Am J Hum Genet 38:235–252, 1986

Human Chromosome Variation: The Discriminatory Power of Q-band Heteromorphism (Variant) Analysis in Distinguishing between Individuals, with Specific Application to Cases of Questionable Paternity

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SUMMARY

The chromosomes from 57 persons were analyzed by means of quinacrine fluorescent staining in order to assess the amount of variation and the discriminatory power of Q-band heteromorphism analysis. Chromosomes 3, 4, 13, 14, 15, 21, 22, and Y of each person were visually compared to those of 56 others, for a total of 1,596 comparisons. No two persons were found to have the same set of variants. The number of differences between chromosomes for each comparison ranged from 2 to 12 out of a possible total of 14 for females and 15 for males. Relatives were also distinguishable, and differences ranged from two to seven. We used the frequency with which each chromosome was useful for telling two people apart, and estimated the probability of finding two persons with the same set of quinacrine variants as .0003. Distinctly different heteromorphisms were found in the 39 unrelated persons for each of the chromosomes examined. In this small population, the number of different sets of variants observed for chromosomes 3, 4, 13, 14, 15, 21, 22, and Y were six, seven, 27, 16, 20, 15, 24, and five, respectively, for a total number of possible combinations of 1.14×10^{15}.

As a test of the usefulness of chromosome heteromorphisms in paternity cases, 12 father-mother-child trios of virtually certain paternity, owing to the father-child segregation of a rare structural rearrangement, were coded and recombined at random to produce 120 cases of uncertain paternity. When the code was broken, 108 "alleged fathers" had been excluded correctly and the 12 biological fathers had been included correctly.

Received March 15, 1984; revised July 12, 1985.

This research was supported in part by grants from Maternal and Child Health Services 920, and the N. L. Tartar Trust, Medical Research Foundation of Oregon.

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Variants for the 39 unrelated persons were scored according to a modified version of the system proposed at the Paris Conference. We calculated the probability of excluding the wrongfully accused man for each chromosome based on the frequencies of specific variants found in this population. The calculations took into consideration not only the observed sets of variants, but also all possible combinations of the specific variants. The individual probability of exclusion for each chromosome was then used to calculate a cumulative probability of exclusion for all of these chromosomes of 1.0000.

INTRODUCTION

Chromosome variability among normal people has been acknowledged and examined by many investigators [1–7]. With improved methods for chromosome preparation and the availability of multiple staining techniques, it seems likely that each person will prove to be unique [3]. Differences in the heterochromatic regions as distinguished through specific stains have been utilized to identify chromosomes for several purposes. These include establishing parental origin of chromosome abnormalities [8–15], establishing the parthenogenetic origin of an ovarian teratoma [16], and distinguishing maternal from fetal cells in amniotic fluid cultures [17] and donor from recipient cells in bone marrow transplants [18, 19].

Recently, this cytogenetic technology has proved useful in excluding wrongfully accused men in paternity disputes [20–24]. Other polymorphic systems that are routinely tested are red cell enzymes and antigens, serum proteins, and HLA. The first three are often referred to as extended red cell testing. The more systems that are examined, the greater the chances are of excluding a falsely accused man. Using a combination of extended red cell and HLA testing, there is the potential for excluding a wrongfully accused man approximately 99% of the time [25]. The addition of chromosome heteromorphism analysis is making it possible to achieve a higher probability of exclusion.

As with routine paternity testing involving blood groups and HLA, chromosomal exclusions are fairly straightforward. There is difficulty, however, in drawing conclusions in a case when there is no exclusion. In evaluating extended red cell and HLA testing results to estimate a probability of paternity when there is no exclusion, one must rely on gene frequencies in the population. Adequate frequency data are not available for chromosome variants.

We have sampled a small population and have analyzed each individual by means of quinacrine banding to reveal as much information as possible with the technology now available. This procedure has enabled us to draw some conclusions about the amount of variation in this population, as well as to assess the discriminatory capacity of Q-banded chromosomes in distinguishing between individuals. In addition, we have tested the system on cases of known paternity and nonpaternity. Finally, we have calculated the probability of excluding a

wrongfully accused man with Q-banded chromosomes on the basis of frequencies of specific variants in this population.

MATERIALS AND METHODS

Three approaches were used to assess the amount of variation in a population, the degree of ability to distinguish between individuals, and the probability of exclusion in cases of disputed paternity: (1) visual comparison of chromosome variants from 57 people and calculation, based on the ability to distinguish between them, of the chance of finding two people with the same set of variants; (2) creation of paternity cases by randomly recombining fathers from trios of near-certain paternity (due to the presence in father and child of a rare chromosome translocation) with all mother-child pairs, and blindly assessing paternity; and (3) compilation of frequencies of all quinacrine variants in a population of 39 unrelated people and subsequent calculation of probability of exclusion using all combinations of these variants.

Identification of Subjects

Two groups of subjects were examined chromosomally. The first group comprised 57 Caucasian persons, 39 of whom were unrelated, from three laboratories at the Oregon Health Sciences University (OHSU): clinical cytogenetics, paternity testing, and infertility. Families were chosen at random from the first two laboratories, and individual patients were chosen at random from the latter. This group was used to examine the degree of chromosome variability between individuals and to estimate a cumulative probability of exclusion (CPE) on the basis of the variability within this population.

