Studies on African Pygmies. I. A Pilot Investigation of Babinga Pygmies in the Central African Republic (with an Analysis of Genetic Distances)

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There are still a few human populations which live a life of hunting and food gathering, as must have been true of all human beings throughout the Pleistocene. Among them, African Pygmies are perhaps the most important group. It was considered of interest to investigate their population structure, demography, and genetics, since the destruction of the forest in which they live, or other causes, may soon result in the disappearance of these people, or at least of their culture.

African Pygmies are interesting not only because their origin and position in African anthropology are still uncertain but, in addition, they are an example of a population which still lives according to a highly primitive economy. This may have an influence on genetic structure and therefore on evolutionary rates. Naturally, it is difficult to expect that their culture is unchanged since the Pleistocene. It may however be possible to understand what changes have been due to contact with neighboring cultures.

Pygmies may have in the past occupied the whole tropical forest belt of Africa. Today there are several scattered groups of Pygmies with large gaps between them, but they still have extensive cultural similarities. It is likely that they were once a single group, but the time of separation is not known. Whenever they have been in contact with other African cultures, their own culture has been affected to some extent. Some groups, and especially the peripheral parts of most groups, are undergoing acculturation, mostly in the sense of a transition to a simple type of agriculture.

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FIG.1.—Location of principal Pygmy groups in Africa. Area covered by the forest is shaded. Major Pygmy groups are circled with solid lines and minor groups with dotted lines. *1*, Babinga; *2*, Baka; *3*, Badjelli; *4*, Bekwa; *5*, Akoa; *6*, Akka; *7*, Efe; *8*, Baswa; *9*, Batswa; *10*, Batwa. (From Biasutti, 1959, modified.)

Contacts with, and therefore influence of, Western civilization have been almost negligible.

One large group of Pygmies, named Babingas, live in an area at the boundaries between the Central African Republic, Cameroun, and Congo Brazza (Fig. 1). Little is known of this group, which may number on the order of 15,000 to 30,000. (Only very incomplete censuses are available.) Survival of the group and their culture, and the ignorance concerning them, must be largely a consequence of the relative inaccessibility of the region they inhabit.

Previous knowledge of this group is limited to anthropometric studies (see Vallois, 1940; Lalouel, 1949; Cresta, 1964). Blood group and sickle cell hemoglobin data are available for related Pygmy groups living at some distance from the group investigated by us (Olivier, 1947; Miletto, 1951; Ravisse, 1952).

The object of this paper is to report the results of a pilot study made there in the winter of 1966, mostly with a view to selecting targets for further research. During this visit, information on a number of genetic markers of a sample of Babinga

TABLE 1

REPORTED AGES OF SUBJECT

	Age (in Years)						
	<25	25–30	30–35	35–40	40–45	45–50	>50
Males	3	11	4	10	21	22	23
Females	3	6	8	22	12	7	2

Pygmies was collected. Since then, further information has become available from the continuation of the study, but its analysis will require time. Meanwhile, it is believed that the publication of this material can be useful.

SUBJECTS

The present sample was formed of adults of the population of the prefecture of M'Baiki and was obtained from two villages, 32 from Grima, immediately north of the Lobaye River (4°1′ N., 17°3′ E.), and the rest (142) from three Pygmy settlements in the Bagandou region, just south of the Lobaye (3°45′ N., 17°48′ E.).

Pictures were taken of all Pygmies sampled, and blood was collected, using blood donor sets, in vacuum tubes that had no anticoagulant (for serum) or that had ethylenediaminetetraacetic acid (EDTA) or acid-citrate-dextrose (ACD) (for plasma). Blood samples were kept in a battery-operated refrigerator, where they were stored immediately after collection. Samples were centrifuged to obtain serum and were subsequently sent by air freight to Amsterdam in thermostatic containers to insure arrival of the samples in excellent condition.

The age of the subjects was estimated by local "experts," as the Pygmies were unable to state their ages. The distribution of ages thus obtained is given in Table 1, but it is difficult to evaluate the reliability.

The average height did not differ significantly in the four villages. It is given in Table 2 and compares well with that obtained by other authors for Babingas. As a comparison, data from Mbuti Pygmies (Fig. 1) (living in the northeastern Congo and referred to hereafter as Eastern Pygmies) show the latter to be smaller.

CHROMOSOME STUDIES

Chromosome analyses were performed on whole blood cultures. About 0.5 ml of blood was inoculated into tubes containing 9 ml of culture medium (lactalbumin medium plus 15% calf serum) and heparin at a final concentration of 0.1 mg/ml. In previous experiments, it was found that under these conditions the ability of the lymphocytes to undergo mitosis is maintained for at least seven days of storage at room temperature, before starting the cultures.

Blood samples were sent by ordinary airmail to Pavia. Two different shipments totaling 17 samples reached the laboratory three and four days after collection,

TABLE 2

MEAN STATURE OF PYGMIES

	♂♂ (cm)	♀♀ (cm)	Notes
Babinga.....	153.5±0.67	144.7±0.70	Present study: mean ±SE of 105 adult males and 78 adult females
Babinga.....	148.9–157.2	139.0–154.7	Range of means observed in eight earlier studies (Vallois, 1940; Lalouel, 1949; Cresta, 1964)
Mbuti.....	141.7–144.0	135.5–137.0	Range of means observed in three studies (Gusinde, 1948)

respectively. Three tubes broke. The remaining 14 cultures were set up by centrifuging the cells of each sample and resuspending them in 9 ml of fresh medium to which 0.3 ml of Difco phytohemagglutinin solution was added. Cultures were incubated at 37° C for three days, and slides were prepared following a modification of the technique of Moorhead *et al.* (1960).

Two cultures did not show any mitotic activity; in the other 12, the number of satisfactory metaphases was adequate for chromosome analysis. The karyotype was normal in all cases; in five individuals the sex chromosome constitution was XY and in the others it was XX, in agreement with phenotypic sex.

HEMATOLOGICAL PICTURE

A total of 53 samples were subjected to a complete hematological investigation, including red cell number, hematocrit, hemoglobin concentration, and red cell fragility. Means and standard deviations are given in Table 3.

It is clear that this population, unlike other primitive groups (e.g., from India; Bernini *et al.*, 1969), is in good shape insofar as the over-all blood picture is concerned. Hematocrit and hemoglobin values are similar to those observed in Eastern Pygmies (Mann *et al.*, 1962).

