GENETIC STRUCTURE OF HUMAN POPULATIONS

I. LOCAL DIFFERENTIATION OF BLOOD GROUP GENE FREQUENCIES IN JAPAN *

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Received 26.iv.65

1. INTRODUCTION

HUMAN populations are usually considered to be composed of many subpopulations, within each of which random mating takes place approximately. These subpopulations have been called isolates (Wahlund, 1928) or neighbourhoods (Wright, 1943). As shown by Wright (1943), this type of mating structure necessarily leads to a local differentiation of gene frequencies, even if there are no local differences of selection intensity for the genes. If such exist, the differentiation of gene frequencies may be further enhanced.

Blood groups in man are favourable traits for the study of genetic structure of populations, since they are typical polymorphic characters and the gene frequencies are easily estimated. Thus, Cavalli-Sforza (1959, 1963) studied the local variation of various blood group gene frequencies in a region of Italy and attempted to estimate the effective population size of the region.

In this paper we will examine the degree of local differentiation of the ABO and MN blood group gene frequencies in Japan and analyse the possible causes of the differentiation using the method described by Nei (1965). The bearings of these results on the genetic structure of the Japanese population will also be discussed.

2. BLOOD GROUP FREQUENCY DATA

The Japanese have long inhabited the three major islands of Honshu, Kyushu and Shikoku, as well as other small islands surrounding them. Another major island, Hokkaido, has been inhabited by the Japanese only recently. History indicates that only a small proportion of people have immigrated into or emigrated from Japan at least for these 2000 years. Thus, the Japanese population appears to be suitable for the study of population structure. The present inhabitants of Hokkaido are a mixture of people from various regions of Japan, so that the data on them were not used in this analysis. The data for the Ryuku islands also were omitted because of the possible intermarriage with other ethnic groups. So too were those for the nearby Tanegashima island (Kagoshima Prefecture).

* This investigation was supported in part by a grant from the Scientific Research Fund of the Ministry of Education, Japan.

(i) *ABO blood groups*

Since around 1930 extensive data have been collected on the ABO blood group frequencies in Japan, the total number of reported observations amounting to about 850,000. Of the 850,000 observations about 250,000 were excluded from our analysis for reasons given above. The final number employed for the present analysis was then 599,342. These are the data compiled from 340 different reports (cf. table 1).

FIG. 1.—Subdivision of Japan according to Prefecture. The number in each subdivision refers to the Prefecture number in tables 1 and 2.

In a recent survey of the ABO blood group data, Tanaka (1959) compiled more than one million observations, but his compilation includes some 300,000 unpublished observations as well as the data on the Japanese people living outside Japan.

The final data were divided into 45 different groups or sub-populations (table 1). The groups correspond to the 45 different Prefectures—administrative units of Japan (fig. 1). As seen from this figure, the area is not the same for all Prefectures but the population size in 1900-45, during which the majority of tested individuals were born, was nearly the same (*ca.* 0.6-2.0 millions) for all Prefectures save for several urban districts such as Tokyo and Osaka. In view of the population movement before and during 1900-45, the unit of random mating group appears to be much smaller than Prefectures and thus each Prefecture is considered to comprise many neighbourhoods.

TABLE 1

Data on the blood groups O, A, B and AB according to Prefecture

Pref. no.	Prefecture	No. of observations	Per cent. frequency				Author index*
			O	A	B	AB	
1	Aomori . .	5,066	32.19	33.16	26.06	8.59	2, 26, 37, 59
2	Akita . .	2,842	31.24	34.24	25.16	9.36	37
3	Iwate . .	1,897	35.79	35.58	21.14	7.49	37
4	Yamagata . .	5,942	30.21	37.14	23.73	8.92	37
5	Miyagi . .	13,518	31.89	37.22	21.49	9.40	37
6	Fukushima . .	4,954	29.98	35.63	23.33	11.06	9, 37
7	Tochigi . .	1,829	31.93	35.16	24.22	8.69	37
8	Ibaragi . .	2,764	31.51	34.84	23.19	10.46	37
9	Gumma . .	8,414	29.86	37.10	22.77	10.27	37
10	Saitama . .	4,026	29.16	39.39	21.69	9.76	37
11	Tokyo . .	65,743	31.06	37.83	22.07	9.04	32, 33, 37, 48, 58, 63, 66, 77
12	Chiba . .	49,487	33.23	36.33	22.28	8.16	34, 37, 67
13	Kanagawa . .	6,931	31.47	37.57	21.03	9.93	37, 64
14	Niigata . .	26,291	29.70	37.34	22.81	10.15	1, 37, 46, 71
15	Nagano . .	24,490	27.21	36.91	24.78	11.10	19, 31, 37, 65, 74
16	Yamanashi . .	2,750	29.64	39.64	20.87	9.85	37
17	Shizuoka . .	9,113	31.63	37.48	22.56	8.33	37, 62
18	Aichi . .	31,873	30.55	37.91	22.28	9.26	6, 17, 24, 37
19	Gifu . .	11,008	32.33	39.73	20.28	7.66	37, 79
20	Toyama . .	6,126	30.92	37.81	21.69	9.58	37
21	Ishikawa . .	49,866	30.59	37.12	22.50	9.79	9, 37
22	Fukui . .	3,957	33.06	37.17	21.41	8.36	37
23	Shiga . .	2,744	27.70	39.03	22.85	10.42	25, 37, 60
24	Mie . .	4,629	30.11	36.19	23.78	9.92	37
25	Nara . .	2,709	31.34	39.46	19.68	9.52	37, 41, 45, 60
26	Wakayama . .	6,922	31.38	37.27	22.31	9.04	37, 60
27	Kyoto . .	16,047	29.35	30.72	22.18	9.75	11, 37, 56, 60, 73
28	Osaka . .	28,289	31.61	39.81	20.27	8.31	35, 37, 60, 69
29	Hyogo . .	9,153	30.57	38.91	20.99	9.53	37, 57, 60
30	Okayama . .	8,846	29.07	38.40	22.04	10.49	37, 60
31	Tottori . .	1,591	30.11	40.60	20.36	8.93	37, 60
32	Shimane . .	4,965	28.46	40.34	20.97	10.23	37, 47, 60, 76
33	Hiroshima . .	14,683	29.45	40.31	21.26	8.98	37, 42, 43, 44, 60, 78
34	Yamaguchi . .	7,303	30.69	38.98	20.81	9.52	37, 38, 60
35	Tokushima . .	9,266	29.25	42.48	20.03	8.24	37, 53, 60
36	Ehime . .	7,369	27.78	41.02	20.97	10.23	37, 60
37	Kagawa . .	5,436	30.63	39.57	20.97	8.83	37, 60
38	Kochi . .	2,061	28.14	40.32	21.88	9.66	37, 60
39	Fukuoka . .	33,576	28.61	41.53	20.62	9.24	5, 37, 60
40	Oita . .	4,417	28.03	39.91	21.15	10.91	37, 60
41	Saga . .	3,887	28.25	38.72	22.64	10.39	37
42	Nagasaki . .	37,623	29.40	39.24	21.11	10.25	28, 29, 37, 39, 40
43	Kumamoto . .	11,802	30.14	39.87	20.26	9.73	37, 60
44	Miyazaki . .	3,201	30.05	39.96	19.34	10.65	27, 37, 60
45	Kagoshima . .	27,936	30.79	39.70	20.49	9.02	37, 50, 54, 60, 61
Total . .		599,342	30.44	38.36	21.82	9.38	

