ONLINE RESOURCES

Distribution of MN blood group types in local populations in Philippines

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The classification of MN system of human blood type is based on the presence of either glycophorin A (GPA) or glycophorin B (GPB) in the erythrocyte membrane, leading to the expression of the M or N antigen for GPA and only the N antigen for GPB (Kudo and Fukuda 1994). The MN system has been known to indicate specific kinds of immune responsiveness as well as serve as viable genetic markers for particular health conditions and diseases (Delanghe *et al.* 1995). The mode of inheritance of the agglutinogens M and N is through a single set of allelomorphic alleles wherein the *M* and *N* alleles are codominant to each other. This allows the heterozygote *MN* to have a distinguishable phenotype from the homozygous *MM* and *NN* (Mourant *et al.* 1976).

The codominant nature of the *M* and *N* alleles makes it convenient to test whether a population exhibits Hardy–Weinberg equilibrium (HWE) at the MN blood group locus. MN blood groups have shown to exhibit differences in allele frequency in different populations, but are typically in HWE (Furuhata *et al.* 1954; Morton and Chung 1959; Lee 1965; Breguet *et al.* 1986). Thus, genetic drift is a likely reason for the differences in the frequencies of the *M* and *N* alleles between human populations.

Despite the significance of the MN blood groups in the fields of genetics and health, only two prior published data sets on the MN blood group in Philippines are available; one study was restricted to Manila (Fraser *et al.* 1964), while the other compared the frequency distribution of *M* and *N* alleles between a population based in Manila and another in a rural town of Northern Luzon (Guzman *et al.* 2009). Through surveying of the MN blood types, the present study aimed to (i) produce the first comprehensive pool of data on the distribution of the MN blood groups in Philippines; (ii) determine the frequencies of the *M* and *N* alleles in various populations from the major Philippine island groups of Luzon, Visayas, Mindanao and Palawan; and (iii) test whether or not these populations are at HWE.

Seven populations, representing the major regions of Philippines were selected for standard blood sampling (figure 1). Populations from Luzon included: (i) Pozzorubio, Pangasinan; (ii) metropolitan Manila; and (iii) Pili, Camarines Sur. Visayas was represented by (iv) Naga, Cebu while Mindanao included (v) Butuan City, Butuan and (vi) Matanao, Davao del Sur. Palawan was represented by (vii) Puerto Princesa. Data from a recent study of Guzman *et al.* (2009) from Cabagan, Isabela (Luzon) and Metropolitan Manila were also pooled with the data from this study, bringing a total of eight populations and 541 individuals. Following the protocol of Guzman *et al.* (2009), respondents of this study were asked to fill out a questionnaire that incorporated questions about the place of birth and address of the respondent and his/her parents. The rate of migration for each population, which measures how many people come into the local population, was then computed as the number of respondents who were not born in the area surveyed over the total respondents. The place of birth and the ethnolinguistic identity of each migrant were also noted.

The simple, noninvasive procedure for testing blood types involved obtaining two drops of blood from each participant through a single prick with a sterile lancet. Each drop of blood was placed on a spot plate with either the anti-M or anti-N of Cypress Diagnostics (Langdorp, Belgium). The presence of agglutination was determined after probing the mixture gently. A clotting reaction with the test serum indicated that the participant was positive for that particular blood grouping antigen. The records of the participants' blood types were consolidated and were subjected to statistical analysis.

Analysis of variance (ANOVA) with Duncan's post-hoc analysis was applied to the genotypic frequencies obtained from different populations to determine significant differences and the degree of associations among the populations. Through two-tailed *t*-tests, the MN blood type distribution of each local population was also compared with the metropolitan population of Metro Manila, which is expected to approximate the distribution of a mixed Philippine population, to

