm (Fig. 1). The pedigree shown almost certainly underestimates the level of inbreeding that has occurred in this family, since detailed records were only available on males in the family; moreover, inbreeding had probably occurred for many generations before the pedigree begins. Four multi-locus probes, 33.6 and 33.15 (Jeffreys et al. 1985a), (CAC)<sub>3</sub> (Schafer et al. 1988) and M13 (Vassart et al. 1987) were used to produce DNA fingerprints of all or part of the Gaza family. British families were used as outbred controls. Another multilocus probe (TTGGGG)<sub>3</sub> (Hastie and Allshire 1989) was used to compare fingerprints of Indian and Caucasian populations.

## Materials and methods

DNA extraction, restriction enzyme digestion and Southern transfer conditions have been described previously (Inglehearn et al. 1990). Digested DNA was size fractionated by electrophoresis through a 40 cm, 0.7% agarose gel at 2 V/cm for 52 h with recircu-

lation of buffer (0.089 M TRIS-borate, 0.89 M boric acid, pH8.2) and then transferred to Hybond N membranes (Amersham). Probes 33.6 and 33.15 were labelled with ( $\alpha$ - $^{32}$ P)dTTP by the method of Jeffreys et al. (1985a). M13 was labelled by the random-priming method (Feinberg and Vogelstein 1983). The oligomers (CAC)<sub>5</sub> and (TTGGGG)<sub>5</sub> were labelled by ( $\gamma$ - $^{32}$ P)ATP end labelling (Chaconas and Van der Sande 1980).

**Table 1.** Expected mean band sharing frequency, ( $x_e$ ) for first degree relatives was calculated as by Georges et al. (1988). Expected mean band sharing frequency for first degree relatives is  $(1 + q - q^2)/(2 - q)$  where  $q$  is the mean allele frequency.  $q$  is related to the mean band sharing frequency between unrelated individuals ( $x$ ) by  $x = 2q - q^2$ .  $x$  was estimated by pairwise comparisons between the DNA fingerprints of unrelated individuals run in adjacent lanes, using the same hybridization conditions as for the inbred and outbred families. Since  $x$  varies with experimental conditions, we felt it important to make our own estimates of band sharing, rather than use figures obtained in other laboratories. These values with their standard deviations are shown in **b** under columns  $x$  and  $sd$ .

respectively. Observed mean band sharing ( $x_o$ ) was calculated by comparisons between DNA fingerprints in adjacent lanes. The number of degrees of freedom was equal to the number of pairwise comparisons minus one. The two-tailed  $t$  value was calculated by  $t = (x_o - x_e)/(SD/n^{0.5})$  (where  $SD$  = standard deviation of  $x_o$ , and  $n$  = mean number of bands scored). The probability in the final column is that of observing this difference, if there is no real difference between the observed and expected band sharing. Effectively unrelated individuals are those who are no more closely related than third cousins. The coefficient of relatedness for third cousins is 1/128; this should have a negligible effect on mean band sharing

**a** Observed v expected band sharing for first degree relatives in the Gaza family

Probe	Size range	$x_e$	$x_o$	SD	$t$	$df$	Probability
M13	>9	0.543	0.618	0.342	1.33	40	NS
	9-4	0.613	0.754	0.132	6.48	40	< 0.0001
	4-2	0.692	0.842	0.071	12.77	40	< 0.0001
	Total	0.639	0.794	0.073	12.88	40	< 0.0001
(CAC) <sub>5</sub>	>9	0.572	0.585	0.251	0.392	41	NS
	9-4	0.641	0.769	0.093	8.95	41	< 0.0001
	4-2	0.725	0.867	0.052	17.85	41	< 0.0001
	Total	0.666	0.781	0.067	11.13	41	< 0.0001
33.6	>9	0.582	0.552	0.336	0.578	41	NS
	9-4	0.632	0.737	0.115	5.96	42	< 0.0001
	4-2	0.702	0.818	0.080	9.48	42	< 0.0001
	Total	0.660	0.758	0.062	9.11	41	< 0.0001
33.15	>9	0.569	0.571	0.208	0.041	17	NS
	9-4	0.656	0.791	0.102	5.60	17	< 0.0001
	4-2	0.680	0.900	0.057	16.39	17	< 0.0001
	Total	0.654	0.809	0.074	8.92	17	< 0.0001