* Author index refers to that of the papers listed in the Appendix to this paper. Index number 37, i.e. Kobayashi's (1940) paper, includes the data compiled from 261 papers, none of which is listed in the Appendix.

(ii) *MN blood groups*

Data on the MN blood groups have been obtained in only 21 Prefectures (table 2). The total number of observations is 70,487, of

TABLE 2

Data on the blood groups M, MN and N according to Prefecture

Pref. no.	Prefecture	No. of observations	Per cent. frequency			Author index*
			M	MN	N	
1	Aomori . . .	841	27.71	38.05	34.24	2
9	Gumma . . .	3,203	28.32	50.45	21.23	4, 12
11	Tokyo . . .	6,179	29.94	48.97	21.09	3, 13, 32, 63, 72
12	Chiba . . .	4,100	29.61	51.51	18.88	12, 14, 67
13	Kanagawa . . .	951	27.02	49.95	23.03	64
14	Niigata . . .	2,028	32.74	47.78	19.48	21, 46
15	Nagano . . .	2,460	28.38	50.85	20.77	31
20	Toyama . . .	1,071	33.62	50.23	16.15	22, 52, 70
21	Ishikawa . . .	1,564	30.56	48.79	20.65	10, 15, 36, 51
22	Fukui . . .	1,219	31.99	47.58	20.43	20
23	Shiga . . .	646	32.04	43.04	24.92	25
25	Nara . . .	743	25.17	53.83	21.00	45
26	Wakayama . . .	202	30.20	46.04	23.76	68
27	Kyoto . . .	1,814	31.97	46.64	21.39	8, 11, 16, 49, 56
28	Osaka . . .	1,723	27.80	53.22	18.98	23, 30, 69
32	Shimane . . .	1,465	35.70	44.16	20.14	47, 76
33	Hiroshima . . .	1,961	32.02	49.26	18.72	42
39	Fukuoka . . .	311	32.80	48.55	18.65	7
42	Nagasaki . . .	12,032	27.82	50.01	22.17	28, 29, 40, 75
43	Kumamoto . . .	1,975	32.81	48.20	18.99	55
45	Kagoshima . . .	2,121	27.16	52.90	19.94	50
	Total . . .	48,609	29.60	49.46	20.94	

* Author index refers to that of the papers listed in the Appendix to this paper.

which 21,878 were discarded again because they pertained to Hokkaido or the Ryukyus or the location of tested individuals could not be identified. The final number used was 48,609.

3. ANALYSIS OF DATA ON THE ABO BLOOD GROUPS

(i) *Variation and covariation of gene frequencies*

The frequencies of the genes I^A , I^B and I^O controlling the ABO blood groups were estimated by the well-known formula developed by Bernstein (1930), namely,

$$\text{Frequency of } I^A: p = p' \left(1 + \frac{D}{2} \right)$$

$$\text{Frequency of } I^B: q = q' \left(1 + \frac{D}{2} \right)$$

$$\text{Frequency of } I^O: r = \left(r' + \frac{D}{2} \right) \left(1 + \frac{D}{2} \right)$$

where $p' = 1 - \sqrt{(n_B + n_O)/n}$, $q' = 1 - \sqrt{(n_A + n_O)/n}$, $r' = \sqrt{n_O/n}$, and $D = 1 - p' - q' - r'$, n_O , n_A , n_B , and n representing the observed numbers

