GENETIC POLYMORPHISMS IN ISOLATED COMMUNITIES

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The Japanese Subcommittee for the International Biological Program (Matsunaga, 1975) has compiled more than 40 kinds of polymorphic traits. The current issue for "The Distribution of the Human Blood Groups and Other Polymorphisms" (Mourant et al., 1976) contains data on over 60 kinds of polymorphic traits, based upon newly developed immunochemical and electrophoretic techniques. The study of genetic polymorphisms has greatly contributed to our knowledge not only of the genetic characteristics of a given individual and population but of our understandings of evolutionary changes of protein molecules and population structure. In addition, the disease susceptibility occurring in conjunction with a given polymorphic trait has been studied.

DATA OBTAINED

1. Gene frequency of various genetic polymorphic traits in isolated communities

We have surveyed genetic polymorphisms in several isolated communities in Japan (Fig. 1 and Table 1) (Fujiki et al., 1968; Yamamoto et al., 1972). We examined blood types; ABO, MN, Rh, red cell enzyme types; AK, Dia, GOT, GPT, AcP, EsD, 6PGD, PGM, and serum protein types; Hp, Tf, Gc, and estimated their gene frequencies (Tables 2 and 3) (Fujiki and Nishigaki, 1980).

Each isolated community revealed different values of gene frequencies, compared with neighboring communities, owning to such factors as geographic distribution, adaptability to environmental agents, consanguinity, isolation and small sample size. In an infinitely large population, gene frequencies may be only slightly affected by mutation and selections, while in an isolated population remarkable alterations due to genetic drift and founder's effect may be observed. Disasters such as epidemic, famine and flood, abruptly change gene frequencies by chance. One of the authors (Nishigaki, 1978) estimated the mean heterozygosity value and standard deviation for gene frequencies of ten polymorphic traits. He obtained mean heterozygosity value as high as 19.4% in To village, which was higher than that of 17.7% for all of Japan estimated by Ishimoto and 18.1% for England estimated by Harris. At present our unexpectedly high value is inexplicable.
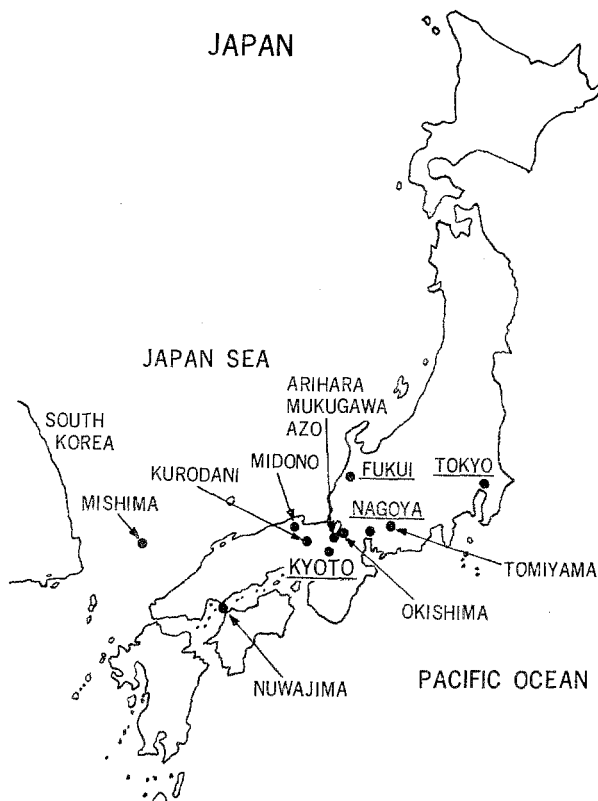
Fig. 1. Communities surveyed.

Table 1. Consanguinity rates and mean inbreeding coefficients in isolates.

Area		Popula-tion	Couple	Inbred couple	Inbred rate	1C	1.5C	2C	Other	Mean inbreeding coefficient	
										Marital unit	Sib unit
M	H	1,975	517	92	17.8	52	13	15	14	0.00771	0.00506
Is.	U	737	192	46	24.0	23	9	4	12	0.01165	0.01164
N	M	1,191	340	52	15.3	23	8	11	6	0.00620	0.00536
Is.	K	648	175	15	8.6	7	2	2	4	0.00304	0.00480
O	Is.	686	286	82	28.7	40	11	19	12	0.01135	0.01065
Mi	Vill.	161	54	14	25.9	7	0	2	6	0.01125	0.01770
K	Ku	243	85	8	9.4	5	3	0	0	0.00489	0.00728
Vill.	Ok	136	48	16	33.3	6	6	2	2	0.01628	0.01462
Ar	Vill.	181	69	40	58.0	13	9	11	7	0.01881	0.02427
Mu	Vill.	235	83	29	34.9	6	3	10	11	0.00901	0.00519
Az	Vill.	251	95	20	21.1	13	3	1	5	0.01053	0.00915
To	Vill.	245	156	48	30.8	20	12	11	5	0.00807	0.00736