When 102 of these individuals were tested for osmotic fragility with 0.37%

NaCl, only four samples were abnormally resistant. Only one of these proved to be thalassemic based on a distinctly increased A_2 level, while another had an A_2 level suspiciously high. The finding of normal red cell fragility, apart from these two individuals presumed to be beta-thalassemic, and of normal mean corpuscular hemoglobin concentration indicates normal iron uptake, but no direct estimation of iron in serum was done.

THALASSEMIA, HEMOGLOBIN VARIANTS, AND RED CELL ENZYMES

It has already been mentioned that one or, more probably, two thalassemics were found among 102 individuals tested by osmotic fragility and further checked by increased A_2. This is a low level of thalassemia. Other polymorphisms involved in malaria resistance were also fairly low, especially if compared with values from neighboring groups. Thus, the incidence of G6PD deficiency among males was two

TABLE 3

RESULTS OF HEMATOLOGICAL STUDIES OF PYGMIES

Measure	Mean ± SE	SD
Hemoglobin (g/100 ml)	12.5 ±0.4	2.64
No. of red cells (millions/mm³)	4.76±0.1	0.52
Hematocrit	41.7 ±0.9	6.15
Mean corpuscle volume (μ^3)	88.2 ±0.9	6.23
Mean corpuscle hemoglobin concentration (%)	30.5 ±0.3	1.91

of 71 tested, while there were three heterozygous females of 23 tested. Deficiency was always of the African type: Gd(−), A−. Among the normals there were 8 Gd(+),A and 61 Gd(+),B males and 5 Gd(+),AB as well as 15 Gd(+),B females. Nomenclature used is that recommended by the World Health Organization (1967).

Hemoglobin S was found in heterozygous combination in 16 of 96 individuals. The identity of the hemoglobin was checked by fingerprinting on the pooled samples. Of a total of 95 hemolyzates examined in starch gel, there were found three heterozygous carriers of a delta-chain variant, with mobility equivalent to that of Flatbush hemoglobin (Jones *et al.*, 1966), and one carrier of an altogether new delta chain mutant named Hb Babinga. Again, the nature of these hemoglobins was checked by fingerprinting (De Jong and Bernini, 1968). One clear instance of elevated fetal hemoglobin (5.3%) and four cases of slight elevation (average of 1.8%) were found among the 110 tested. One of the four cases had an elevated A_2.

The red cell acid phosphatase patterns were determined according to the technique of Hopkinson *et al.* (1963). The distribution obtained is given in Table 4.

BLOOD GROUP ANTIGENS

One hundred sixty-three samples of citrated blood were investigated. A portion of the red cells of each sample was used for blood group typing, and the rest was stored in liquid nitrogen. Nitrogen-stored red cells were used later to test the s factor of the MNSs blood group system.

Blood group testing was performed, using tube techniques, with the following

antisera: anti-A, anti-B, and anti-A+B (group O serum); most of the A and AB samples were subtyped with anti-A_1 (extract of *Dolichos biflorus* seeds); two anti-M sera (rabbit), two anti-N reagents (one rabbit serum and an extract of *Vicia graminea* seeds), anti-He (rabbit), two anti-S sera (one saline agglutinating and one used in the indirect antiglobulin test), anti-s (antiglobulin test); two anti-P_1 sera (saline agglutinating); anti-C (saline agglutinating serum) containing incomplete anti-D; positive reactions were repeated with a second anti-C serum; part of the C positives were scored with anti-C serum with mainly anti-rh_i specificity, which was, however, known to react with part of the r'^n antigens (Nijenhuis and Gemser-Runiak, 1965), negative and low scoring blood samples are rh_i negative; two anti-D sera (one saline agglutinating, one used in the bromelin tube test); D-negatives were tested with a third, incomplete anti-D serum in the indirect antiglobulin test in order to detect D^u antigens; anti-E, anti-c, and anti-C^w (all bromelin tube test); E-positive blood samples were tested with anti-e (bromelin); two anti-K sera, anti-Fy^a, and anti-Fy^b, and anti-Jk^a (indirect antiglobulin test); two anti-Lu^a sera (saline agglutinating)

TABLE 4

ACID PHOSPHATASE IN PYGMIES

Phenotype	*N*
A	2
BA	5
B	85
Total	92

NOTE.—The P^c gene is absent or very rare, as observed among American Negroes. Gene frequencies are: $P^a = .0489$ and $P^b = .9511$.

and anti-Wr^a (bromelin tube test); part of the samples were tested with anti-Di^a (provided by Dr. M. Layrisse, Caracas), anti-Lan, anti-Vr (all three indirect antiglobulin test), anti-I (saline agglutinating), and anti-Le^a and anti-Le^b (both in the two-step anticomplement test).

ABO gene frequencies (Table 5) were computed by standard maximum likelihood. They are in agreement with Hardy-Weinberg equilibrium.

In Table 5, there appear fractional observed frequencies for the following reason. One A (of 44) and two AB (of four individuals) were not subtyped for A_1. The A individuals not typed for A_1 were distributed among A_1 and A_2 in the ratio suggested by the results in the individuals that were typed. The AB individuals not typed for A_1 were distributed proportionate to gene frequencies calculated approximately.

MNSs gene frequencies (Table 6) were computed by a generalized minimum χ^2 analysis. A minority of individuals (17 of 163) were not tested with anti-s. They were distributed into s+ and s− classes in the same proportions in which these two types appear in the corresponding MNSs He group. Therefore, here also are observed fractional frequencies. The numbers of individuals actually tested with anti-s in each class are given in parentheses in the same column. Six of 163 (3.7%) individuals were Henshaw-positive, and of the six, one was MS+s+, one was MNS+s−, two were MNS−s+, one was MNS−s−, and one MNS−.

Individuals who were S−s− were considered provisionally as S^uS^u, but no test

TABLE 5

ABO BLOOD GROUP DISTRIBUTION

Phenotype	Observed Frequency		Corrected Observed Frequency	Expected Frequency
A_1	36	44	36.84	35.49
A_2	7		7.16	7.87
A_1B	1	4	2.53	4.38
A_2B	1		1.47	1.08
B		27	.....	26.29
O		88	.....	87.89
Total		163	.....	163.00

Gene Frequencies

A_1	.1301
A_2	.0316
B	.1017
O	.7366

NOTE.—Hardy-Weinberg equilibrium: $\chi^2_{[2]} = 1.0489$; $P \sim .50$.