TABLE 3
ABO and MN blood group gene frequencies in each Prefecture

Prefecture number	ABO blood groups				MN blood groups		
	p	q	r	χ^2	p	f_s	χ^2
1	0.2368	0.1930	0.5702	0.67	0.4673	0.2357	46.72
2	0.2491	0.1909	0.5600	0.11	...	...	...
3	0.2456	0.1553	0.5991	0.06	...	...	...
4	0.2663	0.1798	0.5539	4.52	...	...	...
5	0.2690	0.1685	0.5625	2.84	...	...	...
6	0.2689	0.1893	0.5418	6.31	...	...	...
7	0.2511	0.1812	0.5677	0.54	...	...	...
8	0.2595	0.1847	0.5558	3.61	...	...	...
9	0.2742	0.1815	0.5443	3.40	0.5354	-0.0141	0.64
10	0.2871	0.1721	0.5408	0.12	...	...	...
11	0.2713	0.1702	0.5585	4.64	0.5443	0.0128	1.02
12	0.2553	0.1662	0.5785	9.79	0.5537	-0.0422	7.30
13	0.2747	0.1686	0.5567	5.46	0.5200	-0.0005	0.0002
14	0.2752	0.1811	0.5437	1.60	0.5663	0.0273	1.51
15	0.2790	0.1993	0.5217	0.01	0.5380	-0.0230	1.30
16	0.2891	0.1676	0.5433	0.13	...	...	...
17	0.2646	0.1691	0.5663	7.18	...	...	...
18	0.2733	0.1727	0.5540	2.08	...	...	...
19	0.2754	0.1515	0.5731	10.54	...	...	...
20	0.2744	0.1709	0.5547	0.45	0.5873	-0.0363	1.41
21	0.2711	0.1770	0.5519	3.19	0.5496	0.0146	0.33
22	0.2621	0.1621	0.5758	0.14	0.5578	0.0355	1.54
23	0.2892	0.1833	0.5275	0.15	0.5356	0.1349	11.76
24	0.2657	0.1858	0.5485	0.02	...	...	...
25	0.2852	0.1583	0.5565	1.27	0.5209	-0.0786	4.59
26	0.2674	0.1715	0.5611	0.22	0.5322	0.0754	1.15
27	0.2823	0.1751	0.5426	0.38	0.5529	0.0567	5.83
28	0.2802	0.1551	0.5647	8.11	0.5441	-0.0728	9.13
29	0.2818	0.1664	0.5518	0.46	...	...	...
30	0.2847	0.1784	0.5369	1.61	...	...	...
31	0.2900	0.1593	0.5507	0.28	...	...	...
32	0.2969	0.1704	0.5327	0.11	0.5778	0.0948	13.17
33	0.2886	0.1651	0.5463	8.09	0.5665	-0.0003	0.02
34	0.2821	0.1652	0.5527	0.48	...	...	...
35	0.2992	0.1537	0.5471	15.68	...	...	...
36	0.3019	0.1705	0.5276	0.06	...	...	...
37	0.2821	0.1624	0.5555	1.04	...	...	...
38	0.2933	0.1730	0.5337	0.84	...	...	...
39	0.2990	0.1628	0.5382	14.28	0.5707	0.0091	0.03
40	0.2982	0.1754	0.5264	1.49	...	...	...
41	0.2866	0.1817	0.5317	0.004	...	...	...
42	0.2889	0.1712	0.5399	8.07	0.5283	-0.0034	0.14
43	0.2897	0.1631	0.5472	2.50	0.5691	0.0172	0.58
44	0.2959	0.1626	0.5415	5.94	...	...	...
45	0.2840	0.1605	0.5555	0.47	0.5361	-0.0635	8.56
Mean	0.2773	0.1706	0.5521	5.47	0.5433	0.0021	0.58

of blood groups O, A, B, and the total number of observations respectively. These estimates of gene frequencies are given in table 3.

The departure of the observed blood group frequencies from the

Hardy-Weinberg equilibrium was tested by $\chi^2 = 2n(1+r/pq)D^2$ with one degree of freedom. The value of χ^2 thus obtained exceeded the 5 per cent. level of significance in 13 Prefectures (29 per cent.) out of 45 and the 1 per cent. level in 8 Prefectures (18 per cent.) (table 3). These departures could result from various factors such as the non-random sampling of data, technical errors in diagnosis, inbreeding within Prefectures, overdominance, maternal-fetal incompatibility and meiotic drive. At present it is not known which of these is the most important, but it appears that at least some parts are

FIG. 2.—Distributions of the blood group gene frequencies.

due to the inbreeding within Prefectures. The reasons are as follows: (1) Marriages are usually contracted within areas smaller than Prefectures and consanguineous marriages are fairly common in Japan. (2) The value of χ^2 obtained for each sample within Prefectures reached the significance level less frequently than that for Prefecture. This may indicate that the high values of χ^2 for Prefectures are at least partly due to the subdivisions of mating groups within Prefectures. (3) The simultaneous estimation of gene frequencies and inbreeding coefficient from the total frequencies of O, A, B and AB blood groups for all Prefectures gives a positive value for the estimate of inbreeding coefficient (see below).

If the inbreeding within Prefectures is a significant factor, then the simultaneous estimation of gene frequencies and inbreeding coefficient for each Prefecture is desired (cf. Schull *et al.*, 1962). This simultaneous estimation is, however, very sensitive to other factors, and the inbreeding within Prefectures appears to be small, so that no account was made of the inbreeding in the estimation of gene frequencies.