The second group included 12 father-mother-child trios from 10 families in which a paternal translocation was segregating. These families, in which father and child had the same rare translocation, constituted a group of virtually certain paternities. They were selected from patients studied in the Clinical Cytogenetics Laboratory, OHSU.

Methods

Peripheral blood was cultured according to standard methods. Slides were prepared, stained with quinacrine [26], and examined under a Zeiss photoscope III. Ten to 14 well-spread, well-stained metaphases were photographed. One full karyotype and two to eight serially printed composites [27] of chromosomes 3, 4, 13, 14, 15, 21, 22, and Y (in males) were prepared for each of the 57 persons.

The heteromorphic regions of the chromosomes of each person were compared to those of the other 56. A conservative approach was taken in designating two variant regions as different. When a question arose as to the similarity between variants, the variants were considered the same. In an actual paternity case, further serially printed cells would be examined that would give a more accurate representation of the variants and possibly allow a distinction between the two. The number of differences present in each comparison and the range of variation for each of the seven chromosomes analyzed in this population were determined.

The 12 translocation families were examined for consistent Mendelian inheritance of variant chromosomes. The rearrangements did not involve chromosome regions containing the variants being compared. The partial karyotypes of the translocation fathers were changed at random and coded by a cytogeneticist not involved in the study, who kept the proper mother-child pairs together so that the paternity in the 120 resulting triads was in question. Cases were independently analyzed by two cytogeneticists. The accuracy of the chromosome analysis results was then confirmed by examination of the records, which indicated whether the alleged father and child in each triad were carriers of the same translocation.

Fluorescent chromosome variants in the group of unrelated persons were scored according to a modified version of the system proposed in 1971 at the Paris Conference

TABLE 1

SCORING OF HUMAN CHROMOSOME HETEROMORPHISMS STAINED WITH QUINACRINE

Score	Satellite	Stalk	Internal standard
Size:			
0	Absent	Absent	...
1	Very small	Very short	...
2	Small	Short	...
3	Intermediate	Intermediate	Yp
4	Large	Long	18p
5	Very large	Very long	...
Intensity:			
0	No fluorescence	...	...
1	Almost no fluorescence	...	Distal 1p
2	Pale	...	...
3	Medium	...	Two broad bands, 9q
4	Intense	...	...
5	Brilliant	...	Yq
6	Visible by serials only	...	...

NOTE: Scoring was modified from Paris Conference, 1971.

(table 1). Frequencies were calculated for specific variants in the juxtacentromeric region of chromosomes 3 and 4; the short arm (p11), stalk (p12), and satellites (p13) of chromosomes 13, 14, 15, 21, and 22 (see fig. 1); and the Y long arm. Categories were combined for the scores that were most often confused on rescoring.

The probability of exclusion (PE) was calculated for each "locus" on the eight different chromosomes by the following formula:

$$PE = \sum_{i=1}^{n} P_i(1 - P_i)^2 + \sum_{\substack{i,j \\ i<j}}^{n} (P_iP_j)^2(3P_i + 3P_j - 4) \ ,$$

[28], where P is the frequency of an allele designated i, j . . . n. The PE, or average power of exclusion, A, as described by Garber and Morris [28], measures the probability that a

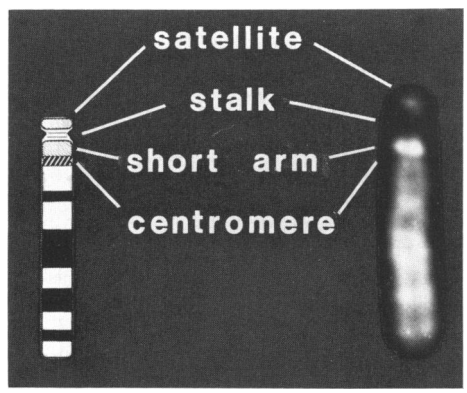

FIG. 1.—Ideogram and photograph of chromosome 13 illustrating independently varying regions of acrocentric chromosomes. The variants depicted here are scored 11 (satellite), 3 (stalk), and 24 (short arm). See table 1 for scoring system.

polymorphic locus will exclude a wrongfully accused man, and, therefore, indicates the usefulness of the system. It is dependent only on the allele frequencies in a population and not on the specific information in any given paternity case. (For derivation of formula, see [28]).

Using each independently varying polymorphic segment as a locus, the cumulative probability of exclusion (CPE) for each chromosome, as well as for all eight chromosomes, was then calculated with the formula: CPE = PE1 + PE2 − (PE1 × PE2). This represents the probability of excluding a man at locus 1 or locus 2, subtracting the chance of excluding at both loci. The CPE for an individual chromosome indicates the probability of excluding a falsely accused man by using the variants present on that chromosome alone. The CPE for all chromosomes examined demonstrates the likelihood of excluding a falsely accused man by considering all variant regions on those chromosomes.