Table 2. Gene frequencies of polymorphic traits in isolates (I)
——Blood groups and serum protein types——

Area		ABO			MN		Rh		Hp		Tf		Gc	
		I^A	I^B	I^O	G^M	G^N	D	d	Hp^1	Hp^2	Tf^C	Tf^D	Gc^1	Gc^2
M	Is.	0.246	0.178	0.553	0.533	0.467	—	—	—	—	—	—	—	—
N	Is.	0.220	0.205	0.597	0.547	0.453	—	—	—	—	—	—	—	—
O	Is.	0.333	0.170	0.479	0.672	0.328	(407)	(3)	0.383	0.617	(250)	(1)	0.619	0.381
Mi	Vill.	0.285	0.457	0.258	—	—	—	—	—	—	—	—	—	—
K	Vill.	0.243	0.187	0.675	0.613	0.387	(73)	(0)	0.205	0.795	(142)	(0)	—	—
Ar	Vill.	0.317	0.198	0.485	0.278	0.722	(90)	(0)	0.117	0.852	(89)	(1)	—	—
Mu	Vill.	0.365	0.137	0.498	0.517	0.483	(149)	(0)	0.212a	0.788a	(121)	(0)	—	—
Az	Vill.	0.337	0.154	0.509	0.524	0.476	(143)	(0)	0.298	0.702	(115)	(0)	—	—
To	Vill.	0.224	0.227	0.549	0.586	0.414	(185)	(0)	0.170	0.830	0.907	0.093	—	—
Kinki area		0.278	0.182	0.540	0.558	0.442	0.965	0.035	0.279	0.721	0.988	0.012	0.737	0.263

( ), observed no.   a variant.

Table 3. Gene frequencies of polymorphic traits in isolates (II)
——Red cell enzyme types——

AcP		PGM_1			G6PD		6PGD	
p^a	p^b	PGM_1^1	PGM_1^2	PGM_1^7	B^+	B^-	PGD^A	PGD^C
—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
0.181	0.819	—	—	—	(112)	(0)	(112)	(0)
—	—	—	—	—	—	—	—	—
0.079	0.921	0.851	0.149	0.000	(73)	(0)	(72)	(1)
0.219	0.781a	0.856	0.144	0.000	(90)	(0)	(990)	(0)
0.156	0.844	0.829	0.171	0.000	(149)	(0)	(149)	(0)
0.231	0.829	0.930	0.070	0.000	(143)	(0)	(143)	(0)
0.235	0.765	0.704	0.261	0.035a	(112)	(0)	(112)	(0)
0.210	0.790	0.772	0.217	0.011	1.000		0.915	0.085

ADA		EsD		GPT	
ADA_r	ADA^2	EsD^1	EsD^2	Gpt^1	Gpt^2
—	—	—	—	—	—
—	—	—	—	—	—
0.993	0.007	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
0.986	0.014	0.638	0.362	0.667a	0.333a
0.972	0.028	0.649	0.351	0.623	0.377

To Vill.:   AK 1 (185), GOT 1 (184) 2-1 (1), Dia 1 (185).   ( ), observed no.   a variant.

2. Variant forms of polymorphic traits

Our study of consanguinity in isolated communities can not only clarify the genetic constitutions of a given individual and population, but also demonstrate rare variants such as PGM_1 7-7 homozygotes or Gpt^0 heterozygotes, according to electrophoretic and enzyme assay on the basis of family survey.

The distribution of PGM phenotypes of 171 individuals was determined in To village, where the frequencies of the PGM_1^7 allele was 0.0380, while among neighboring populations it was 0.0114. Moreover, one rare PGM_1^7 homozygote (PGM 7-7) and one heterozygote (7-2) were detected. The existence of the homozygote PGM_1^7 is most likely attributable to the specific genetic structure of this community with high consanguinity (Table 4) (Nishigaki et al., 1978).

The quantitative difference between three common phenotypes of the red cell enzyme GPT was demonstrated in To village (Table 5). The mean activity of this enzyme was 6.47 μ/g Hb for GPT 1, 4.52 for GPT 2-1, and 2.65 for GPT 2 (theoretically 3 : 2 : 1), which is compatible with the finding that the red cell Gpt^1 gene product has a catalytic activity about 2.5 times higher than that of Gpt^2.