Fig. 2 shows the frequency distributions of p , q and r estimated by the above method. It is seen that the shape of the frequency distribution is nearly the same for all gene frequencies, though the variance is not the same (cf. table 4). The χ^2 value for the heterogeneity of gene frequencies among Prefectures was computed to be 1536.6 with 88 degrees of freedom, thus indicating a highly significant differentiation of gene frequencies among Prefectures. Partition of the χ^2 showed that this differentiation has occurred for every gene frequency. Accordingly, these gene frequency data were subjected to the genetic analysis described by Nei (1965).

In practice any gene frequency is estimated from a sample of finite size, thus being subject to sampling variation. If p is the true frequency of gene I^A in a subpopulation and δp is the sampling deviation of the observed (p_0) from the true value, then the variance of observed gene frequency among subpopulations is given by $\sigma_{p_0}^2 = \sigma_p^2 + \sigma_{\delta p}^2$, where σ_p^2 is the variance of true value and $\sigma_{\delta p}^2$ the sampling variance. The value of $\sigma_{\delta p}^2$ for a sample or subpopulation is obtained by:

$$\sigma_{\delta p}^2 = \frac{I_{qq}}{I_{pp}I_{qq} - I_{pq}^2}$$

where I_{pp} , I_{pq} and I_{qq} are the elements of the information matrix for the estimates of p and q . The sampling variance $\sigma_{\delta p}^2$ is a function of p , q and the number of observations, n , but in this case $\sigma_{\delta p}^2$ is determined almost exclusively by n , since p and q do not vary so widely. Hence, an approximate formula for $\sigma_{\delta p}^2$, which depends only on n , was derived, using the weighted means of p and q as given in table 3. The formula obtained is

$$\sigma_{\delta p}^2 = 0.11867/n.$$

The mean sampling variance is then obtained by

$$\begin{aligned}\sigma_{\delta p}^2 &= \Sigma n \frac{0.11867}{n} / \Sigma n \\ &= 0.11867/\bar{n}\end{aligned}$$

where $\bar{n}$ is the mean number of observations for Prefectures. The sampling variances of q and r and sampling covariances between p , q and r were obtained in the same way. The values thus obtained are set out in table 4.

The variances and covariances of observed gene frequencies are obtained in the following way. For example,

$$\begin{aligned}\sigma_{p_0}^2 &= \frac{\Sigma n(p_0 - \bar{p}_0)^2}{\Sigma n} \\ \sigma_{p_0 q_0} &= \frac{\Sigma n(p_0 - \bar{p}_0)(q_0 - \bar{q}_0)}{\Sigma n}.\end{aligned}$$

The true or genetic variances and covariances of p , q and r are then obtained by the subtraction of their respective sampling variances and covariances from the observed values. These estimates of σ_p^2 , σ_{pq} , etc. are also given in table 4.

TABLE 4
Variances, covariances and degree of differentiation of the ABO blood group gene frequencies

Gene frequency concerned	Variance or covariance		f_{ST}
	Genetic	Sampling	
p	0.0001456	0.0000089	0.0007265
q	0.0000921	0.0000058	0.0006507
r	0.0001826	0.0000113	0.0007383
$p \cdot q$	-0.0000275	-0.0000017	0.0005822
$p \cdot r$	-0.0001180	-0.0000072	0.0007710
$q \cdot r$	-0.0000645	-0.0000041	0.0006850
Mean	...	...	0.0006923

Table 4 shows that the genetic variances and covariances are much larger than the sampling variances and covariances. This again indicates that there is a significant differentiation of gene frequencies among Prefectures.

(ii) Degree and form of the differentiation

Nei (1965) has shown that the degree of differentiation of subpopulations as measured by the inbreeding coefficient can be obtained by

$$f_{ST} = \frac{\sigma_j^2}{\bar{p}_j(1 - \bar{p}_j)} \quad \text{or} \quad f_{ST} = \frac{-\sigma_{jk}}{\bar{p}_j \bar{p}_k}$$

provided that the differentiation has occurred at random. Here, $\bar{p}_j$, σ_j^2 and σ_{jk} denote the mean and variance of the j th allele frequency, and the covariance between the frequencies of the j th and k th alleles, respectively. The inbreeding coefficients obtained by the above formulæ are given in the last column of table 4. It is seen from this table that the degree of differentiation is of small magnitude. Note, however, that the subpopulations employed in this analysis are not the smallest units of random mating. Table 4 also shows that all the estimates of inbreeding coefficient are remarkably similar, though obtained from different variances and covariances. This homogeneity of f_{ST} suggests that the differentiation of ABO blood group gene frequencies has occurred largely at random (cf. Nei, 1965).

The randomness of differentiation can be tested by another method, *i.e.* by the comparison of the observed with the expected correlations

between gene frequencies (Nei, 1965). The expected correlation between the frequencies of the j th and k th alleles is given by

$$r(j, k) = -\sqrt{\frac{\bar{p}_j \bar{p}_k}{(1 - \bar{p}_j)(1 - \bar{p}_k)}}$$

if differentiation has occurred at random. The expected correlations thus obtained are given in table 5, together with the observed correlations. The *direct* and *corrected* values of observed correlations refer

TABLE 5
Correlations between the ABO blood group gene frequencies

Gene frequencies concerned	Expected	Observed	
		Direct	Corrected
$p \cdot q$	-0.2810	-0.2381	-0.2379
$p \cdot r$	-0.6877	-0.7235	-0.7240
$q \cdot r$	-0.5036	-0.4982	-0.4978

to those obtained from the observed variances and covariances and from the genetic variances and covariances, respectively. Statistical test indicates that there is no significant difference between the expected and *direct* observed values in any pair of gene frequencies. It is highly probable that the differences between the expected and *corrected* observed values are also insignificant, since there are such small differences between the *direct* and *corrected* values of correlations. This again indicates that the differentiation of ABO gene frequencies is not much deviated from randomness.