TABLE 6

MNSs BLOOD GROUP DISTRIBUTIONS

Phenotype	Observed Frequency	Expected Frequency
MS+s+	4(4)	3.69
MNS+s−	12.2(10)	8.41
MS+s−	2(2)	2.42
MS−s+	40.8(37)	36.38
MS−s−	2.2(2)	5.95
MNS+s+	8.8(7)	13.51
MNS−s+	53.4(50)	53.52
MNS−s−	6.6(6)	5.40
NS+s+	10.7(7)	8.75
NS+s−	1.3(1)	4.47
NS−s+	19.9(19)	19.28
NS−s−	1.1(1)	1.23

Gene Frequencies

MS	0.03477
Ms	0.32207
MS^u	0.19102
NS	0.09902
Ns	0.26797
NS^u	0.08516

NOTE.—Hardy-Weinberg equilibrium: $\chi^2_{[6]} = 9.311$; $P \sim .20$. In the absence of tests with anti-U serum, alleles were considered provisionally to be S^u when they did not react with either anti-S or anti-s. Numbers in parentheses are the numbers tested with anti-s.

with anti-U was performed. The agreement with Hardy-Weinberg equilibrium is good (Table 6).

The distribution of Rh blood groups is given in Table 7. The R^0 chromosome is almost the only chromosome present. An analysis by generalized minimum χ^2 gave a very low frequency of R^1 (<.0001), and thus this chromosome is not indicated among those present. With this hypothesis, χ^2 for Hardy-Weinberg equilibrium is good.

It is possible, on the other hand, that there actually are some *CDe* alleles. In fact, six individuals who were C-positive, when tested for rh_i, gave the following:

TABLE 7

Rh BLOOD GROUP DISTRIBUTIONS

Phenotype	Observed Frequency	Expected Frequency
ccddee	2	2.13
ccDee	143	142.94
ccDEe	4	3.71
Ccddee	2	1.63
CcDee	12	11.83
ccddEe+ccddEE+ ccDEE+CcddEe+ CcDEe+CCddee(?)	0	.75
Total	163	162.99

Gene Frequencies	
cde	.1144
cdE	.0060*
cDE	.0065
Cde	.0437
cDe	.8294

NOTE.—Hardy-Weinberg equilibrium: $\chi^2_{[1]} = .867$; $P \sim .50$.

* There is only statistical evidence in this series of data for the existence of this allele.

one Ccddee (rh_i-), four CcDee (rh_i-), and one CcDee (rh_i+). On this basis, one-sixth of all genes given as *Cde* might instead be *CDe*. The expectations for the phenotypes change almost imperceptibly.

D^u individuals were included with the respective D types. The ultimate criterion for D-negative was an indirect antiglobulin test with a potent polyvalent anti-D serum.

Results of all other tests on blood groups are shown in Table 8.

HAPTOGLOBINS, TRANSFERRINS, GROUP-SPECIFIC COMPONENT

The distribution of haptoglobins, examined by conventional starch gel electrophoresis according to Poulik (1957), is given in Table 9. The very large number of apparent ahaptoglobinemics (72/160, or 45%) is among the highest so far found. It is impossible to say on the basis of the present data to what extent this phenomenon

TABLE 8

OTHER BLOOD GROUPS TESTED IN BABINGA PYGMIES

P	No.	%	Kell	No.	%	Duffy	No.	%
P_1+	161	98.77	K−	161	98.77	Fy(a+b−)	0	0
P_1-	2	1.23	K+	2	1.23	Fy(a+b+)	0	0
	(163)			(163)		Fy(a−b+)	0	0
						Fy(a−b−)	163	100
							(163)	
Kidd	No.	%	Lutheran	No.	%	Diego	No.	%
Jk(a+)	162	99.4	Lu(a+)	1	0.6	Di(a+)	0	0
Jk(a−)	1	0.6	Lu(a−)	162	99.4	Di(a−)	95	100
	(163)			(163)			(95)	

Lan	No.	%	Vr	No.	%	I	No.	%	Wright	No.	%
Lan−	0	0	Vr+	0	0	I+	59	100	Wr(a+)	0	0
Lan+	53	100	Vr−	68	100	I−	0	0	Wr(a−)	163	100
	(53)			(68)			(59)			(163)	

Lewis*	Groups O, A_2, and B	Groups A_1 and A
Le(a+b−)	9	4
Le(a−b+)	27	3
Le(a−b−)	9	12
	(45)	(19)

NOTE.—Gene frequencies: *L*, 0.55; *l*, 0.45; *Se*, 0.50; *se*, 0.50.

* Total Le(a+) = 13/64, or 20.3%.

TABLE 9

FREQUENCY OF HAPTOGLOBIN TYPES

Phenotype	Observed Frequency		Expected Frequency*
Hp 0	72		
Hp 2−2	25	31	33.13
Hp 2−2 weak	6		
Hp 2−1	35		
Hp 2−1 weak	9	46	41.73
Hp 2−1 mod.	2		
Hp 1−1	10	11	13.14
Hp 1−1 weak	1		
Total	160	88	

* Hardy-Weinberg equilibrium, excluding Hp 0: $\chi^2_{[1]} = .922$ $P \sim .40$.

is caused by genetic factors. The presence of "weak" phenotypes suggests a participation of environmental factors, such as hemolysis or reduced protein synthesis.

The distribution of phenotypes is in any case altered in such a way as to make the computation of gene frequencies unsatisfactory. Hp 1-1 phenotypes may be less likely than other types to appear as ahaptoglobinemics under the effect of environmental factors. In these phenotypes, the protein is not subdivided into many bands as for hp 2 combinations. Moreover, hp 2 polymers are known to have a lower hemoglobin binding capacity per gram of protein. On the basis of these considerations, gene frequency estimation and the testing for Hardy-Weinberg equilibrium should be considered as tentative.

Phenotype Hp 2-1 modified involves an Hp^2 allele which is believed to confer a lower rate of synthesis of hp 2 protein (Bernini *et al.*, 1966). It seems less frequent than in other African populations.

TABLE 10

FREQUENCY OF TRANSFERRIN TYPES

Type	Observed	Expected
C	122	119.02
CD_1	32	37.95
D_1	6	3.02
Total	160	.....

TABLE 11

FREQUENCY OF Gc TYPES

Gc 1-1	66
Gc 2-1	3
Gc 2-2	1
	70

Both Hp 1-fast and Hp 1-slow monomers have been found when a pool of Hp 1-1 and Hp 2-1 sera have been submitted to subtyping according to Connell *et al.* (1962).