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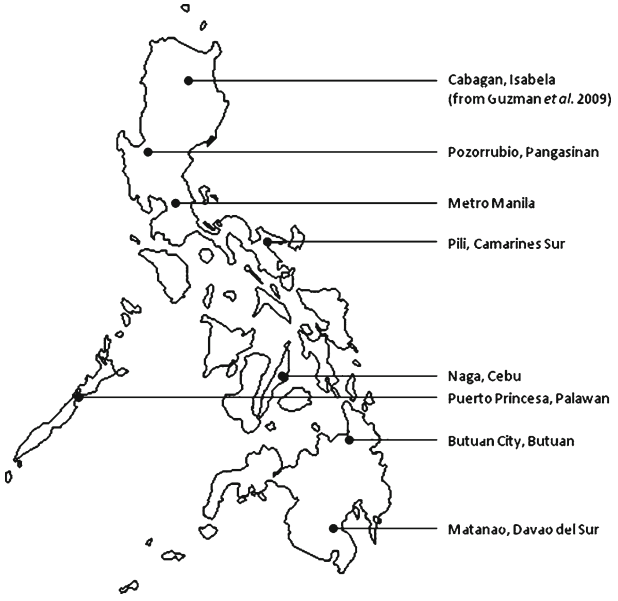

Figure 1. Distribution of sample populations surveyed in this study. Data from Cabagan, Isabela were obtained from the study of Guzman *et al.* (2009).

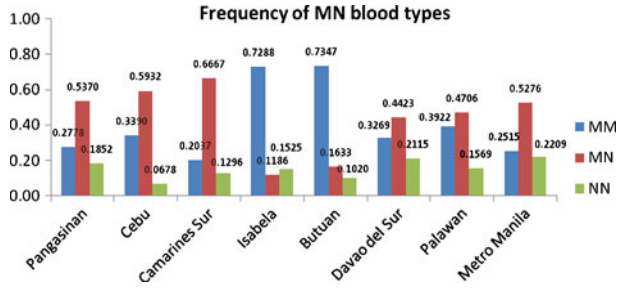

Figure 2. Frequency distribution of the MN blood groups in various types of populations in the Philippines. *NN* was of the lowest frequency in all the populations observed.

determine if there is significant departure from admixture among the different populations. To test for Hardy–Weinberg equilibrium in the local populations, the allelic frequencies for the MN blood groups were subjected to Pearson's chi-square test. Departure from Hardy–Weinberg equilibrium (DHW) was also tested using Wright's inbreeding coefficient, which was computed for each population as follows: 1-(H_o/H_e) (Wright 1922). A positive value indicates a deficit of heterozygotes and a negative value indicates an excess of heterozygotes compared to a population at HWE. All statistical tests were conducted using the Statistical Package for Social Sciences (SPSS) version 18.0 (Chicago, USA) and where $P \leq 0.05$ was the level used for determining statistical significance.

National data revealed an excess of *MN* (45.84%) followed by *MM* (37.52%) and *NN* (16.64%), leading to a higher frequency of *M* allele (60.44%) over *N* (39.56%). However, there were significant differences found in the frequency distribution of the MN blood types across the sampled populations. While MN appeared as the predominant blood types and N the least common in Metropolitan Manila, Pangasinan, Cebu, Camarines Sur, Matanao, and Palawan, M was the predominant blood type in Isabela (72.89%) and Butuan (73.47%) (figure 2). The last two populations also had the highest frequency of *M* allele, with 78.81% and 81.63%, respectively (figure 3). The percentage of migrants for each population is shown in figure 4, with Butuan exhibiting the lowest percentage (4.08%) and Camarines Sur exhibiting the highest (62.96%). Wright's inbreeding coefficient (figure 5) demonstrated extreme heterozygote deficit for Isabela (0.6448) and Butuan (0.4556). Analysis of variance with Duncan's post-hoc test ($P < 0.05$) also placed these two populations under a subset, which was significantly different from the other subset containing all the other Philippine populations (table 1). Two-tailed *t*-tests comparing all the populations with the Metro Manila population showed that the Isabela and Butuan populations were likewise significantly different (table 2). All the populations observed in this study were in HWE except for the local populations of Pili in Camarines Sur, Cabagan in Isabela, and Butuan based on Pearson's chi-square tests.