RESULTS

Population Data

In this study, 57 persons (39 unrelated) were compared with one another for a total of 1,596 comparisons. Each was found to be unique in the combination of quinacrine heteromorphisms present on chromosomes 3, 4, 13, 14, 15, 21, 22, and Y. Table 2 lists the total number of comparisons in which specific chromosomes differed between individuals. In comparing a specific pair of chromosomes in two different people, a total of four homologous chromosomes was being considered. The four chromosomes have, therefore, been referred to as homologs. For each chromosome, the totals have been broken down into those comparisons in which two or three homologs differed and those in which all four homologs differed. Chromosomes 15 and 22 were most often informative in attempts to distinguish between two persons, and chromosomes 3, 4, and Y proved to be the least informative. Comparisons of related persons have been included and show that even they can be distinguished.

TABLE 2

COMPARISON OF 57 PERSONS

	No. distinguishable comparisons				
Chromosome	Two to three different chromosomes	All four different chromosomes	Total	P	1 − P
3	434 (11) [2]	90	524	.328	.672
4	260 (3)	33	293	.184	.816
13	853 (21) [2]	420	1,273	.798	.202
14	931 (17) [1]	155	1,086	.680	.320
15	839 (25) [2]	463	1,302	.816	.184
21	869 (20) [1]	210	1,079	.676	.324
22	919 (25)	414 [1]	1,333	.835	.165
Y	...	...	136	.229	.771

NOTE: Total comparisons = 1,596 (595 male-male). Symbols: (), child-parent; [], sib-sib. Abbreviations: P, proportion of comparisons in which chromosome was informative: 1 − P, proportion of comparisons in which chromosome was not informative. $(1 - P_3)(1 - P_4)(1 - P_{13})(1 - P_{14})(1 - P_{15})(1 - P_{21})(1 - P_{22})(1 - P_Y)$ = theoretical proportion of comparisons in which none of the eight chromosomes would be informative = .0003.

The number of informative chromosomes per comparison between individuals differed from one comparison to another. Table 3 illustrates the distribution of the number of informative chromosomes per comparison. No comparison had less than two informative chromosomes. The range was 2 to 12 differences out of a possible 14 in females and 15 in males. Even though no two persons were identical, we were interested in estimating the frequency with which two persons might possess the same set of quinacrine variants. We arrived at this frequency by using the figures for the frequency with which each chromosome distinguished between two persons. We designated P as the proportion of comparisons out of 1,596 in which a specific chromosome was informative, and 1 − P as the frequency of its being noninformative. On this basis, $(1 - P_3)(1 - P_4)(1 - P_{13})(1 - P_{14})(1 - P_{15})(1 - P_{21})(1 - P_{22})(1 - P_Y)$ equaled the expected frequency of no informative chromosomes. In this population, the expected frequency of zero differences was .0003 (table 2).

For each acrocentric chromosome, there are four independent regions of continual variation: the centromere, short arm, stalk, and satellite. These regions may vary in size, shape, and intensity (fig. 1). The different variants in each of these regions can be considered comparable to alleles at each of four loci. Similarly, the juxtacentromeric regions of chromosomes 3 and 4 and the Y long arm may be considered alleles of varying size and intensity. Figures 2–7 illustrate distinct heteromorphisms in the population of 39 unrelated persons. By noting the number of different "alleles" in this population, we were able to estimate a possible number of combinations. To calculate a minimum estimate for quinacrine variants, we treated each set of four variable regions for each acrocentric as a whole. Table 4 lists the number of observed variants. The possible number of combinations for each chromosome was then calculated by

TABLE 3

COMPARISON OF 57 PERSONS

No. informative chromosomes per comparison	Total comparisons
2	19 (3)
3	185 (8)
4	258 (10) [1]
5	320 (7)
6	314 (2) [1]
7	238 (1)
8	158
9	62
10	34
11	6
12	2
	1,596

NOTE: Symbols: (), child-parent; [], sib-sib.

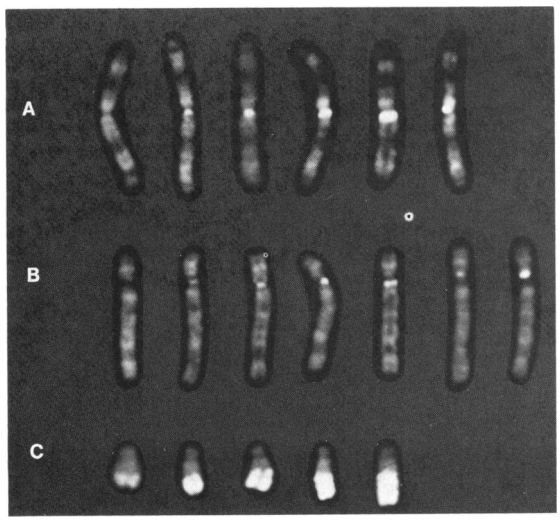

FIG. 2.—Variants of chromosomes 3 (*A*), 4 (*B*), and Y (*C*) from a population of 39 unrelated individuals. Chromosomes 3 and 4 vary in the juxtacentromeric region. *A*-6, *B*-6, and *B*-7 have brightly fluorescing heterochromatin inverted into the short arm. The scores range from 00 (*A*-1) to inv 25 (*A*-6) for chromosome 3 and from 00 (*B*-1) to 24 (*B*-7) for chromosome 4. The fluorescent portion of the Y long arm varies between males, with scores ranging from 1 (*C*-1) to 5 (*C*-5).