(III) Correlation of the gene frequencies between different localities

If differentiation occurs at random by means of genetic drift, the gene frequencies of close localities tend to be more alike than those of

TABLE 6
Numbers of locality pairs for various units of distance

Distance unit	1	2	3	4	5	6	7	8	9	10	11	12	13
ABO blood groups	95	142	156	134	111	94	86	67	50	31	16	6	2
MN blood groups	20	25	31	37	20	18	20	16	10	7	4	1	1

localities far apart. Kimura and Weiss (1964) have shown that with their stepping stone model the correlation coefficient of the gene frequencies between two localities or colonies decreases with distance

irrespective of linear or areal distribution, though the rate of decrease depends on the distributional dimension. Thus, if the correlation of ABO blood group gene frequencies decreases with distance, then it would become another evidence for the random differentiation.

FIG. 3.—Correlations of the gene frequencies between different localities for the ABO blood groups. Closed circle refers to the intraclass-like correlation and open circle the ordinary correlation.

As mentioned previously, the unit of locality employed in this investigation is Prefecture, which possibly includes many neighbourhoods, and these neighbourhoods correspond to the colonies of Kimura and Weiss (1964), disregarding the apparent continuous distribution. Further, the area or population size is not the same for all Prefectures.

Thus, the fitting of stepping stone model is only an approximation and no exact fitting of the mathematical model developed by Kimura and Weiss (1964) will be attempted here. The distance between two localities may be measured by the number of steps or intervening colonies as was theoretically studied by Kimura and Weiss (1964) or by the total length of roads connecting the two localities. In this investigation the distance was measured by the number of intervening localities plus unity. When there were many ways of counting the intervening localities, the smallest number was used. The number of

FIG. 4.—Intraclass-like correlation of the I^A gene frequency for one unit of distance.

pairs of localities is not, of course, the same for all units of distance. The actual numbers of locality pairs for various distance units are shown in table 6. It is seen from this table that the number of pairs for 10 or more units of distance is less than 60. Hence, no correlations were computed for the pairs of these distances, since they are considered to be subject to a large sampling error. Note that the actual number of observations of gene frequency is 45, though the number of pairs of observations is large. Further, when distance is remote, not all observations are used for taking pairs. For example, with the 10 units of distance only 20 observations could be used for taking pairs of localities (cf. fig. 1).

FIG. 5.—Intraclass-like correlation of the I^A gene frequency for nine units of distance.

FIG. 6.—Cline as seen for the I^A gene frequency.

Two kinds of correlation were computed to measure the similarity of gene frequencies. One is that which looks like the so-called intra-class correlation and obtained as follows:

$$r = \frac{2\sum xy - (\sum x + \sum y)^2/N}{\sum x^2 + \sum y^2 - (\sum x + \sum y)^2/N}$$

where x and y are the gene frequencies of the localities with the smaller and larger Prefecture number *in pair* respectively (cf. table 1 and fig. 1), and N is the number of locality pairs. The correlation coefficients obtained are graphically presented in fig. 3. This figure shows that the correlation for gene I^A decreases gradually with increasing distance until around 5 units of distance, as is theoretically expected. Thereafter, however, the correlation increases in the negative direction. A similar but less obvious trend is also observed for genes I^B and I^O . A detailed examination revealed that these unexpected correlations for high units of distance are due to the symmetrical property of the correlation used interacting with the cline of gene frequencies, which will be considered in the next section. This situation can be seen from figs. 4 and 5, in which the correlations for 1 and 9 units of distance are shown.

In order to remove the artifact negative correlation, another type of correlation, *i.e.* the ordinary correlation between x and y was computed. The results obtained are given also in fig. 3. It is seen from this figure that the correlation of the gene frequencies between two localities tends to decrease with increasing distance for any of the genes I^A , I^B and I^O . The linear regressions representing the decrease of correlation for the genes I^A , I^B and I^O are

$$r^A = 0.7314 - 0.0678X$$

$$r^B = 0.1124 - 0.0182X$$

$$r^O = 0.1958 - 0.0062X$$

respectively, where X represents the distance units. The regression coefficient for I^A is significant at the 1 per cent. level. These results suggest that the differentiation of ABO blood group gene frequencies has occurred nearly at random.

(iv) *Cline of gene frequencies*

So far the analysis has suggested that the differentiation of the ABO blood group gene frequencies has occurred nearly at random. However, early workers such as Furuhashi (1933), Kobayashi (1940) and Tanaka (1959) have noted that the frequency of the gene I^A is higher in the south-western part of Japan than in the north-eastern part, while the frequency of I^B is reciprocally distributed. Thus, a quantitative study was carried out on the cline of gene frequencies.

Geographically, Japan is a long country from north-east to south-west, the people thus being distributed semi-linearly. In this investigation this semi-linear distribution was regarded as a linear one and the

FIG. 7.—Cline as seen for the I^B gene frequency.

FIG. 8.—Cline as seen for the I^O gene frequency.

distance of each locality from an arbitrary origin, Aomori, was measured in kilometres along the National Railways. In this measurement the location of each Prefecture was represented by that of the Prefecture capital. The relationships between the distance from Aomori and gene frequencies of I^A , I^B and I^O are given in figs. 6-8.