Transferrins show a polymorphism limited to the D phenotype (Table 10). The characterization of the D transferrin, done by Dr. H. E. Sutton by fingerprinting, corresponds with the ordinary African D_1 variant.

The frequency of Tf^{D^1} is 0.1375; $\chi^2_{[1]}$ for Hardy-Weinberg is 3.948, just over the 5% significance limit. The smallness of the expected frequency in the Tf^{D^1}/Tf^{D^1} class throws doubt on the validity of this result, but there may be here, as for other markers, a slight deficiency of heterozygotes. It is impossible at present to decide among the various possible explanations.

The Gc system shows the existence of two alleles, with a low frequency of the Gc^2 allele (Table 11). The presence of one homozygote Gc^2Gc^2, which has a low expectation, is subject to considerations similar to those already made for transferrins.

Gm and Inv determinations

The reagents used are listed in Table 12. The alphabetical notation given in the right-hand column of the table is used in this paper for reasons given by Van Loghem and Mårtensson (1967).

All the anti-Gm reagents made use of established test systems that were inhibited by Gm(+) sera diluted 1:50 or more, but not by Gm(−) sera, even when undiluted. The tests were performed in microtitrator plates. Each serum was tested in two dilutions, 1:10 and 1:30. Equal volumes of anti-Gm or anti-Inv serum dilution and 0.5% sensitized cell suspension were added. The reaction was read macroscopically after one hour at 18° C. Appropriate controls were included for all determinations.

A total of 162 sera were tested for all factors noted in Table 12, except for Gm(g),

TABLE 12

Reagents Used in Gm and Inv Testing

Gm Reagents		Numerical Nomenclature	Alphabetical Nomenclature
Anti-Gm	Anti-Rh		
2130	2300	1	a
2405	2397	2	x
A.J.	2300	3,4	f
5306*	Perd	21	g
2357	2127	11	b^0
2247	2127	5,12	b^1
2277	2127	10,13	b^3
Bu†	2127	14	b^4
Bu	Vai	.....	b^5
Nij	Vai	.....	s
vDijk	Vai	.....	s
Prie	Vai	.....	t
2359	Baal	6	c^3
Vers	Baal	6	c^3
Stie	Baal	6	c^5
Hess	Baal	6	c^5
Inv Reagents		**Numerical Nomenclature**	**Alphabetical Nomenclature**
Anti-Inv	Anti-Rh		
2151	2290	1	1
Virm	2290	2	a

Note.—The numerical nomenclature was suggested by the WHO Scientific Group on Genes, Genotypes and Allotypes of immunoglobulins 1965. We use the designation Gm (1), Gm(b^1), and Gm(b^3). Different Gm(c) (5) reagents give discordant results; we use the designation Gm(c^3) and (c^5) because of the relations of these specificities to Gm(b^3) and (b^5) (see WHO, 1965).

* Rabbit antiserum obtained by immunization with Gm(g+) paraprotein diluted with 1:10 Gm(a− g− f+ b+) normal serum.

† This serum was diluted with 1:10 Gm(a+ x− f− g+ b^1− b^3+ b − b^5+ s+ t+ c^3− c^5−) serum.

(b^4), and (b^5). These factors were determined in 20 sera selected from 162 sera. Three different Gm phenotypes were found:

5 Gm(a+ x− f− b^0+ b^1+ b^3− s− t− c^3+ c^5+),
38 Gm(a+ x− f− b^0+ b^1+ b^3+ s− t− c^3+ c^5+),
119 Gm(a+ x− f− b^0+ b^1+ b^3+ s− t− c^3− c^5−).

The first group of 5 sera and 15 of the group of 119 sera were also tested for Gm(g), (b^4), and (b^5); they were found to be Gm(g− b^4− b^5−) and Gm(g− b^4+ b^5+), respectively.

In Negroes, the most common allele is $Gm^{a,b0,b1,b3,b4,b5}$ (Steinberg *et al.*, 1960). In Surinam Negroes, two alleles determining one or more Gm(c) factors are present: $Gm^{a,b0,b1,c3,c5}$ and $Gm^{a,b0,b1,c3,b4,b5}$ (Van Loghem and Mårtensson 1967). The data obtained in this study indicate that two alleles occur in the Pygmies tested: $Gm^{a,b0,b1,b3,b4,b5}$ and $Gm^{a,b0,b1,c3,c5}$, with frequencies of 0.85 and 0.15, respectively.

Three alleles determining Inv factors are known: Inv^{1a}, Inv^{1}, and Inv^{b}. Ninety-six sera were found to be Inv(1+ a+) and 68 sera Inv(1− a−). No reagents were available to test for Inv(b). The phenotype Inv(1+ a−) was not found. It seems probable, therefore, that only two alleles, Inv^{1a} and Inv^{b}, occur in Pygmies, with frequencies of 0.35 and 0.65, respectively.

ORIGIN OF THE PYGMIES AND ANALYSIS OF GENETIC DISTANCES

The origin of African Pygmies has been the source of much speculation. Early authors were struck by the presence of small-sized individuals in forest areas outside Africa (e.g., Negritos) and conjectured a common origin with African Pygmies. However, the consideration of blood group data made this interpretation very unlikely (Boyd, 1963). To use Buettner-Janusch's (1966) expression, "the blood group gene frequencies suggest that Papuan Pygmies are simply short Papuans and African Pygmies are short Africans."

In addition to being small, Pygmies have a lighter skin color than their neighbors and have other peculiarities (prognathism, e.g.). These facts prompted Gates (1958) to make Pygmies the descendants of a hypothetical, extinct race having normal stature but other Pygmy characteristics. Coon (1965) suggested that, if this is true, such a race might descend from Rhodesian man.

Data on genetic markers have been scarce. It was thus impossible to draw definite conclusions on the origins of Pygmies. While this investigation was in progress, however, there appeared a series of papers on Eastern Pygmies (from the Ituri forest). In these papers, many blood group systems were analyzed along with other markers. The relevant data are summarized in Tables 13 and 14.

The authors (Fraser *et al.*, 1966) concluded: "The Pygmies may represent a survival of a primitive race with high S^u and R^0 and low V and Js^a, part of which mixed, in times when the population of Africa was sparse, with other non-Pygmy populations of different gene composition to form the ancestral Negro population."