In addition, we found one pedigree which did not show the typical segregation pattern of this codominant gene Gpt in To village. There was a discrepancy between the parents, II-7 and II-8 and their son III-5. Other examined genetic markers did not show such discrepancies from the Mendelian pattern, so that parental exclusion was not considered responsible for this single anomaly. An alternative explanation is the presence of a silent gene allele which would not participate in the determina-

Table 4. Distribution of phenotypes and allele frequencies for PGM_1 in the community surveyed.

	1	2-1	2	7-1	7-2	7	Total
Observed	83	65	11	10	1	1	171
Percentage	48.5	38.0	6.4	5.9	0.6	0.6	100.0
Expected	84.9	62.0	11.3	9.2	3.3	0.2	170.9
Allele frequency	PGM_1^1=0.7047		PGM_1^2=0.2573		PGM_1^7=0.0380		

χ^2=5.0682; 3 d.f.; 0.25>p>0.10.

Table 5. Mean levels of red cell GPT activity classified according to phenotypes.

	Total	1	2-1	2	1-0	2-0
Number	376	138	167	69	1	1
Percent	100.0	36.7	44.4	18.3	0.3	0.3
GPT activity (units/g Hb)		6.47	4.52	2.65	2.22	0.98

□ ○ Alived (602)
■ ● Died (830)
♀ ♀ Move out
♀ ♂ Move -in
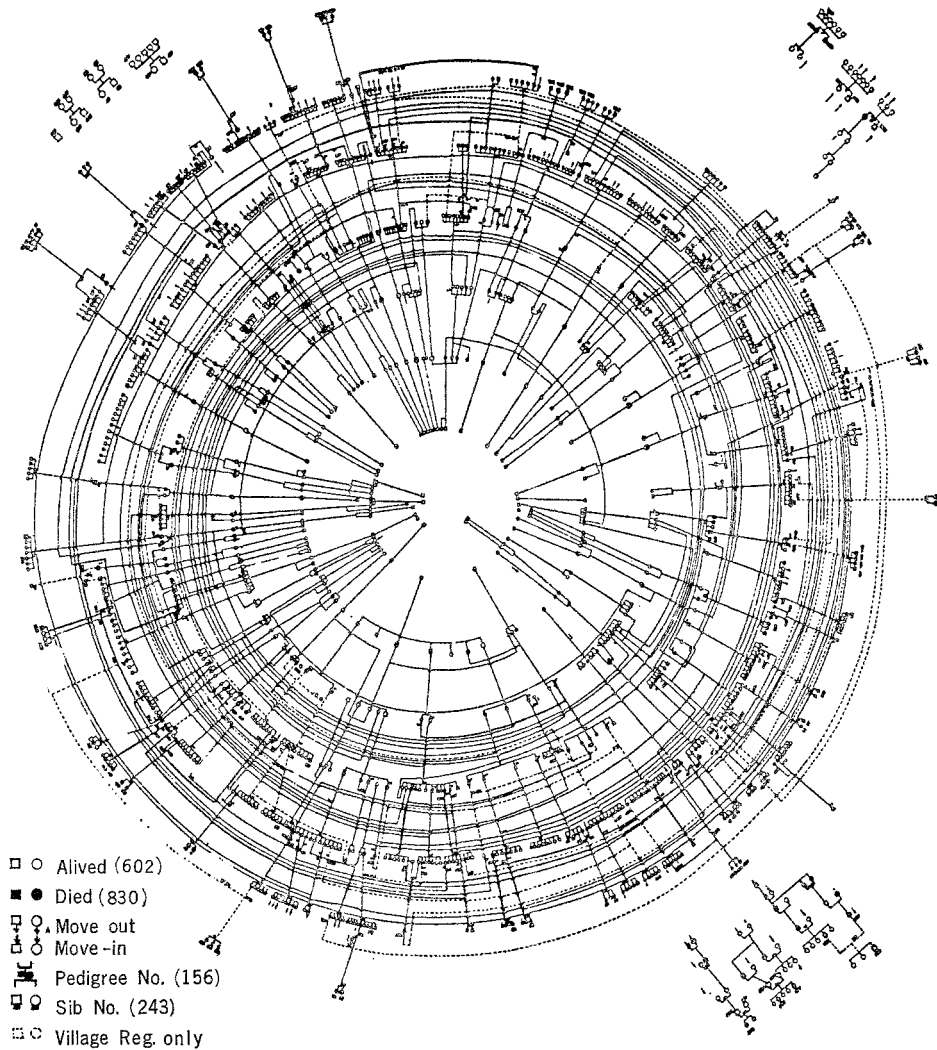Pedigree No. (156)
♀ ♀ Sib No. (243)
□ ○ Village Reg. only