It is seen from these figures that the frequency of gene I^A shows a typical cline from north to south. The correlation coefficient between the distance and p is 0.783, the linear regression coefficient of p on the distance (1000 km.) being 0.0243 ± 0.0029 . The clines of frequencies of the genes I^B and I^O are less typical, though the correlation coefficients between the distance and q and r are -0.416 and -0.480 respectively, which are both significant at the 1 per cent. level. The regression coefficients of q and r on the distance are -0.0093 ± 0.0031 and -0.0150 ± 0.0042 respectively.

As mentioned previously, Japan is a long country from north-east to south-west. So, one might have the idea that the apparent correlation between the gene frequencies and the distance from Aomori is due to the primary correlation between the gene frequencies and latitude. In practice, the correlations between the latitude and p , q and r are -0.753 , 0.428 and 0.442 respectively. Thus, this possibility cannot be excluded.

As will be discussed later, the cline of gene frequency may arise by two factors, *i.e.* the local differences in selection intensity and the mixture of two races. If the cline is caused by the former, it indicates that the differentiation of gene frequencies has not occurred completely at random. Further, the fact that p is positively correlated with the distance while q and r are negatively correlated suggests that the selection determining the cline has operated as if there were two alleles, *i.e.* I^A versus I^O and I^B . A more elaborate analysis of this cline is now in progress.

4. ANALYSIS OF DATA ON THE MN BLOOD GROUPS

The analysis of data on the MN blood groups is much simpler than that of data on the ABO blood groups, since there are only two alleles concerned and all the genotypes can be identified phenotypically. The gene frequency and inbreeding coefficient or deviation from Hardy-Weinberg equilibrium can easily be estimated by the maximum likelihood method. The estimates of the m gene frequency (p) and inbreeding coefficient (f_s) from 21 Prefectures are given in table 3. The mean of gene frequency weighted for the number of observations is obtained to be 0.5433. The gene frequency of Aomori Prefecture is considerably deviated from the mean. This deviation may be due to some technical error in diagnosis. The high value of χ^2 for this Prefecture also indicates the possibility of technical error. Some of the estimates of inbreeding coefficient are negative, which must have arisen either by sampling error or the real excess of heterozygotes. χ^2 -test has shown that 3 out of the 10 negative estimates cannot be accounted for by sampling error alone at the 1 per cent. level of significance (table 3). It appears that there is some mechanism causing the excess of heterozygotes, such as overdominance.

At any rate, the mean of f_s weighted for the number of observations is 0.0021, if the datum of Aomori is included, and -0.0021 , if this is

excluded. These values are too small to be used for an estimate of inbreeding coefficient within Prefectures concerning neutral genes, for even the inbreeding coefficient estimated from the frequencies of consanguineous marriages amounts to about 0.3-0.4 per cent. in middle-sized cities of Japan (Schull, 1958; Tanaka, 1963). The small or negative value of f_s may again be due to the existence of some mechanism causing the excess of MN heterozygotes.

The genetic and sampling variances of gene frequency were obtained in two ways, *i.e.* including and excluding the datum of Aomori, by the same principle as that of the ABO blood groups. The results obtained are shown in table 7. The χ^2 for the heterogeneity of

TABLE 7

Variance and degree of differentiation of the MN blood group gene frequency

	Variance		f_{ST}	f_s	f_T	f'_T *
	Genetic	Sampling				
Whole Japan	0.000303	0.000054	0.00122	0.00206	0.00328	0.0034
Aomori excluded	0.000207	0.000052	0.00084	-0.00205	-0.00121	-0.0011

* f'_T refers to the estimate of the total inbreeding obtained from the total frequencies of M, MN and N.

gene frequency among all subpopulations is 139.7 with 20 degrees of freedom, which indicates a significant differentiation of subpopulations. If the datum of Aomori is excluded, the heterogeneity χ^2 becomes 99.9 with 19 degrees of freedom, which is again statistically significant at the 1 per cent. level.

The inbreeding coefficient measuring the differentiation is 0.0012 if the datum of Aomori is included, and 0.00084 if this is excluded. The former is about two times larger than the value obtained from the ABO blood group data, whereas the latter is almost identical with this. The total inbreeding in Japan can be estimated by

$$(1 - f_T) = (1 - f_{ST})(1 - f_s)$$

though no correction is made for the local variation in population size (Wright, 1943). Using $f_s = 0.0021$, we have $f_T = 0.0033$, whereas $f_s = -0.0021$ gives $f_T = -0.0012$. The value of f_T can also be estimated from the total frequencies of M, MN and N blood groups. This estimate becomes 0.0034 if all data are used and -0.0011 if the datum of Aomori is excluded, thus both agreeing with the former estimates.

In order to see whether the differentiation observed has occurred at random or not, the correlation of the gene frequencies between two

localities was examined as with the case of ABO blood groups. The results obtained are shown in fig. 9. Inspection of this figure shows that the correlation is almost independent of the distance of localities

FIG. 9.—Correlations of the gene frequencies between different localities for the MN blood groups. Closed circle refers to the intraclass-like correlation and open circle the ordinary correlation.

in pair, though the correlation is subject to a large sampling error because of the small number of Prefectures employed. Further, the examination of the relation between geographical location and gene frequency has indicated that there is no cline in the *m* gene frequency.

5. DISCUSSION

Examining the departure of the MN blood group frequencies from the Hardy-Weinberg equilibrium in 55 different samples obtained from various parts of the world, Taylor and Prior (1939) found that the departure was statistically significant at the 1 per cent. level in eight samples (12.7 per cent.). They attributed these departures to the technical errors in diagnosis, indicating that the technical errors occur in both positive and negative directions. The same pattern of departures from the Hardy-Weinberg equilibrium has been observed also in our analysis of the MN blood groups. These departures, however, need not all be attributed to the technical errors. If a sample is taken from a random mating group and there is overdominance operating in the foetal and childhood stages, as was suggested by Morton and Chung (1959), the frequency of MN heterozygotes is expected to be higher than the Hardy-Weinberg frequency.