the formula $[N(N - 1)/2] + N$, where N equals the number of different variants for a particular chromosome. (Note: N must be added to the general formula for ordered combinations to account for those instances in which both homologs appear to have the same variants.) By multiplying the latter together, we arrived at a conservative estimate of 1.14×10^{15} possible combinations. We also calculated a theoretical number of possible combinations using the scoring categories from table 1. This figure was 6.76×10^{42}, treating each of the four variable regions in the acrocentric chromosomes independently.

The translocation families we studied demonstrated Mendelian inheritance of all chromosome variants, with one exception. The child in one family had one chromosome 14 variant consistent with inheritance of this homolog from his father. The other homolog had a large, bright satellite that was inconsistent with inheritance of either of the mother's chromosomes 14. The mother had a chromosome 14 with a satellite that was as bright, but only half the size. New slides stained with quinacrine produced the same results. All other variants in the child were consistent with Mendelian inheritance of chromosomes from both parents. We postulated that the child's large, bright satellite was a new heteromorphism resulting from duplication of the mother's heterochromatin.

Cases of Unknown Paternity

One hundred twenty cases of unknown paternity were created by assorting chromosome sets of the translocation fathers with all 12 mother-child pairs, so that all possible combinations were represented. Cases were coded so that the

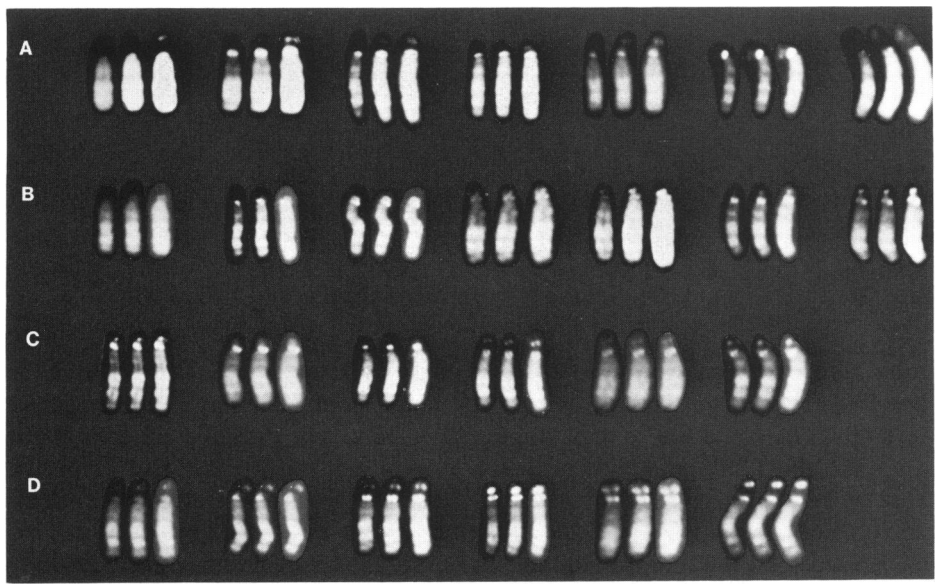

FIG. 3.—Twenty-six different variants of chromosome 13 from a population of 39 unrelated people. Each chromosome is serially printed to reveal heteromorphisms not visible at an exposure generally chosen to define the overall banding pattern. *A*-1–*A*-7 have satellites that would not have been observed at a routine exposure. Scores are determined by comparisons of serial prints against standards, including an internal standard. Very short, medium to intense short arms (scored 13, 14, and 15) as in *C*-1 or *D*-3 are relatively common on chromosomes 13, whereas bright short arms on chromosomes 14 and 15 are less frequent.

identities of the persons were unknown. Two cytogeneticists independently decided whether or not a particular man was the biological father of a child and agreed upon 108 exclusions and 12 inclusions. The code was broken to reveal that all conclusions were correct.

Heteromorphism Frequency Data

Frequency results for each "allele," or variable region, observed in the 39 unrelated persons are listed in table 5. The first number is the score for size, the second is for intensity. For example, a satellite with a score of 23 is small (2) in size and of medium (3) intensity. Variants that were relatively more common included very short, intermediate to bright intensity short arms on chromosome 13 (scored 13, 14, and 15) and intense (4) to brilliant (5) intensity satellites on chromosome 15. Bright heterochromatin was more often inverted into the short arm in chromosome 4 (13 of 39 people) than in chromosome 3 (three of 39 people). Large to very large (4–5) and intense to brilliant intensity (4–5) satellites were uncommon for any acrocentric chromosome. Serial printing revealed satellites on acrocentrics that would otherwise have gone undetected in 6% of the acrocentric homologs.