Our data confirm and extend the conclusion that Pygmies are "African"; indeed,

TABLE 13

GENE FREQUENCIES FOR EIGHT HUMAN GROUPS

Group	A_1	A_2	B	O	cDe	CDe	cDE	cde	Cde	CDE	cdE	Hp^1	Hp^2
1. Caucasian	.236	.068	.066	.630	.053	.415	.110	.404	.008	.004	.005	.384	.616
2. American Indian	0	0	0	1.000	.016	.533	.439	0	0	.012	0	.731	.269
3. Australian aborigine	.258	0	.009	.733	.077	.644	.233	0	0	.046	0	.260	.740
4. Forest Negro	.105	.052	.150	.693	.625	.082	.073	.188	.026	0	.006	.624	.376
5. Bushmen	.197		.052	.751	.890	.090	.020	0	0	0	0	.299	.701
6. W. Pygmies	.126	.035	.100	.739	.830	.007	.007	.114	.036	0	.006	.386	.614
7. E. Pygmies	.246		.104	.650	.951	.004	.045	0	0	0	0	.398	.602
8. Congo	.158		.137	.705	.676	.084	.040	.192	.008	0	0	.581	.419

Group	MS	MS^u	Ms	NS	NS^u	Ns	Tf^C	Tf^B	Tf^D	Gm^a	Gm^b	Gm^{ax}	Gm^{ab}	Gm^{abc}	Gd^-	Hb^S
1. Caucasian	.270	0	.310	.076	0	.344	.994	.006	0	.204	.704	.092	0	0	0	0
2. American Indian	.236	0	.462	.107	0	.195	.996	.002	.002	.775	0	.123	.102	0	.067	0
3. Australian aborigine	0	0	.509	0	0	.491	.989	0	.001	.654	0	.263	.083	0	0	0
4. Forest Negro	.118	.052	.358	.058	.078	.336	.939	0	.061	0	0	0	.786	.214	.170	.066
5. Bushmen	.068	....	.523	.018	....	.391	.946	0	.054	.189	0	0	.770	.041	.034	0
6. W. Pygmies	.035	.191	.322	.099	.085	.268	.863	0	.137	0	0	0	.851	.149	.042	.083
7. E. Pygmies	.067	.402		.071	.460		.967	0	.033	0	0	0	.971	.029	.040	.183
8. Congo	.203		.365	.128		.304	.970	0	.030	0	0	0	.697	.303	.166	.091

NOTE—Sources of the figures are as follows: for groups 1, 2, and 4, Krieger *et al.* (1965), except for Fy in group 4, which is taken from Race and Sanger (1962). Australian data are from Kirk (1965), except for MNS, which come from Simmons *et al.* (1962). Bushmen are from Zoutendyk *et al.* (1953) for ABO, MNS, Rh, K, Fy, and Lu; from Jenkins and Steinberg (1966) for Hp, Tf, Inv, and Gm; from Baker and Weiner (1966) for Di, P, Hb S, and G6PD. Congo and Eastern Pygmies are from Fraser *et al.* (1966), Giblett *et al.* (1966), Motulsky *et al.* (1966), and Steinberg *et al.* (1961). Whenever data were available for more than one population belonging to the same group, weighted averages were taken. MNS of Eastern Pygmies are taken from Ikin and Mourant (1952). Comparable figures from Motulsky *et al.* (1966) are: frequency of gene $M = .504$; frequency of S^u ($MS^u + NS^u$) = .591. In the computation of genetic distances, it was necessary to pool all alleles of the MNS and ABO systems for which no distinction was made by the testing procedure and whose frequencies are therefore given cumulatively.

they favor their being a proto-African race. A summary of most data obtained in the present paper is given in Tables 13 and 14, where Babinga Pygmies are referred to as Western Pygmies. For comparison, data have been added to Tables 13 and 14 for the same genetic systems in three other major human ethnic groups (Caucasoids, American Indians, Australian aborigines) as well as data from other African populations. The averages for the "Forest Negro" come, with slight modifications, from the paper by Krieger *et al.* (1965). The "Congo" frequencies are averages of six groups, mostly Bantu (Fraser *et al.*, 1966), living in various places in the Congo and Ruanda-Urundi. These tables were prepared for computing the coefficients of genetic distance given later.

From the point of view of the African origin of Pygmies, it is significant that among them, R^0 is very high, and Eastern Pygmies show the highest observed frequencies. But also, another allele considered even more typically African than R^0, namely, *Fy*, shows in our data that Western Pygmies are "more African" than all other African groups (Table 14). Several other markers show the same trend, namely, a higher frequency among Pygmies of the allele more often represented in Africans. Thus, among markers not given in Tables 13 or 14, Jk(a+) has a frequency of almost 100% among Western Pygmies (Table 8), 93% in New York Negroes (R. E. Rosenfield, cited by Race and Sanger 1962), 95% in West Africans (Mourant, 1954), 50% in Caucasoids (Race and Sanger). P_1 is almost 99% among Pygmies (Table 8), over 95% in west Africans, and about 78% in Caucasoids (Race and Sanger 1962). In the case of acid phosphatase, P^b reaches 95% among Pygmies (Table 4), 80% in Africans, and less among Caucasoids (Modiano *et al.*, 1967).

Many of the smaller differences may, however, be trivial, so that a global consideration seems in order. It was carried out by the methods given by Cavalli-Sforza and Edwards (1967), slightly modified as follows.

To compute genetic distances between two populations, the quantity D is computed first for each locus:

$$D = 1 - \cos\theta = 1 - S\sqrt{p_{ai}p_{aj}},$$

(where the p values are the gene frequencies for allele a in the two populations i and j), extending the sum S to all alleles at the locus.

A weighted average is computed for all loci from

$$\bar{D} = SD_k/S(a_k - 1),$$

where a_k is the number of alleles and D_k is the D value for the kth locus.

The quantity

$$f_\theta = 4D \simeq \sigma_p^2/p\bar{q}$$

is an estimate of the mean kinship coefficient with respect to the genes which have been used to compute it (Cavalli-Sforza, 1969). In the formula, σ_p^2 is the variance of gene frequency, $\bar{p}$ its mean, $\bar{q} = 1 - \bar{p}$. For reasons that will be discussed later, the quantities given as "genetic distances" d in the table are the square roots of f_θ. The standard error of d has been computed from the variation between loci.

The values of genetic distances thus calculated are given in Tables 15 and 16.