**b** Observed v expected band sharing for effectively unrelated members of the Gaza family

Probe	Size range	$x$	$sd$	$x_e$	$SD_e$	$t$	$df$	Probability
M13	>9	0.113	0.106	0.208	0.396	0.679	7	NS
	9-4	0.285	0.110	0.481	0.225	2.46	7	0.043
	4-2	0.468	0.105	0.671	0.056	10.29	7	< 0.0001
	Total	0.348	0.077	0.582	0.069	9.57	7	< 0.0001
(CAC) <sub>5</sub>	>9	0.186	0.194	0.213	0.133	0.729	12	NS
	9-4	0.353	0.074	0.544	0.098	7.03	12	< 0.0001
	4-2	0.539	0.080	0.722	0.080	8.27	12	< 0.0001
	Total	0.410	0.050	0.570	0.087	6.37	12	0.0001
33.6	>9	0.210	0.179	0.140	0.240	0.921	9	NS
	9-4	0.330	0.080	0.521	0.150	4.04	9	0.003
	4-2	0.489	0.094	0.697	0.076	8.63	9	< 0.0001
	Total	0.395	0.059	0.582	0.094	6.28	9	0.0002
33.15	>9	0.178	0.149	0.455	0.309	2.22	4	NS
	9-4	0.387	0.053	0.618	0.081	6.39	4	0.003
	4-2	0.441	0.085	0.641	0.057	7.89	4	0.0014
	Total	0.382	0.042	0.605	0.054	9.23	4	0.0008

Table 1 (continued)

c Observed v expected band sharing for first degree relatives in outbred families							
Probe	Size range	$x_e$	$x_o$	SD	$t$	$df$	Probability
M13	> 9	0.543	0.479	0.366	0.741	17	NS
	9-4	0.613	0.649	0.100	1.53	17	NS
	4-2	0.692	0.718	0.126	0.875	17	NS
	Total	0.639	0.659	0.096	0.888	17	NS
(CAC) <sub>5</sub>	> 9	0.572	0.584	0.207	0.272	21	NS
	9-4	0.641	0.647	0.094	0.298	21	NS
	4-2	0.725	0.745	0.051	1.85	21	NS
	Total	0.666	0.684	0.060	1.41	21	NS
33.6	> 9	0.582	0.599	0.245	0.355	27	NS
	9-4	0.632	0.653	0.113	0.985	27	NS
	4-2	0.702	0.730	0.076	1.96	27	NS
	Total	0.660	0.692	0.063	2.69	27	0.012
33.15	< 9	0.569	0.603	0.171	0.486	5	NS
	9-4	0.656	0.609	0.146	0.786	5	NS
	4-2	0.680	0.670	0.051	0.479	5	NS
	Total	0.654	0.652	0.046	0.107	5	NS

Prehybridization for M13, 33.6 and 33.15 was in 1% bovine serum albumin (BSA), 7% SDS, 0.263 M sodium orthophosphate buffer (pH 7.5) (Church and Gilbert 1984), at 65°C for 2 h, except in the case of M13 where it was carried out overnight. Hybridization with 33.6 and 33.15 was carried out in the same solution overnight at 65°C. M13 was hybridized under the same conditions for about 36 h. The filters were washed in 2 × SSC (1 × SSC = 150 mM NaCl/15 mM sodium citrate, pH 7.0), 0.1% SDS for 2 × 15 min at room temperature with a higher stringency wash at 65°C for 15 min in the same buffer, except for M13 where the third wash was omitted. The filters were rinsed in 3 × SSC before sealing in plastic tubing for autoradiography.

For hybridization with the oligomers, the filters were first prehybridized for 1 h, then hybridized overnight in 5 × Denhardt's (1 × Denhardt's = 0.02% BSA/0.02% polyvinylpyrrolidone/0.02% Ficoll), 5 × SSC, 0.1% SDS, 0.1% sodium pyrophosphate. (CAC)<sub>5</sub> was hybridized at 37°C and (TTGGG)<sub>3</sub> at 48°C. Both were washed for 2 × 20 min at room temperature in 4 × SSC, 0.1% SDS, 0.1% sodium pyrophosphate with a higher stringency wash for 5 min, in the same solution but at the hybridization temperature. We estimate that these conditions should allow hybridization with two or possibly three mismatches.