The frequency data was used to calculate the PE for each chromosome. Table 6 lists these figures, as well as the CPE of 1.0000 for all seven (female)

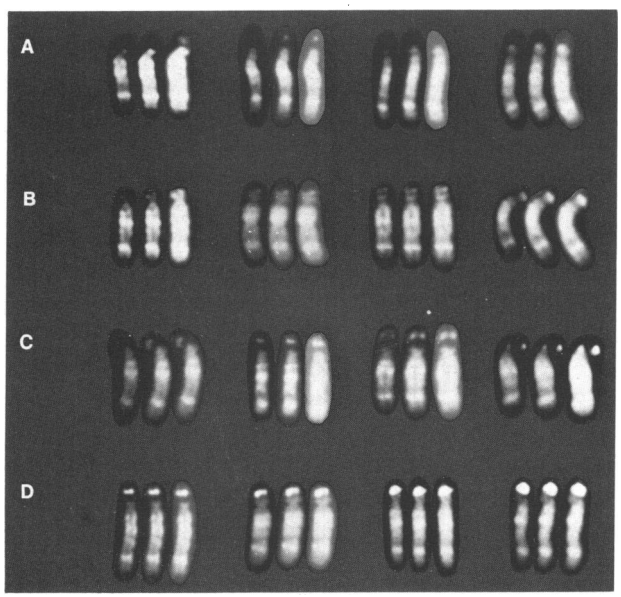

FIG. 4.—Sixteen different variants of chromosome 14 from a population of 39 unrelated people. Each chromosome is serially printed to reveal heteromorphisms not visible at an exposure generally chosen to define the overall banding pattern.

and eight (male) chromosomes. (See MATERIALS AND METHODS section for discussion of formulas used in these calculations.)

DISCUSSION

The finding that each subject had a different combination of chromosome heteromorphisms by Q-banding supports the evidence for the uniqueness of individual karyotypes [2, 3, 6]. Particularly striking is the fact that even the relatives could be distinguished by quinacrine staining alone. As would be expected, the range of differences between family members (2–7) was not as broad as that between unrelated persons (2–12). The frequency with which sibs can be expected to have identical sets of variants is $(1/4)^7$ or 6.1×10^{-5} (except for identical twins, in which case the frequency is 1.00). Two sib-sib comparisons in this study and two others not reported here showed differences of six, six, four, and six chromosomes. The chromosomal data on one set of identical twins was consistent with the evidence for identical sets of variants, that is, the twins shared not only variants of those chromosomes studied here, but also a fragile 16q.

In their paper on the use of chromosome variants in distinguishing maternal from fetal cells in amniotic fluid cultures, Hauge et al. [17] made 50 mother-fetus comparisons. In the 10 chromosome pairs examined, differences were found between six or more chromosomes in 56% of the comparisons. No mother-fetus pair had less than two differences. We had a similar experience in our group of 1,596 comparisons: we demonstrated six or more differences in

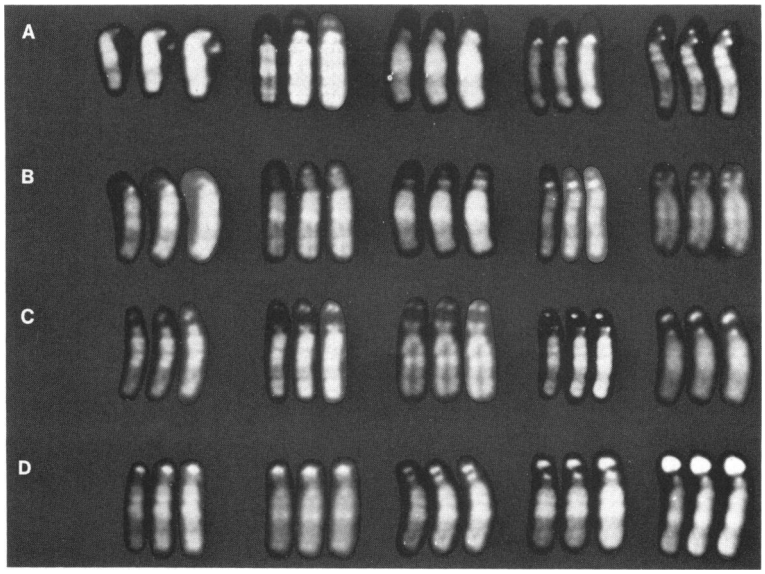

FIG. 5.—Twenty different variants of chromosome 15 from a population of 39 unrelated people. Each chromosome is serially printed to reveal heteromorphisms not visible at an exposure generally chosen to define the overall banding pattern. The large, bright satellites in *D*-5 (scored 55) are relatively uncommon and were present in only one person in the population studied.

51% of the comparisons and no less than two differences between individuals. In the subset of parent-child comparisons, 10 of 21 (48%) had six or more differences and none showed less than two.