TABLE 14

CONTINUATION OF TABLE 13 FOR MARKERS NOT AVAILABLE FOR THE LAST TWO POPULATIONS IN TABLE 13

Group	Di^a	Di^b	P^1	p	K	k	Lu^a	Lu^b	Inv^a	Inv^b	Fy^a	Fy^b	Fy
Caucasian	0	1.000	.542	.458	.048	.952	.036	.964	.100	.900	.421	.549	.030
American Indian	.170	.830	.430	.570	0	1.000	0	1.000	.307	.693	.682	.318	
Australian aborigine	0	1.000	.213	.787	0	1.000	0	1.000	.267	.733	1.000	0	0
Forest Negro	0	1.000	.754	.246	.005	.995	.036	.964	.316	.684	0	.056	.944
Bushmen	0	1.000	.334	.666	.053	.947	0	1.000	.331	.669	.082	.918	
W. Pygmies	0	1.000	.889	.111	.008	.992	.003	.997	.352	.648	0	0	1.000

TABLE 15

GENETIC DISTANCES BETWEEN THE EIGHT POPULATIONS GIVEN IN TABLE 13 FOR THE LOCI *ABO, Rh, Hp, MN, Tf, Gm*

Group	American Indian	Australian Aborigine	Forest Negro	Bushmen	Western Pygmies	Eastern Pygmies	Congo
Caucasian	.589 ± .055	.496 ± .074	.365 ± .092	.650 ± .074	.650 ± .083	.719 ± .084	.635 ± .093
American Indian		.365 ± .068	.687 ± .071	.649 ± .061	.756 ± .063	.777 ± .072	.698 ± .074
Australian aborigine			.647 ± .083	.555 ± .075	.685 ± .082	.697 ± .095	.655 ± .085
Forest Negro				.346 ± .042	.194 ± .028	.309 ± .038	.082 ± .011
Bushmen					.293 ± .041	.261 ± .050	.341 ± .049
W. Pygmies						.238 ± .028	.203 ± .024
E. Pygmies							.316 ± .051

Table 15 refers to all markers known for all the eight populations, the frequencies of which are given in Table 13. Only six loci were used (22 alleles) because G6PD and Hb S were excluded from such computation, as they could outweigh distances of Negro–non-Negro comparisons. It will, however, be noted that the exclusion of these two loci caused a negligible shift of the values of genetic distances (see Fig. 2).

TABLE 16

GENETIC DISTANCES BETWEEN THE SIX POPULATIONS GIVEN IN TABLES 13 AND 14 FOR THE LOCI

ABO, Rh, MNS, Hp, Tf, Gm, Gd, Hb, Di, P, K, Lu, Inv, Fy

Group	American Indians	Australian Aborigines	Forest Negro	Bushmen	Western Pygmies
Caucasian	.519 ± .047	.535 ± .082	.591 ± .074	.570 ± .065	.629 ± .069
American Indian	.....	.419 ± .063	.643 ± .078	.595 ± .061	.696 ± .073
Australian aborigine	.....	.....	.734 ± .141	.636 ± .138	.761 ± .138
Forest Negro	.....	.....	.....	.352 ± .034	.190 ± .023
Bushmen	.....	.....	.....	.....	.339 ± .060

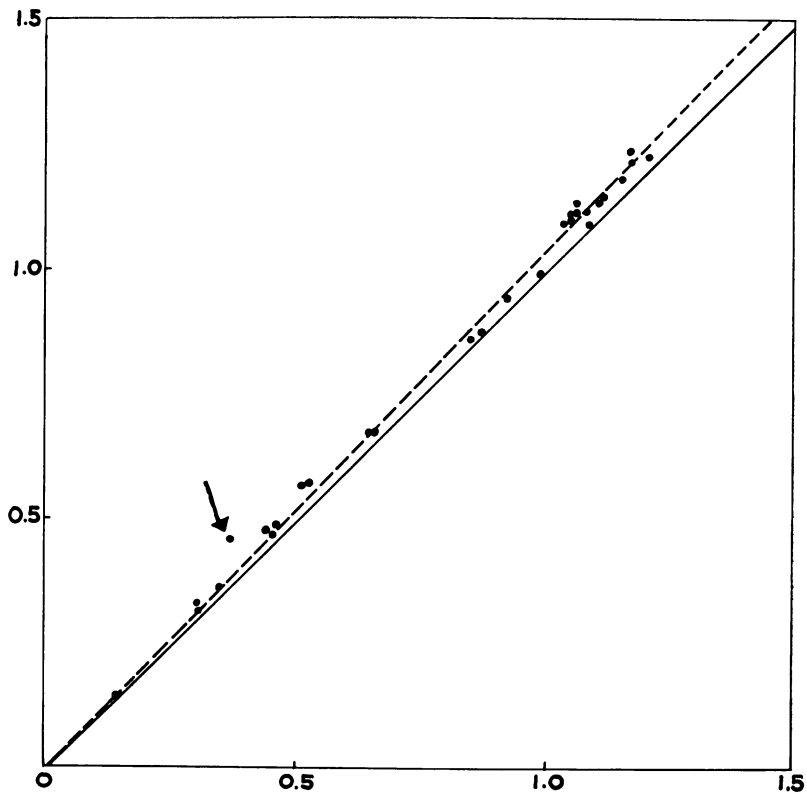


FIG. 2.—The correlation between genetic distances computed excluding (x = abscissa) and including (y = ordinate) G6PD and Hb. Values of x are those given in Table 15. Solid line = equality. Dotted line = proportionality ($y = 1.052x$). The major deviation, indicated by an arrow, is that of the distance between Bushmen and Eastern Pygmies.

The distance values given in Table 15 show a clear-cut bimodal distribution. All distances between any two African populations are smaller than 0.35 and average .258 ± .026, while all distances between African and non-African groups, or between two non-African groups, are higher than 0.35 and average .641 ± .023. Thus, the latter distance values, which are those found between accepted major racial groups, are all clearly higher than all intra-African distances.