Fig. 2.   Pedigree chart of To village.

tion of GPT phenotype.   This fact would suggest the probable existence of a silent allele, Gpt^0.   Therefore, II-7, II-8 and III-5 have genotypes of Gpt^2/Gpt^2, Gpt^1/Gpt^0 and Gpt^2/Gpt^0 respectively, which appeared in the GPT 2, GPT 1 and GPT 2 in phenotypic electrophoretic patterns.   Heterozygotes with Gpt^0 exhibit less than half normal activity (Fig. 3).   At present these heterozygotes are all healthy.   In summary, with these quantitative data and electrophoretic patterns from family studies, the existence of two heterozygotes with the silent allele Gpt^0 was confirmed (Nishigaki et al., 1980).

In haptoglobin survey, three families (two in Mu and one in Ar) with atypical
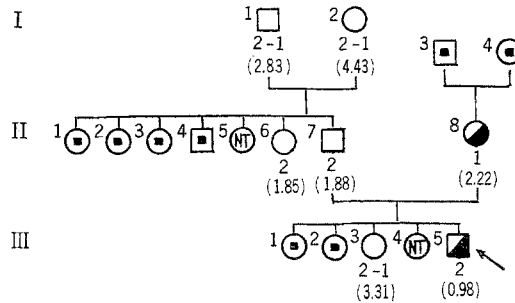
Fig. 3. Pedigree chart of family indicating atypical segregation of GPT phenotypes. (GPT phenotypes and (enzyme activities) are given below each symbol. Half black or square black shows individuals possibly heterozygous for GPT_0 and residents moved-out, respectively. Proband indicated by arrow. NT, not tested.)
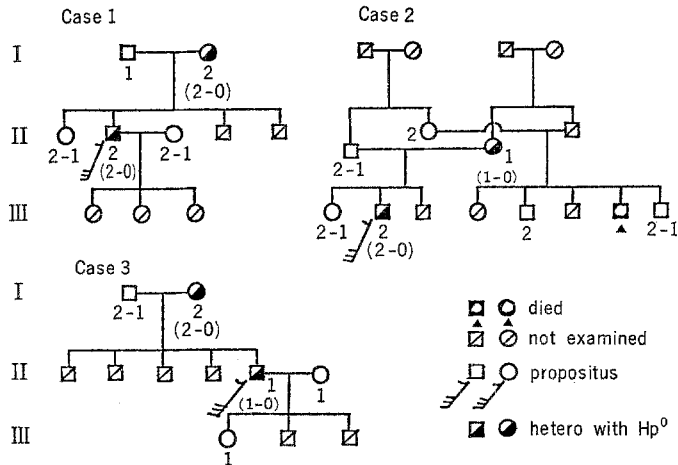

Fig. 4. Atypical segregation of Hp types (Kanazawa, 1971).

segregation of Hp phenotype were encountered. There was strong evidence of the presence of the Hp^0 gene to account for the Hp 1-1 electrophoretic pattern in presumed heterozygotes genotypically Hp^0/Hp^1, and for Hp 2-2 genotypically Hp^0/Hp^2 (Fig. 4). Such a silent Hp^0 allele results in an amount of Hp too low for immunodiffusion determination. These results obtained from this study so far also coincide well with the hypothesis of a silent Hp^0 allele (Kanazawa, 1971).

3. Relations between diseases and genetic susceptibility

In order to clarify the morbidity of some polygenic diseases, various genetic polymorphic traits relating to diseases are examined. For example, genetic determinants in neoplasia may be reflected in a relationship to certain blood types. For the ABO blood types, blood type A is associated with gastric cancer, but no other

Table 6. Relative risk (Woolf's value) of polymorphic traits among blood dyscrasia.

	Phenotype	Apl.	Leuk.
ABO	O	1. 2630	0. 6879*
AcP	B	1. 5938	0. 3337*
ADA	1	0. 7225	0. 2463*
EsD	1	0. 5686	0. 9347
6PGD	A	0. 8233	0. 8035
GPT	1	1. 5548	1. 4148
PGM	1	0. 5591*	0. 6350
GOT	1	0. 1910*	0. 4266
Hp	1	1. 8137	1. 2994
Tf	C	0. 3617	0. 8517

* $p < 0.05$.

consistent correlation has been found. However, wide use of red cell enzyme types and serum protein types may possibly provide new opportunities to detect different associations. Our recent preliminary study suggests relative risk of some poly-morphic traits among blood dyscrasia (Table 6) (Fujiki et al.). A slight associa-tion between Hp 1-1 and lower values of hemoglobin concentration was found in Ar village, where there was also a much higher prevalence of anemia. However, we could not find a close relationship in Ok village, where there was less anemia (Wada, 1978).