Despite the fact that no less than two differences were observed per comparison, the chance of finding two randomly selected persons with an identical set of quinacrine variants was calculated to be .0003 by means of our data on how often each chromosome was useful in distinguishing between two persons in

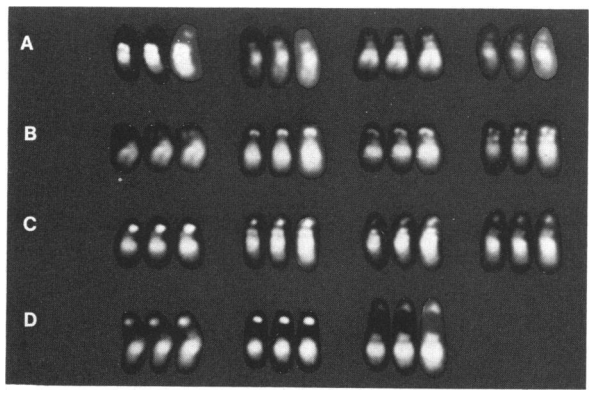

FIG. 6.—Fifteen different variants of chromosome 21 from a population of 39 unrelated people. Each chromosome is serially printed to reveal heteromorphisms not visible at an exposure generally chosen to define the overall banding pattern.

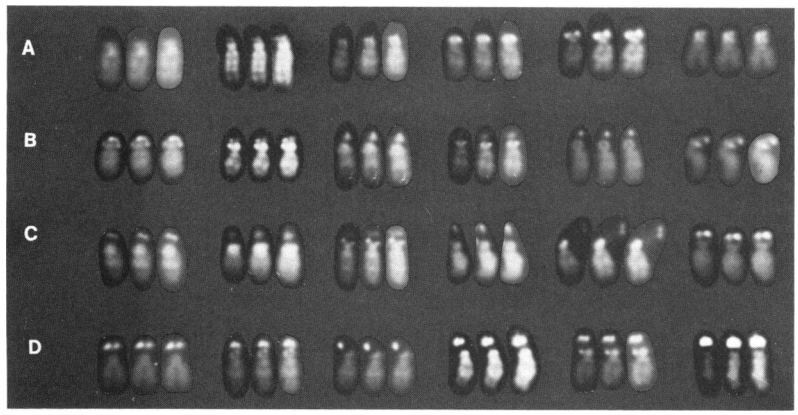

FIG. 7.—Twenty-four different variants of chromosome 22 from a population of 39 unrelated people. Each chromosome is serially printed to reveal heteromorphisms not visible at an exposure generally chosen to define the overall banding pattern. The large, bright satellites in *D*-6 (scored 45) are relatively uncommon and were present in only one person in the population studied.

this study. This means that even with a conservative estimate of the amount of variation, one would expect to distinguish between two persons at least 99.97% of the time. This figure would most likely be higher had more analysis of the variants been undertaken in cases where questions arose as to the similarity of two specific variants, rather than calling them the same.

There is evidence that there is a difference in proportion of specific chromosome variants between populations [29–36]. For example, Lubs et al. [31] found that the U.S. black population that they studied had a greater preponderance of bright Q-band heteromorphisms than did their white population.

TABLE 4

POSSIBLE COMBINATIONS OF HETEROMORPHISMS OBSERVED IN A POPULATION OF 39 PERSONS

Chromosome	No. different variants (N)	Possible combinations (PC)
3	6	21
4	7	28
13	27	378
14	16	136
15	20	210
21	15	120
22	24	300
Y	5	5

NOTE: The four variant regions of chromosomes 13, 14, 15, 21, and 22 were treated as a whole. Abbreviations: N, number of different variants; PC, possible combinations. $PC = [N(N - 1)]/2 + N$. $PC_{Total} = (PC_3)(PC_4)(PC_{13})(PC_{14})(PC_{15})(PC_{21})(PC_{22})(PC_Y) = 1.14 \times 10^{15}$.

TABLE 5

VARIANT REGION SCORES FOR CHROMOSOMES 3, 4, 13, 14, 15, 21, 22, AND Y IN 39 UNRELATED PERSONS

	13p13		13p12		13p11		21p13		21p12		21p11	
	S/I	N(P)	S	N(P)	S/I	N(P)	S/I	N(P)	S	N(P)	S/I	N(P)
	00	9 (.1169)	0	20 (.2547)	12	10 (.1299)	00	6 (.0769)	0	18 (.2308)	11+12	39 (.5000)
	11+12	32 (.4156)	1	27 (.3507)	13	26 (.3377)	11+12	34 (.4359)	1	37 (.4743)	13	36 (.4616)
	13	14 (.1818)	2	17 (.2208)	14+15	31 (.4026)	13	19 (.2436)	2	19 (.2436)	14	1 (.0128)
	14	2 (.0260)	3	9 (.1169)	22	1 (.0130)	14	4 (.0514)	3	3 (.0385)	22	1 (.0128)
	16	7 (.0909)	4	4 (.0519)	23	3 (.0389)	16	3 (.0385)	4	1 (.0128)	23	1 (.0128)
	21+22	4 (.0519)		...	24	5 (.0649)	21+22	2 (.0256)		...		...
	23	4 (.0519)		...	45	1 (.0130)	23	2 (.0256)		...		...
	24+34	3 (.0390)		...		...	24+34+35	7 (.0897)		...		...
	26	1 (.0130)		...		...	45	1 (.0128)		...		...
	45	1 (.0130)		...		...		...		...		...
Total	10	77 (1.0000)	5	77 (1.0000)	7	77 (1.0000)	9	78 (1.0000)	5	78 (1.0000)	5	78 (1.0000)

