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Short Communication

Valongo, genetic studies on an isolated Afro-Brazilian community

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Abstract

A southern Brazilian isolated community of predominantly sub-Saharan African origin, with a total population of 74 individuals and high degree of inbreeding (F = 0.081) was studied. The small sizes of the breeding (35) and effective (21) populations, as well as the very small effective migration rate (4%), suggest a high probability for the occurrence of genetic drift. A sample was typed for fourteen blood genetic systems and most of these systems seem to reveal the founder effect. This evolutionary factor was probably responsible for the absence of some polymorphic alleles frequent in African populations, *i.e.*: *ABO*B, RHD-RHCE*DCe, GPA-GPB*NS (MNSs*NS), GPA-GPB*NS^{U} (MNSs*NS^{U}), HBB*S, HP*2M and ESD*2.* The most unusual allele frequency was that for *BCHE*A,* 0.27, four times higher than its highest estimated frequency and fifty times higher than that those observed in African populations. Considering the allele frequencies of the Sub-Saharan African (**A**) and European (**E**) ancestral populations, the population studied can be quantified as containing 97.33% ± 10.41 of **A** alleles and 2.67% ± 10.41 of **E** alleles.

Key words: isolated community, polymorphism, random genetic drift, blood systems, admixture.

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The isolated Valongo community was founded in the 1880s by seven runaway and freed slaves, plus a white man, in the region called `Sertão de Valongo' (27°12'12'' S, 48°44'30'' W) in the Porto Belo municipality of the southern Brazilian state of Santa Catarina and by 1995 had a population of 74 people of predominantly African origin and has been described in detail by Souza and Culpi (1992). The average inbreeding coefficient is high (F = 0.081), mostly due to the abundance of relatives remaining in the region and to religious (they are Seventh Day Adventists) and racial segregation (neighbouring communities have descended from Catholic Europeans of German, Italian and Portuguese origin). The small size of the breeding (35) and effective (21) population and the effective migration frequency (4%) strongly suggested the possibility of genetic drift.

The aim of the research presented in this paper is to verify the possible effect of genetic drift in the Valongo community by comparing phenotypic and allele frequencies for several different polymorph systems found within this community with those of other populations composed of individuals of sub-Saharan African ancestry. We also undertook to calculate the average heterozygosity and to examine the effect of inbreeding on the phenotype frequencies and quantify the relative contribution of the different ethnic groups to the current gene pool of the Valongo community.

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Blood samples were collected from 49 different individuals (22 women and 27 men, 66% of the inhabitants) whose ages varied from 4 and 90 years. The study was sanctioned by the bioethics commission of our institutions and blood samples only taken after obtaining the informed consent of the individual or, in the case of children, legal guardian. Fourteen loci were tested for, the ABO (*ABO*), Duffy (*FY*) P (*P*), Rh (tested with anti-C, -c, -D, -E, -e for the *RHD-RHCE* loci), MNSs (*GPA-GPB*) and Kell (*KEL*) loci in each blood sample being detected serologically and the hemoglobin (*HBB*), haptoglobin (*HP*), transferrin (*TF*), albumin (*ALB*), esterase D (*ESD*) and carbonic anhydrase 2 (*CA2*) loci using horizontal 10% starch gel electrophoresis (Smithies, 1955 and Harris *et al*., 1960 as modified by Carvalho and Azevedo, 1976). Loci abbreviations are given in parenthesis. The cholinesterase *CHE2* locus was investigated using 1% agar electrophoresis (Robinson *et al*., 1957, adapted by Van Ross and Vervoot, 1973) and the method of

Alcântara *et al.* (1991) was employed to phenotype the butyrilcholinesterase *BCHE* locus.

Allele frequencies were calculated either by gene counting using the square root of the frequency of recessive homozygotes, or by use of a quadratic equation (Race and Sanger, 1975) assuming Hardy-Weinberg (H-W) or Wright equilibrium and the χ^2 values calculated using the CLUMP program (Sham and Curtis, 1995). Nei's (1987) method was employed to calculate the average heterozygosity. The admixture values (*i.e.* the relative contribution of sub-Saharan African and Ibero-European ancestors) were obtained by the weighted least-squares method (Long, 1991a,b) using the ADMIX program (Ota, 1993) and the allele frequencies of the thirteen loci investigated by us plus another 39 alleles belonging to the studied population and its ancestral sub-Saharan African (Bantu and west African) and Ibero-European populations compiled from the literature (Cavalli-Sforza *et al.,* 1994; Bortolini *et al.*, 1995). The 39 additional alleles were chosen because they had previously been used to calculate admixture in isolated southern Brazilian populations which had been located based on the history of the region (Bortolini *et al*., 1992; 1994; 1997). The isolated Valongo population was considered di-híbrid because we knew the history of its foundation.