On the other hand, if a sample is taken from a large population divided into many subpopulations, the frequency of MN heterozygotes could be smaller than the Hardy-Weinberg equilibrium. If these two kinds of samples are mixed, the total frequency might well agree with the Hardy-Weinberg expectation, as in the case of Taylor and Prior (1939). A similar situation may occur also for the ABO blood groups.

Nevertheless, the possibility of the effect of technical errors cannot be excluded completely, especially when the data of many workers are compiled. Different workers may have different techniques for blood group typing, thus their data deviating in different directions. In our case the data on the ABO blood groups are taken from 340 different reports, while the data on the MN blood groups are from 43 different reports, though many of them were obtained by Dr T. Furuhashi and his school. There is, however, one study in which the ABO blood group frequencies are obtained for all Prefectures with use of moderately large samples. This is the study on the Japanese Army (the data were reported by Kobayashi (1940) but appear to have been collected by several workers). The average sample size for a Prefecture is 1935, the individual sample varying from 283 to 5532. If the gene frequencies obtained from these data are positively correlated with those obtained from the rest (the Army data are included in the previous analysis), then it would reflect the importance of the differentiation of gene frequencies among Prefectures. The correlation coefficients of the gene frequencies of I^A , I^B and I^O between these two sets of data are 0.5445, 0.3315 and 0.3254 respectively, which are all statistically significant. Thus, it seems that a large fraction of the variability in the ABO blood group gene frequencies is ascribed to the local differentiation. Note that the correlations between the two sets of data cannot be very high, because the gene frequencies have been estimated from samples of finite size and the two samples for a Prefecture have not necessarily been taken from the same area within the Prefecture.

There are no data for the MN blood groups which can be analysed in the same way as in the case of ABO blood groups. However, as it is likely that the same worker is subject to the same type of technical errors, the significant heterogeneity of gene frequency among Prefectures, for the data obtained by the same worker, would indicate the real differentiation of gene frequency. In order to test this point five samples obtained by Dr H. Kobayashi and his co-workers were subjected to χ^2 -analysis. The sample size and gene frequency estimates are given in table 8. The heterogeneity χ^2 for these samples is obtained to be 18.8 with 4 degrees of freedom, which is highly significant. This indicates that the differentiation of gene frequency observed for the MN blood groups cannot all be ascribed to technical errors.

In a population divided into many random mating subpopulations the frequency of heterozygotes for a locus decreases by the amount equal to twice the variance of gene frequency among subpopulations

compared with that of a single random mating population, provided that there is no disturbance due to gametic or zygotic selection (Wahlund, 1928). If, however, there is some mechanism causing the excess of heterozygotes, the effect of subdivisions may be diluted and in an extreme case the inbreeding coefficient in the total population (f_T) may become negative as in the case of MN blood groups. This negative estimate of inbreeding coefficient does not necessarily indicate that there is no differentiation among subpopulations, since even under the effect of overdominance or other similar agencies the gene frequencies are differentiated among subpopulations unless the effective population size is infinitely large.

As shown in table 3, the value of χ^2 for the departure of the total ABO blood group frequencies from the Hardy-Weinberg equilibrium is statistically significant. Thus, the simultaneous estimation of gene frequencies and inbreeding coefficient (f_T) was undertaken by the

TABLE 8

Heterogeneity of the Prefecture gene frequencies obtained by the same worker

Prefecture	Sample size	Gene frequency	Source
Niigata	1248	0.5645	Kobayashi and Katoh (1957)
Nara	743	0.5209	Kobayashi and Okada (1956)
Hiroshima	1961	0.5665	Kobayashi and Ando (1953)
Shimane	967	0.5646	Kobayashi <i>et al.</i> (1957)
Nagasaki	1673	0.5293	Kobayashi (1952)

Heterogeneity $\chi^2 = 18.8$. DF = 4. $P < 0.001$.

maximum likelihood method. The estimates obtained are $p = 0.2974$, $q = 0.1831$, $r = 0.5195$ and $f_T = 0.1383$ respectively. The estimates of gene frequencies are not much deviated from the mean gene frequencies given in table 3, but the estimate of f_T is extremely large. Such a large value as this may be explained if f_s is large or there is some mechanism which causes the deficiency of heterozygotes such as maternal-fœtal incompatibility. If the former is the case, however, f_s must be 0.1377, which is again unbelievably large. Further, the ABO maternal-fœtal incompatibility as advocated by Matsunaga and Itoh (1958) has not been completely established (see Hiraizumi *et al.*, 1963). Thus, the real cause of the high value of f_T is not known at present. In this connection it may be noted that the simultaneous estimation of the three parameters is very much sensitive to various factors and with some types of blood group frequencies the maximum likelihood estimates do not approach any asymptote even if more than a hundred cycles of approximations are made (Nei and Imaizumi, unpublished). At any rate, care must be exercised in using polymorphic traits to estimate the average inbreeding coefficient for many loci.