Autoradiographic images were obtained without intensification except in the case of oligolabelled M13, where autoradiography was at -70°C with 2 intensifying screens.

Actual mean band sharing between the DNA fingerprints of unrelated individuals and of first degree relatives was calculated by pairwise comparisons between adjacent lanes. Expected mean band sharing between first degree relatives was calculated by the method of Georges et al. (1988), using values of  $x$  that we had calculated previously for each probe by pairwise comparisons between unrelated Caucasians. We did not use estimates of  $x$  obtained by other groups, as this value can be affected by the degree of resolution and number of fragments detected, both of which may vary between different laboratories.

## Results

Figure 2 shows DNA fingerprints of part of the studied Gaza family. Mean band sharing between first degree

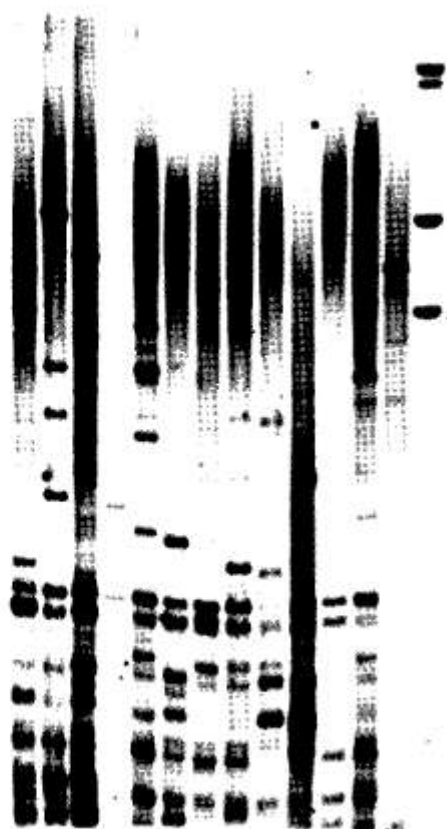
relatives in the Gaza family was significantly higher than expected for first degree relatives from an outbred population notably, in the 9-kb to 4-kb and 4-kb to 2-kb size ranges, for all four multi-locus probes (Table 1a). A similar result was obtained by comparing mean band sharing between effectively unrelated members of the family (those more distant than third cousins), with that expected for an outbred population (Table 1b). Mean band sharing was not significantly increased compared with that expected in the British families (Table 1c).

Mean band sharing in the > 9-kb size range was not higher than expected, probably because there is a higher new mutation rate for larger minisatellites (Jeffreys et al. 1985a, 1988), preventing alleles reaching a high population frequency. Furthermore, as there are a low number of bands detected in this region, the known error in the estimation of band sharing is large.

We also found evidence that the less variable bands can become fixed in a population by comparing the DNA fingerprints of unrelated Indians and Caucasians. A strongly hybridizing doublet of approximately 3-kb, detected by (TTGGG)<sub>3</sub> and M13 in *Hae*III, *Hinf*I and *Pst*I digests, was present in 41 out of 42 Hyderabad Indians (one individual had only one of the bands) and 23 out of 23 Bombay Indians (Fig. 3). None of 20 Europeans had this doublet. No constant bands unique to the Indian DNA samples were seen with probes (CAC)<sub>5</sub>, 33.6 or 33.15.

## Discussion

Increased similarity of human DNA fingerprints in traditionally inbred groups is important in forensic medicine and paternity testing. The probability of falsely accusing an innocent man by DNA fingerprinting is depen-



**Fig. 3.** DNA samples from 12 Hyderabad Indians (lanes 1–12) and one Caucasian (lane 13) digested with *Hae*III and probed with the oligomer (TTGGGG)<sub>3</sub>. Lane 14 contains *Hind*III-digested phage lambda. A strongly hybridizing doublet (approximately 3kb) is present in all the Indian samples, except lane 9 where only one band is present, but neither band is present in the Caucasian DNA sample.