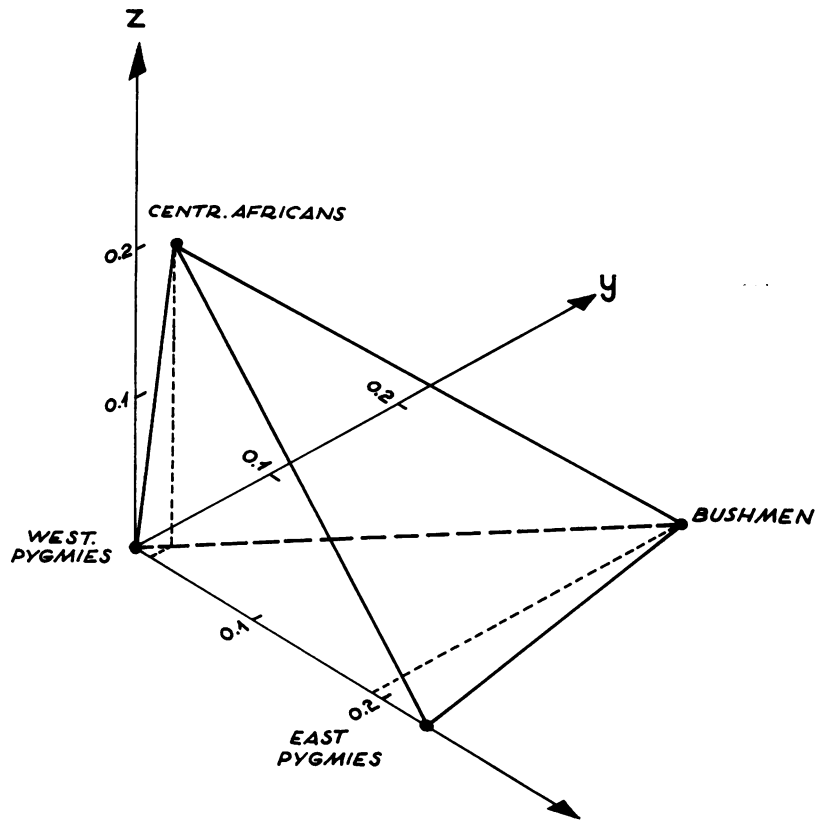


FIG. 3.—The genetic distances, computed from the data of Table 13, among Western and Eastern Pygmies, Bushmen and Central Africans. The last are formed by pooling groups labeled as "Forest Negroes" and "Congo" in Table 13.

Among the intra-African distances, the lowest value is that between "Forest Negroes" and the "Congo" group, an expected result, since there is a large overlap between the two populations. The data from which the computations are made are, however, fully independent. Taking an average of the Forest Negroes and Congo and calling them "Central Africans," the distances between them, the Bushmen, and the Western and Eastern Pygmies are represented graphically in Figure 3. All four groups are almost equidistant from one another. There is a nonsignificantly greater similarity between Central Africans and Western Pygmies on the one hand and Eastern Pygmies and Bushmen on the other. This shows that it is not justified

to separate sharply Bushmen from other African populations. Genetic markers do not seem to warrant the creation of a Khoisanid, Capoid, etc., "race," as some anthropologists have done. Others (Singer and Weiner, 1963; Weiner, 1966) have stressed the fact that Bushmen are essentially African. We note that Bushmen also show a considerable resemblance to Pygmies, especially to the Eastern group (Table 15). This is not perhaps so surprising, in spite of the cultural differences between the two groups. In fact, there are several remnants of Bushmanoid groups north of the area presently inhabited by Bushmen, extending to regions very near that occupied by Eastern Pygmies.

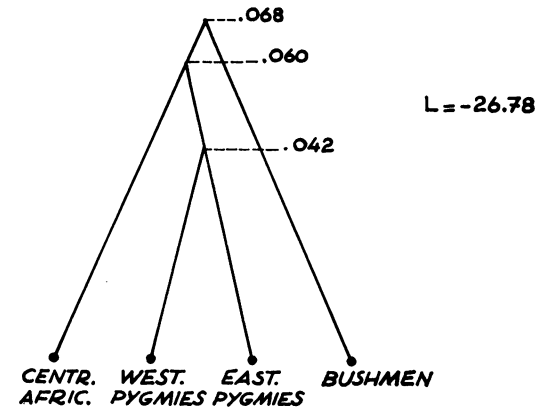
Table 15 includes, however, only markers given in Table 13. When we extend our consideration to the other six loci given in Table 14, information on which is not available for all the populations listed in Table 14, one obtains the matrix of genetic distances given in Table 16. The distances of Table 16 essentially confirm the earlier conclusions. They also increase the similarity of Western Pygmies to Central Africans.

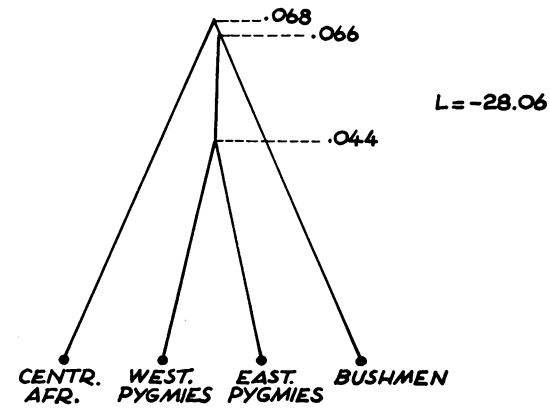
We have analyzed the four African groups defined before on the basis of the distances given in Figure 3, using the method of reconstructing phylogenetic relationships given by Cavalli-Sforza and Edwards (1967). With four groups, there are 15 possible different phylogenetic trees. Only three of them seem sufficiently well adapted to represent the observed distances so that a finite value of maximum likelihood can be obtained. They are given in Figure 4 along with their likelihood value. The tree given at the top of the figure has a slightly higher likelihood than the other two, and it is thus the best of the 15. It is perhaps also the most satisfactory one from the point of view of common sense expectation. Present data are not, however, sufficient to place a firm belief on this phylogeny. The accumulation of further data, both on other markers and on related populations, should improve the resolution to a satisfactory level.

The trees of Figure 4 are based on the assumption that no important exchange among these groups has taken place. This hypothesis may be wrong; in fact, it has been suggested that the Babingas may represent a mixture of a Pygmy stock, of which the Eastern (Mbuti) Pygmies may be more direct descendants, and a Central African prototype. This would account for their somewhat intermediate stature and their intermediacy in other respects (see Vallois, 1940; Cresta, 1964; Cavalli-Sforza, 1966).

One cannot exclude the consideration that important gene flow has contributed to obscuring or altering the original relationships. It should be noted, however, in agreement with earlier authors, that gene flow is likely to take place mostly from Pygmy to non-Pygmy (say, Bantu). Today, at least, social rules make the marriage of Pygmy females to Bantu males possible, but the couple is absorbed into the Bantu culture. The reciprocal marriage is believed to be very rare. As Pygmies are, at present at least, a minority group, their contribution to the genetic constitution of Bantu people must be fairly small.