	14p13		14p12		14p11		22p13		22p12		22p11	
	S/I	N(P)	S	N(P)	S/I	N(P)	S/I	N(P)	S	N(P)	S/I	N(P)
	00	13 (.1688)	0	27 (.3507)	11+12	40 (.5194)	00	7 (.0897)	0	30 (.3847)	11+12	29 (.3718)
	11+12	24 (.3117)	1	35 (.4545)	13	16 (.2078)	11+12	31 (.3973)	1	31 (.3973)	13	27 (.3462)
	13	23 (.2987)	2	11 (.1429)	14+15	2 (.0260)	13	22 (.2824)	2	12 (.1539)	14	11 (.1411)
	14	5 (.0649)	3	4 (.0519)	21+22+32	11 (.1429)	14	7 (.0897)	3	3 (.0385)	22	7 (.0897)
	16	7 (.0909)		...	23+33	8 (.1039)	21+22	2 (.0256)	4	1 (.0128)	23	2 (.0256)

	21+22	2 (.0260)	...	...	23	2 (.0256)	5	1 (.0128)	24+25	2 (.0256)		
	24	1 (.0130)	...	...	24+25	6 (.0769)		...		...		
	45+55	2 (.0260)	...	...	45	1 (.0128)		...		...		
Total	8	77 (1.0000)	4	77 (1.0000)	5	77 (1.0000)	8	78 (1.0000)	6	78 (1.0000)	6	78 (1.0000)

	15p13		15p12		15p11		3q11		4q11		Yq12	
	S/I	N(P)	S	N(P)	S/I	N(P)	S/I	N(P)	S/I	N(P)	S	N(P)
	00	4 (.0514)	0	29 (.3718)	11+12	35 (.4487)	00	5 (.0641)	00	29 (.3718)	1+2	19 (.7600)
	11+12	31 (.3973)	1	35 (.4487)	13	14 (.1795)	11	1 (.0128)	11	2 (.0256)	3	4 (.1600)
	13	12 (.1539)	2	11 (.1411)	14	1 (.0128)	12	2 (.0256)	13	19 (.2436)	4	1 (.0400)
	14	10 (.1282)	3	2 (.0256)	21+22+31	18 (.2308)	13	21 (.2693)	14	12 (.1539)	5	1 (.0400)
	16	5 (.0641)	4	1 (.0128)	24	1 (.0128)	14	10 (.1282)	23	1 (.0128)		...
	22	1 (.0128)		...	23+33	9 (.1154)	23	2 (.0256)	24	1 (.0128)		...
	23	2 (.0256)		...		...	24	16 (.2052)	34	1 (.0128)		...
	24+25+34+35	12 (.1539)		...		...	25	10 (.1282)	inv 13	4 (.0514)		...
	55	1 (.0128)		...		...	34	2 (.0256)	inv 14	7 (.0897)		...
		...		...		...	35	4 (.0514)	inv 24	1 (.0128)		...
		...		...		...	45	2 (.0256)	inv 35	1 (.0128)		...
		...		...		...	inv 24	1 (.0128)		...		...
		...		...		...	inv 25	2 (.0256)		...		...
Total	9	78 (1.0000)	5	78 (1.0000)	6	78 (1.0000)	13	78 (1.0000)	11	78 (1.0000)	5	25 (1.0000)

NOTE: Abbreviations: S, size; I, intensity; N, no. homologs; P, proportion; p13, satellite; p12, stalk; p11, short arm; q11, long-arm juxtacentromeric band; Yq12, fluorescent portion of Yq.

TABLE 6

PROBABILITY OF EXCLUSION FOR EIGHT CHROMOSOMES

Chromosome	PE	CPE
3	.6925	.6925
4	.5662	.8666
13	.8888	.9852
14	.8421	.9977
15	.8561	.9997
21	.7819	.9999
22	.8500	1.0000
Y	.3808	1.0000

NOTE: Abbreviations and formulas: PE, probability of exclusion; CPE, cumulative probability of exclusion;

$$PE = \sum_{i=1}^{n} P_i(1 - P_i)^2 + \sum_{\substack{i,j \\ i<j}}^{n} (P_iP_j)^2(3P_i + 3P_j - 4)$$

[26]; CPE = PE1 + PE2 − (PE1 × PE2).

Ibraimov and Mirrakhimov [35] found differences in the frequency of the bright inverted Q-heterochromatin band in chromosome 3 between Russian (6.0%) and Asian Mongoloid (0.3%–3.0%) populations. The significance of these differences, as well as the role of heterochromatin in general, is unknown.