This survey provides the first national data on the distribution of the MN blood groups in the Philippines, with representatives from the major island groups (Luzon, Visayas, Palawan and Mindanao). Overall, the frequency distribution of the MN blood groups (37.52% M, 45.84% MN and 16.64% N) is very similar to that of the Chinese at 33.2%, 48.6% and 18.2%, respectively (Boyd 1950). The frequency of the MN is also smaller than the global data of 50% (Ravi *et al.* 2005), whereas the frequency of MM is higher than the global data of only 22% (Ravi *et al.* 2005). The *M* allelic frequency of 60.44% for the Philippines is comparable to those of the Finns at 64% (Nevanlinna 1972), the Iranians at 63% (Mourant *et al.* 1976), the Northern Swedes at 61.7% (Cedergren *et al.* 1981), and the Balinese at 68.3% (Breguet *et al.* 1986). In contrast, the Japanese have only 30.9% (Furuhata *et al.* 1954) while the Koreans have 50.3% (Lee 1965). Further, the Philippine MN blood types did not have an uniform distribution across the eight populations. The Isabela and Butuan populations had an excess

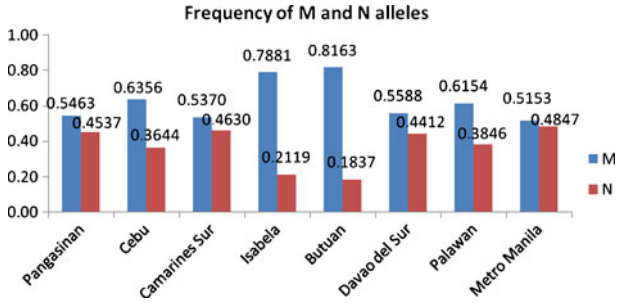

Figure 3. Frequency distribution of the *M* and *N* alleles in the Philippine populations sampled in the study.

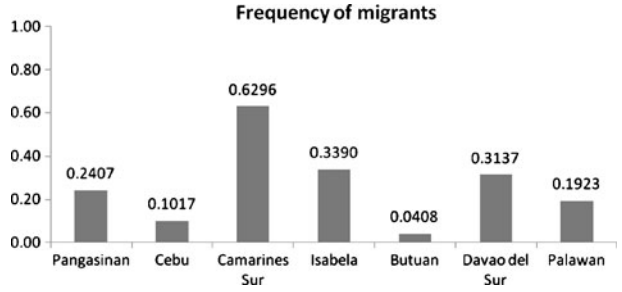

Figure 4. Frequency of migrants in the Philippine populations. Note that the Metro Manila population data for migrants were not included.

of *MM* individuals compared to the six populations, leading to a very high *M* allelic frequency in Isabela and Butuan (78.81% and 81.63%, respectively). Such high *M* allele frequencies echoed those of North American Indians (83.6%) (Gershowitz 1959), Apache Indians in the United States of America (72.0%), and inhabitants of Mexico City (71.0%) (Mourant *et al.* 1976). Other populations that have a high proportion of *MM* individuals include Chilean Indians (about 60%) and East Greenlanders (83.5%) (Ottensooser and Pasqualin 1949), which so far hold the highest frequency of *MM* in the world.

The distribution of the *M* and *N* alleles are clearly not uniform across the country. The Isabela and Butuan populations had an excess of *M* allele, leading to an excess of *MM* and a deficit of the heterozygote *MN*. Morton and Chung (1959) predicted that any fragmentation of a population into local isolates would tend to increase homozygosity. Both populations approximate isolated populations. In case of Butuan, this is reflected by the lowest rate of migration across the eight populations. In fact, of all of the 49 respondents in Butuan City, only two respondents were not born in Butuan City and only two individuals came from a family with at least one parent who is not a native resident of Butuan. The city has a sizable indigenous population with more than 5000 households consisting of members of the Manobo or Lapaknons and the Higaonons. In case of Isabela, the high migration rate of 33.9% belies the fact that most of these migrants