dent on the mean band sharing frequency between unrelated individuals. If a suspect or accused father comes from an inbred population, then the reduced variability of DNA fingerprints must be considered when calculating the probability of a random match having occurred. For example, in the Gaza population, the average total mean band sharing for unrelated individuals with probe 33.6 was 0.582, so the probability of two random unrelated individuals having identical DNA fingerprints is only  $5.2 \times 10^{-8}$  ( $0.582^{31}$ ), assuming an average of 31 bands are detected by this probe. This is 100000 fold higher than the same probability for an outbred population ( $0.395^{31} = 3.1 \times 10^{-13}$ ). This difference will be most important in highly isolated communities where false inclusions could occur if increased band sharing is not considered; this is particularly true in paternity cases, where the probabilities are higher. When DNA fingerprinting for a legal case involves a member of a highly inbred community, an investigation into the variability of DNA fingerprints in the population is required. If this is too time-consuming or difficult, caution should be employed when quoting probability statements in court and highly conservative estimates of band sharing should be used.

We have shown that a moderately inbred human population can be recognised by comparing mean band sharing in DNA fingerprints with a known outbred population. However, we agree with Lynch (1988) that the relationship between a single pair of individuals cannot be determined using DNA fingerprints. In addition, further research is required before it can be concluded that comparisons between species can be made to investigate the level of inbreeding. Our results imply that DNA fingerprinting can be used to identify moderate levels of inbreeding in natural or zoo populations; this might be useful in managing viable populations for conservation. Georges et al. (1988) compared the DNA fingerprints of four domestic species with four multi-locus probes and found that there was variation between species with respect to those species showing most band sharing following studies with each probe. This suggests that comparisons cannot be made between species; however, this may not be the case, as their study involved only a small sample and so their results were not statistically significant. Moreover, domestic species have undergone very rapid selection pressure and may not be suitable models for zoo or wild populations.

**Acknowledgements.** We are grateful to Dr. Renu Wadwha and Dr. Surinder Papiha for the Indian DNA samples and to Ros Richardson for technical assistance. We gratefully acknowledge the American Retinitis Pigmentosa Society and George Gund Foundation for generous support. R.J.B. is supported by an MRC grant and C.F.I. is a recipient of a Wellcome Fellowship. A.J.J. is a Lister Institute Research Fellow. In addition, we would like to thank St. John's Ophthalmic Hospital, Jerusalem for providing facilities for the collection and delivery of the blood samples. Probes 33.6 and 33.15 are the subject of patent applications. Commercial enquiries should be addressed to Cellmark Diagnostics, 8 Blacklands Way, Abingdon, Oxon, OX14 1DY, UK.

## References

- Bell GI, Selby MJ, Rutter WJ (1982) The highly polymorphic region near the insulin gene is composed of simple tandemly repeating sequences. *Nature* 295:31–35
- Burke T, Davies NB, Bruford MW, Hatchwell BJ (1989) Parental care and mating behaviour of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting. *Nature* 338:249–251
- Capon DJ, Chen EY, Levinson AD, Seeburg PH, Goeddel DV (1983) Complete nucleotide sequences of the T24 human bladder carcinoma oncogene and its normal homologue. *Nature* 302:33–37
- Chaconas G, Van der Sande JM (1980) 5'-<sup>32</sup>P labelling of RNA and DNA restriction fragments. *Methods Enzymol* 65:75–85
- Curch GM, Gilbert W (1984) Genomic sequencing. *Proc Natl Acad Sci USA* 81:1991–1995
- Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction fragments to high specific activity. *Anal Biochem* 132:6–13
- Gill P, Jeffreys AJ, Werrett DJ (1985) Forensic application of DNA "fingerprints". *Nature* 318:577–579
- Georges M, Lequarre A-S, Castelli M, Hanset R, Vassart G (1988) DNA fingerprinting in domestic animals using four different minisatellite probes. *Cytogenet Cell Genet* 47:127–131
- Hastie ND, Allshire RC (1989) Human telomeres: fusion and interstitial sites. *Trends Genet* 5:326–331
- Helminen P, Ehnholm C, Lokki M-L, Jeffreys A, Peltonen L (1988) Application of DNA "fingerprints" to paternity determinations. *Lancet* i:574–576