Our second approach to assessing the usefulness of chromosome analysis as a discriminatory tool was to create 120 cases of questionable paternity from families of known paternity. The problem of defining such families was acknowledged as early as 1922 by Ottenberg in the second of a series of papers on blood testing and its application to answering medicolegal questions [37]. He emphasized the dangers of "secret illegitimacy" in any study of human heredity. The estimated rate of illegitimate births in the U.S. is 17% (National Center for Health Statistics, DHHS, 1978). In addressing this problem, we have restricted our families to those in which paternity was virtually certain due to the father-child transmission of a rare chromosome translocation. One hundred eight alleged fathers were correctly excluded, and 12 were correctly included for a 100% accuracy. The probability of arriving at these conclusions by chance alone is 1/10! or 2.8×10^{-7} (two pairs of sibs had the same father).

Our third approach to arriving at an accurate estimate of the power of heteromorphism analysis extends beyond the finite number of variants in this population. We have observed, as have others, other combinations of the variants reported here, as well as many heteromorphisms not represented in our data. These other possibilities can be considered using accepted statistical methods.

Using frequencies of variants in a defined population, one can decide the overall usefulness of a test system, or the PE. This figure indicates what the chances are that a wrongfully accused man will be exonerated by means of

the particular polymorphic locus. Such figures have been calculated for the blood group and HLA systems based on gene frequencies in the population. The individual PEs for each locus are added for a combined PE (CPE). (See MATERIALS AND METHODS section for formulas.) The CPEs in the white population (with black CPEs in parentheses) for red cell antigens, serum proteins, red cell enzymes, HLA (A, B), and, finally, all systems combined are: .72 (.61), .76 (.69), .71 (.65), .87 (.87), and .99 (.99), respectively [25].

Recognizing the small sample size, we estimated the frequencies of specific variants in this population. A CPE of 1.0000 for chromosomes 3, 4, 13, 14, 15, 21, and 22 was calculated. This considers all possible combinations of the variants observed in the population studied, although examples of all of these combinations were not present. Our figure is in contrast to Niebuhr and Gürtler's figures of .72 for females and .74 for males [38]. It is difficult to compare our variant frequencies with those of this group because different criteria were used in the scoring. Our system is modified from that proposed in the Paris nomenclature and is similar to that used by McKenzie and Lubs [2] and Van Dyke et al. [6]. This difference in procedure illustrates one of the problems in compiling adequate population frequency data. Not only are there varied methods of scoring, but there also is a lack of uniformity in the quality of the material being scored. Recent improvements in cytogenetic techniques have allowed better visualization of the variants present on these chromosomes. We believe that each analyzed chromosome contains variants, whether there is a bright marker or not. The absence of a satellite may be just as informative as the presence of one, depending on the other variants present in the karyotypes of each person involved in a particular case.

Taking into consideration the evidence presented here and in the literature for the uniqueness of the individual and the calculations based on our population data, it seems reasonable to estimate the probability of distinguishing between two people by quinacrine-stained chromosomes to be 99.97%–100% and the CPE to approach 100%.

Even with high-quality material, the variation seen in chromosome heteromorphisms is a continuum and it is difficult to assign these variants to specific categories. It is our experience that certain variants, such as short, short arms, are more frequently scored differently on blind rescoring. Other variants (for example, the length of stalks) cover such a wide range that discrepancies seldom occur. For this reason, we have not made it a general rule to use a two-score difference for distinguishing true differences between variants, but have, rather, applied it to specific categories. Many investigators have scored, then blindly rescored individuals to show a discrepancy in a small proportion of their cases [2, 6, 38]. However, those subjects most frequently scored differently were the ones for which the chromosome preparations were of poor to mediocre quality. The fact remains that in any scoring system, information is lost. Individual cases of questionable parentage are best resolved through visual comparison of variants from a number of serially printed metaphases rather than by comparison of scores given to those variants.

Although chromosome heteromorphism analysis has not yet been accepted

as a routine test in paternity disputes, it is being used in a limited number of cases in which neither HLA nor blood group testing has led to an exclusion. In a recent case, we were able to exclude one of two brothers named as alleged fathers after no exclusions were found with HLA, red cell enzymes and antigens, and serum proteins [24]. This illustrates the value of chromosome analysis in cases involving related alleged fathers or alleged fathers related to the mother.

Owing to the time and expertise involved in preparing and analyzing chromosomes in paternity cases, the cost is quite high in comparison with other methods of testing. We, therefore, suggest a stepwise protocol in which chromosome heteromorphism analysis is done only after no exclusions are found with red cell antigens and enzymes, serum proteins, and HLA.

The legal community is very receptive to new scientific developments that will aid in the establishment of paternity and subsequent collection of child support. Mathon [39] suggested that there be legislation allowing all proven scientific testing results admitted as evidence into the courts, rather than specifying particular tests by name. This allows for the acceptance of inevitable scientific advancements and seems extremely pertinent in light of the data presented here.

ACKNOWLEDGMENTS

We thank Drs. Nancy Alexander, Robert Koler, David Linder, Kuo Hwa Lu, and Jeffrey Morris for their helpful discussions and constructive criticisms. We appreciate greatly the technical assistance of Leland Allen, Sande Barton, Libby Clark, Lore Friend, Jeff Inahara, Robert Sheehy, Diane Tomar, and Shelley Winn. We are grateful to Drs. Lynn Ryals, James Geyer, and Jerry Morrisey, Roche Biomedical Laboratory, for sending us the paternity case involving two brothers.

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