It has been shown that the differentiation of the ABO blood group gene frequencies in Japan has occurred largely at random, though there is a cline from north to south. This does not, of course, mean that the ABO blood group genes are neutral with respect to natural selection. The random differentiation of gene frequency may occur even under overdominant selection, if the effective population size is sufficiently small. The decreasing correlation of gene frequencies between localities with distance suggests that at least some part of the differentiation of gene frequencies has occurred by genetic random drift. On the other hand, the correlation of gene frequencies between localities for the MN blood groups did not decrease with increasing distance. This may be due to the small sample size and small number of Prefectures employed or the random geographical variation in selection intensity. If the latter is the case, of course, we cannot ascribe all the variation of the gene frequency to the small effective population size.

The cline of gene frequency in man has often been explained by the migration and intermarriage of different races. But it may arise also by the geographical differences in selection intensity with the diffusion effect of migration, as shown by Haldane (1948) and Fisher (1950), although in the present case it seems reasonable to assume that, contrary to Haldane's or Fisher's model, the polymorphism is maintained primarily by a heterozygote advantage and the cline is caused by the gradient of selection intensity and migration within the population. Note that the recent investigations on the selective mechanisms of the ABO and MN blood groups indicates that these traits are never independent of natural selection (cf. Morton and Chung, 1959; Chung and Morton, 1961; Chung *et al.*, 1961; and others). In practice, however, it is difficult to distinguish between the two factors responsible for a cline, unless the history of the population is well known. In our case it appears, as mentioned previously, that only a small magnitude of migration has occurred between Japan and her surrounding countries at least for these 2000 years. Further, the populations surrounding the Japanese all have the ABO and MN blood group gene frequencies considerably different from those of the Japanese (cf. Tanaka, 1959). These suggest that the clines observed for the ABO blood group gene frequencies have occurred by the geographical differences in selection intensity with the diffusion effect of migration. Since there are significant correlations between gene frequencies and latitude, the selection intensity may be primarily dependent on the temperature.

This view is supported by further evidence, *i.e.* the negative correlations observed between p , q and r . If the clines of these gene frequencies are caused by migration and intermarriage, then the correlation of q and r should be positive, as will be seen from fig. 10. Note that this type of cline is only transient and destined to disappear at equilibrium. On the other hand, if the geographical variation in selection intensity is responsible for the clines, together with the effect

of random genetic drift, then all the correlations are expected to become negative, in so far as the difference in gene frequency between the two extremes is small as in the present data.

In conclusion it may be said that the Japanese population is not a single random mating population but divided into many small sub-populations or neighbourhoods in Wright's sense. The effective size

FIG. 10.—Schematic representation of the clines observed for the ABO blood group gene frequencies.

of these neighbourhoods is not known at present, but it is considered to be so small as to give an appreciable local differentiation of gene frequency even for polymorphic traits, which are possibly maintained by overdominance or other similar agencies. It is then expected that the frequencies of neutral genes, if any, or deleterious recessive genes maintained by mutation and selection are much more locally differentiated. The large local variation of the acatalasemia gene frequency reported by Neel *et al.* (1963) may reflect this circumstance.

6. SUMMARY

1. The geographical variations of ABO and MN blood group frequencies in Japan were subjected to a genetic analysis with the aim of contributing to the genetics of human populations. The data employed for the present analysis were compiled from 340 publications on the ABO blood groups and 43 publications on the MN blood groups. The total number of observations used was 599,342 for the ABO and 48,609 for the MN blood groups.

2. The geographical subdivision was made according to Prefecture (administrative unit of Japan) and gene frequencies were

estimated for each Prefecture. Heterogeneity tests have shown that there is a significant differentiation of gene frequency among Prefectures both for the ABO and MN blood groups.

3. The degree of differentiation of gene frequency as measured by inbreeding coefficient is of the same order for all of the three alleles controlling the ABO blood groups. The inbreeding coefficient obtained from the covariation of gene frequencies is also of the same order. Further, the MN blood group gene frequency shows almost the same degree of Prefectural differentiation as those of the ABO blood group gene frequencies, though in this case the gene frequency is available only for 21 Prefectures out of 45 in total because of the paucity of data.

4. Comparison of the observed and expected correlations between gene frequencies suggests that the differentiation of ABO blood group gene frequencies has occurred largely at random. The random differentiation of these gene frequencies is also suggested by the decrease of correlation of gene frequencies between localities or Prefectures with distance.

5. There is, however, a typical cline for the frequency of gene I^A from north to south and a less typical cline for genes I^B and I^O . It was suggested that the ABO polymorphism is primarily maintained by heterozygote advantage and these clines have occurred by a geographical gradient of selection intensity operating for the ABO blood groups together with the diffusion effect of migration within the population. Since there are significant correlations between gene frequencies and latitude, the selection intensity may be originally dependent on the temperature.

6. There is no tendency of decrease with distance in the correlation of MN blood group gene frequencies between localities. This appears due to either the relatively small sample size for the MN blood groups or the random geographical variation of selection intensity. The MN blood group gene frequency shows no such cline as is observed for the ABO blood group gene frequencies.

7. It was concluded that the Japanese population consists of many small subpopulations or neighbourhoods in Wright's sense. The effective size of these neighbourhoods is so small as to give an appreciable local differentiation of gene frequency even for polymorphic traits, which are possibly maintained by overdominance or other similar agencies.

Acknowledgment.—We are indebted to Professor J. V. Neel for reading the manuscript and correcting the English.

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APPENDIX

SOURCE OF THE DATA EMPLOYED

In addition to the following papers the data obtained from 261 reports were employed in the present analysis, which are listed in Kobayashi's (1940) paper. All the following papers except Shigeno (1931) are written in Japanese and only a few have English title and summary. Therefore, many of the titles of the following papers were translated from Japanese into English, for which the authors are responsible.

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