4. Application of polymorphic data

We reported previously the study of polymorphisms as applied to population genetics. As an example, we estimated, in Ar village, the inbreeding coefficient, effective population size, and genetic distance. Here we briefly introduce the re-sults.

i) Estimation of inbreeding coefficients. Although reports have been published now to estimate inbreeding coefficients using Koseki records as an information source, this pedigree study method is hardly possible to ascertain remote consan-guinity (Fujiki, 1981). Kinship bioassay method and correlation method are re-garded as alternatives to pedigree study. Through the former method the inbreeding coefficient can be estimated as the degree of deviation from the Hardy-Weinberg equilibrium. Through the latter method the coefficient of kinship can be estimated by distance or isonomy. The latter method was originally invented to apply to a large-sized population, but we deem it permissible to apply this method even to small populations, unless they are inconsistent with Hardy-Weinberg equilibrium. The mean inbreeding coefficient in Ar village was estimated to be 0.0243 by pedigree

study, 0.0514 by phenotype bioassay and 0.0590 by correlation method. The low estimate from pedigree study may be attributed either to an incomplete ascertainment of inbreeding in the ancestors, while the high estimate by the bioassay and correlation methods may be attributed to sampling errors and unknown technical difficulties. The true estimate probably lies between the extreme values (Yamamoto et al., 1974a).

ii) Effective population size. Effective population size is one of the important characteristics in a given population. We have applied both Yasuda's formula assaying random genetic drift and Morton's model from the evolution of the kinship coefficients to our study of small isolated communities. The gene frequencies of 11 loci of ABO, MN, Hp, AcP, and PGM were transformed in each generation (II–IV) to values expressed as the Arcsine. The variance effective size was 6 in generation III and 18 in generation IV, and the ratio effective to observed size are 0.18 and 0.90 respectively. This result suggests that the magnitude of random genetic drift is different in each generation. This might be due to misclassification of individuals by generations, different migration rate, or too small population size examined (Yamamoto et al., 1974b).

iii) Transitory changes of gene frequency of blood types. The data of blood frequency types in Ok island were investigated 35 years ago. There was a large deviation of the gene frequency of ABO and MN blood types from neighboring populations due to the highly inbred structure on Ok island. Compared with our present data, there is a remarkable difference between present and past frequencies. It is thought that genetic drift, simple sampling error or some selective pressure presumably accounts for the differences.

iv) Genetic distance. As an overview of the genetic relationship between the surveyed communities, Cavalli-Sforza and Edwards' genetic distance test was performed for the gene frequency of 11 polymorphic traits. The phenotypic distribution in our isolated communities deviated somewhat, judging from χ^2 test for heterogenuity among them. We also formulated genetic distance in the form of a dendrogram, which was calculated through Morton's program, using the phenotypic distribution of 5 polymorphic traits, ABO, MN, AcP, PGM and Hp (Fig. 5). Population size may be too small in each community to regard this dendrogram as completely authentic, but the result was consistent with the geographic distance among the communities, reflecting the migration pattern.
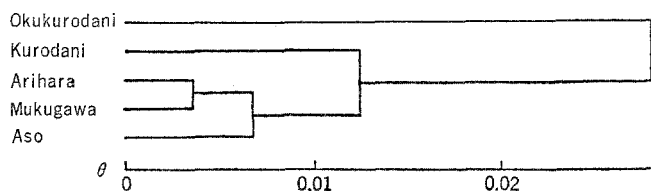

Fig. 5. Dendrogram of isolates surveyed (Yamamoto, 1972).

SUMMARY

Isolated communities offer a unique opportunity for the study of biological and social consequences of consanguinity and migration. The studies of genetic polymorphisms have contributed greatly, not only to knowledge of the genetic constitution of a given individual and population, but also to clarify either relationship between structure and function of polymorphic traits or the susceptibility to multi-factorial diseases, in which interaction between the gene and environment cannot be ignored.

For over 20 years, we have investigated the effect of consanguinity and genetic polymorphisms in 9 isolated communities in western Japan. We reported here different values of gene frequency for each polymorphic trait, compared with the neighboring communities and described how we applied these data to clarification of the genetic constitution of isolated communities as well as of genetic susceptibility to some diseases.

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