- Hill AVS, Jeffreys AJ (1985) Use of minisatellite DNA probes to determine twin zygosity at birth. *Lancet* II: 1394-1395
- Inglehearn CF, Jay M, Lester D, Bashir R, Jay B, Bird AC, Wright AF, Evans HJ, Papiha SS, Bhattacharya SS (1990) No evidence for linkage between autosomal dominant retinitis pigmentosa and chromosome 3 locus D3S47 (C17): evidence for genetic heterogeneity. *Genomics* 6: 168-173
- Jalili IK, Smith NJD (1988) A progressive conerod dystrophy and amelogenesis imperfecta: a new syndrome. *J Med Genet* 25: 738-740
- Jeffreys AJ, Wilson V, Thein SL (1985a) Hypervariable "minisatellite" regions in human DNA. *Nature* 314:67-73
- Jeffreys AJ, Wilson V, Thein SL (1985b) Individual-specific "fingerprints" in human DNA. *Nature* 316:76-79
- Jeffreys AJ, Brookfield SFY, Semeonoff R (1985c) Positive identification of an immigration test-case using human DNA fingerprints. *Nature* 317:818-819
- Jeffreys AJ, Wilson V, Thein SL, Weatherall DJ, Ponder BAJ (1986) DNA "fingerprints" and segregation analysis of multiple markers in human pedigrees. *Am J Hum Genet* 39: 11-24
- Jeffreys AJ, Wilson V, Kelly R, Taylor BA, Bullfield G (1987) Mouse DNA "fingerprints": analysis of chromosome localisation and germline stability of hypervariable loci in recombinant inbred strains. *Nucleic Acids Res* 15:2823-2826
- Jeffreys AJ, Royle NJ, Wilson V, Wong Z (1988) Spontaneous mutation rates to new length alleles at tandem-repetitive loci in human DNA. *Nature* 332:278-281
- Kuhnlein V, Zadworny D, Dawe Y, Fairfull RW, Gavara JS (1990) Assessment of inbreeding by DNA fingerprinting: development of a calibration curve using defined strains of chickens. *Genetics* 125: 161-165
- Litt M, Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am J Hum Genet* 44:397-401
- Lynch M (1988) Estimation of relatedness by DNA fingerprinting. *Mol Biol Evol* 5: 584-599
- Nakamura Y, Leppert M, O'Connell P, Wolff R, Holm T, Calver M, Martin C, Fujimoto E, Hoff M, Kumlika E, White R (1987) Variable number of tandem repeat (VNTR) markers for human gene mapping. *Science* 235: 1616-1622
- Ponder BAJ, Jeffreys AJ, Hartley NE, Carter C, Easton DF, Telenius H, Telenius-Berg M (1987) Application of minisatellite DNA probes to linkage in MEN-2. *Henry Ford Hosp Med J* 35: 161-163
- Reeve HK, Westneat DF, Noon WA, Sherman PW, Aquadro CF (1990) DNA "fingerprinting" reveals high levels of inbreeding in colonies of the cusocial naked mole rat. *Proc Natl Acad Sci USA* 87:2496-2500
- Ryskov AP, Jichardze AG, Prosnjak MI, Ivanov PL, Limborska SA (1988) M13 phage DNA as a universal marker for DNA fingerprinting of animals, plants and microorganisms. *FEBS Lett* 233: 388-392
- Schafer R, Zischler H, Epplen JT (1988) (CAC)<sub>n</sub>, a very informative oligonucleotide probe for DNA fingerprinting. *Nucleic Acids Res* 16:5196
- Thein SL, Jeffreys AJ, Blacklock HA (1986) Identification of post-transplant cell population by DNA fingerprint analysis. *Lancet* II: 37
- Vassart G, Georges M, Monsieur R, Brocas H, Lequarre A-S, Christophe D (1987) A sequence in M13 phage detects hypervariable minisatellites in human and animal DNA. *Science* 235:683-684
- Weitzel JN, Hows JM, Jeffreys AJ, Min GL, Goldman JM (1988) Use of a hypervariable minisatellite probe (33.15) for evaluating engraftment two or more years after bone marrow transplantation for aplastic anaemia. *Br J Haematol* 70:91-97
- Wetton JH, Carter RE, Parkin DT, Walters D (1987) Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature* 327: 147-149