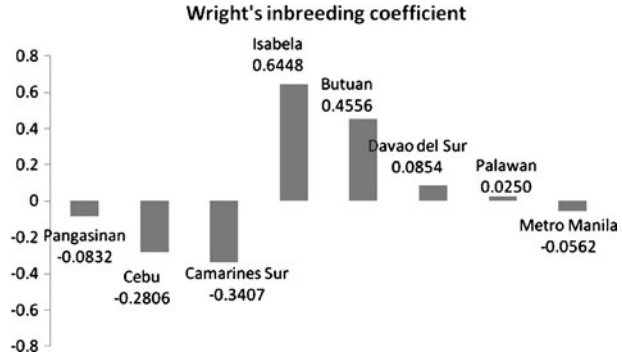

Figure 5. Wright's inbreeding coefficient for the Philippine populations.

Table 1. Analysis of variance with Duncan's post-hoc analysis for the MN blood types showing that Isabela and Butuan belong to a different subset.

Place	N	Subset 1	Subset 2
Butuan	49	1.37	
Isabela	59	1.42	
Cebu	59		1.73
Palawan	52		1.77
Davao	51		1.88
Pangasinan	54		1.91
CamSur	54		1.93
Manila	163		1.97
Significance		0.653	0.094

Means for groups in homogeneous subsets are displayed based on observed means.
The error term is mean square (error) = 0.460.
Uses harmonic mean, sample size = 58.687.
The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed. Alpha = 0.05.

also came from nearby towns, all of which, Isabela included, are composed of individuals from the Ybanag ethnolinguistic minority.

Departures from HWE could be caused by many factors, including migration, nonrandom mating, small population size, selection and mutations. Metro Manila, capital of Philippines, has a population that comprises various ethno–linguistic groups, yet its population, along with five others surveyed, was found to be at HWE. Race and Sanger (1975) also found the same result for London based on 1279 individuals they surveyed, and they speculated that random mating could be a major factor for the population to maintain equilibrium. Not to be discounted is the possibility that different factors that affect genotypic frequencies could cancel each other, thereby leading to a population at HWE (Workman 1969). On the other hand, departure from equilibrium by the Isabela, Butuan and Camarines Sur populations could be attributed to different factors. In case of Isabela and Butuan, the deficit of heterozygotes as shown by Wright's inbreeding coefficient demonstrated nonrandom mating at work. This is supported by the fact that these populations belong to specific ethnolinguistic groups with very little outbreeding taking place, which is clearly evident in the very small migration rate for Butuan, thereby limiting the probability of mating of completely unrelated individuals (Hedrick 2000). In case of Camarines Sur population, the very high migration rate could account for the DHW.

In conclusion, the Philippine population generally has an excess of *MN* heterozygotes while the *NN* genotype occurs the least, albeit a high frequency of *MM* homozygotes had been detected in relatively isolated regional populations. Departures from HWE were detected in three populations, which could be attributed to nonrandom mating for Isabela and Butuan and very high migration rate for Camarines Sur.

Table 2. Results of paired sample *t*-tests showing that Isabela and Butuan are significantly different from the rest of the populations.

		Paired differences							
					95% Confidence interval of the difference				Significance
		Mean	Std. deviation	Std. error mean	Lower	Upper	*t*	df	(2-tailed)
Pair 1	Manila – Pangasinan	0.037	0.931	0.127	-0.217	0.291	0.292	53	0.771
Pair 2	Manila – Cebu	0.203	0.979	0.127	-0.052	0.458	1.596	58	0.116
Pair 3	Manila – Camarines Sur	0.019	1.055	0.144	-0.269	0.306	0.129	53	0.898
Pair 4	Manila – Isabela	0.508	1.006	0.131	0.246	0.771	3.881	58	0.000
Pair 5	Manila – Butuan	0.571	0.979	0.140	0.290	0.853	4.086	48	0.000
Pair 6	Manila – Davao	0.078	1.146	0.160	-0.244	0.401	0.489	50	0.627
Pair 7	Manila – Palawan	0.192	0.991	0.137	-0.084	0.468	1.399	51	0.168

It is recommended that further studies should now focus on determining the MN distribution patterns of the different ethic minorities in the country, which exist mostly as isolated populations.

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