It is possible to test the hypothesis that the Babinga Pygmies are the result of a mixture of Eastern Pygmies and Central Africans by making use of the genetic distances given in Table 15. Genetic distances computed by the method given are





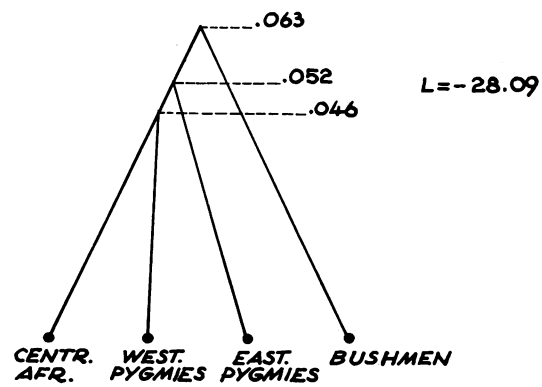


FIG. 4.—Three of 15 possible phylogenies which gave a finite likelihood value on analysis of the set of distances presented graphically in Fig. 2, with their respective likelihood L. Times of separation are given in arbitrary units, as estimated.

approximately linear in the gene frequencies. Therefore, the following approximate relationship holds for a population M made of a mixture in proportions m and $(1 - m)$ from populations A and B:

$$d_{MA} + d_{MB} = d_{AB} ,$$

when d_{MA}, d_{MB}, and d_{AB} are the distances given as before between the populations indicated by the suffixes.

The approximation is of the order of 1% in practice. The proportion m is given very approximately by $d_{MB}/(d_{MA} + d_{MB})$.

In the present case (taking as non-Pygmy ancestral type the "Forest Negro," so as to use directly the data of Table 15), the distance between the two putative ancestral groups, Forest Negroes and Eastern Pygmies, is:

$$d_{AB} = .308 \pm .038 ,$$

while the distances between the two ancestral types and the presumed mixture M (Babingas, i.e., Western Pygmies) are:

$$d_{AM} = .194 \pm .028 ,$$

$$d_{MB} = .238 \pm .027 .$$

The sum of the latter is $.432 \pm .038$. This should be, in the case of a mixture, equal to d_{AB}, but differs from it significantly ($t = 2.31$). Using "Congo" (Table 13) as non-Pygmy ancestors, one finds $d_{AM} + d_{BA} = .441 \pm .036$, giving the same result. Consideration of G6PD and Hb S increases the discrepancy.

For a more exact approach, one can take the pooled Forest Negroes plus Congo= Central Africans as one ancestral type, the Eastern Pygmies as the other ancestral type, compute the gene frequencies of mixtures in all proportions of the two types, and compute the distance between the Babingas and each mixture. The results are plotted in Figure 5. The most likely proportion of mixture is that for which the distance between Babingas and the mixture is smallest, namely, for a mixture having 45% Central African blood. As this distance is several times its standard error (the fiducial region indicated in Fig. 5 corresponds to twice the standard error), the hypothesis of a simple admixture cannot be maintained. Thus, if there has been admixture, either it has taken place in the distant past and the gene frequencies of today's groups have changed considerably since then, in the mixture as well as in the ancestral groups, or the hypothesis of admixture is wrong. Although the data are somewhat in favor of some degree of admixture, the situation is ambiguous.

SUMMARY

A sample of 175 Babinga Pygmies living at the border between the Central African Republic and Congo Brazza were studied for the following genetic markers: A_1A_2BO, MNS, Rh, P, K, Fy, Jk, Lu, Di, Kell, Lewis, Hp, Tf, Gm, Inv, Gc, acid phosphatase, glucose-6-phosphate dehydrogenase (G6PD), and hemoglobins.

An analysis was carried out of the genetic distances between this group and other

African and non-African groups. Calling "Western Pygmies" those analyzed in the present paper and "Eastern Pygmies" the Mbuti group living in the eastern Congo, we found that the two groups of Pygmies are similar, but not more so than, say, Eastern Pygmies and Bushmen or Western Pygmies and Central Africans. The last population was obtained by averaging data from various forest-dwelling tribes and related groups. This favors a unitary origin of these African people and does not warrant the creation of major racial subdivisions among them. The possibility of gene flow is not excluded, but is difficult to take into account on the basis of present data. A tentative phylogeny of the four groups has been computed, and the possibility that Babingas represent a mixture from a Pygmy group and non-Pygmy has been discussed. Although there is some evidence in favor of remote admixture, the results are ambiguous.

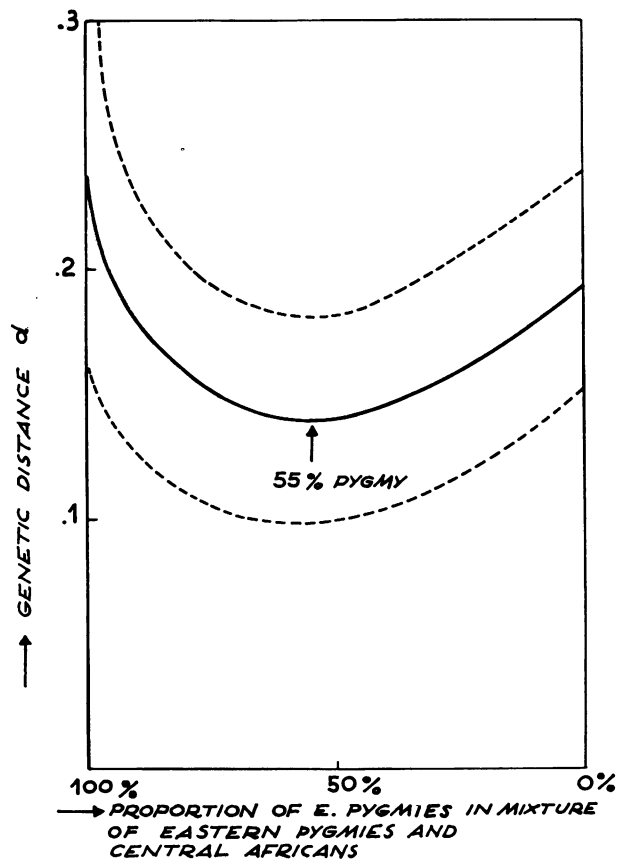


FIG. 5.—Theoretical variation of d (genetic distance) between Babingas and mixtures of Eastern (Mbuti) Pygmy and non-Pygmy population (Central Africans) as a function of the degree of admixture indicated on the abscissa as the proportion of Mbuti genes. The dotted lines indicate 2σ fiducial belts.

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