Demographic studies have shown that from 1985 to 1995 the breeding and effective population of Valongo has increased and the average age of the population decreased despite a decrease in the number of individuals in the population, and this community is essentially young, expanding, and maintaining a low frequency of migration with a high inbreeding coefficient. The F value of the Valongo population has increased from 0.048 in 1985 (Souza and Culpi, 1992) to 0.078 in 1990 (Souza, 1993) and to 0.081 in 1995 and multiple consanguinities have appeared. With regard to the non-consanguineous marriages, generally men were the immigrants while the women belonged to the community. Ethnic, social, cultural and religious factors were very important in the maintenance of the characteristics of this isolated community and the low exogamy coefficient observed turned this small village of little or no social or economic importance into an excellent biological model for studies on genetic drift and evolutionary factors.

Phenotype and allele frequencies for the fourteen alleles studied are shown in Table 1 which also shows the heterozygosity per *locus* (*h*) and the average heterozygosity (*H*). The genotypes were distributed in accordance with expected Hardy-Weinberg and Wright equilibrium. The *h* values varied from zero (*KEL, HBB, ALB, ESD* and *CHE2*)

Table 1 - Phenotype distribution, gene frequencies and heterozygozity (*h*) for 14 *loci* investigated in Valongo. See text for loci and phenotype abbreviation key.

Loci and phenotypes	N	Allele	Frequency	χ^2 H-W and Wright (CLUMP program)	*h*
ABO					
O	45	*ABO*O*	0.958	-	0.080
A	4	*ABO*A1*	0.042		
FY					
a + b-	10	*FY*A*	0.128	NS	0.246
a-b+	1	*FY*B*	0.013		
a-b-	28	*FY*0*	0.859		
P					
P1	25	*P*1*	0.401	-	0.480
P2 + p	14	*P*2 +*p*	0.599		
RHD-RHCE					
DccEE	3	*RHD-RHCE*DcE*	0.158	NS	0.682
DccEe	6	*RHD-RHCE*Dce*	0.257		
Dccee	16	*RHD-RHCE*D^{U}ce*	0.126		
D^{U}ccee	5	*RHD-RHCE*dce*	0.459		
ddccee	8				
GPA-GPB (MNS)					
MS	3	*GPA-GPB*MS*	0.091	NS	0.533
MSs	2	*GPA-GPB*Ms*	0.650		
Ms	24	*GPA-GPB*MS^U*	0.164		
MS-s-	1	*GPA-GPB*Ns*	0.095		
MNSs	3				
MNs	4				
KEL					
K-	37	*KEL*K*	0	-	0

Table 1 (cont.)

Loci and phenotypes	N	Allele	Frequency	χ^2 H-W and Wright (CLUMP program)	*h*
HBB					
A	49	*HBB*A*	1.000	-	0
HP					
1-1	18	*HP*1*	0.653	NS	0.453
2-1	28	*HP*2*	0.347		
2-2	3				
TF					
C	35	*TF*C*	0.865	NS	0.234
CD	13	*TF*D1*	0.135		
ALB					
A	49	*ALB*A*	1.000	-	0
ESD					
1-1	49	*ESD1*1*	1.000	-	0
CA2					
1-1	34	*CA2*1*	0.833	NS	0.278
2-1	12	*CA2*2*	0.167		
2-2	2				
BCHE					
U	23	*BCHE*U*	0.729	NS	0.396
UA	24	*BCHE*A*	0.271		
A	1				
CHE2					
C5-	49	*CHE2*C5-*	1.000	-	0

NS = not significant.
Average heterozygosity (*H*) = 0.242 (s.e.± 0.063).
Mean number of alleles per *locus* = 2.000 (s.e.± 0.277).
Percentage of polymorphic *loci* = 64.3%.

to 0.682 (*RH*) and the *H* value was 0.242 ± 0.063, this *H* value agreeing with those calculated for other Afro-Brazilian isolated populations, *i.e.* 0.177 ± 0.044 for the Trombetas population (Schneider *et al.,* 1987), 0.192 ± 0.049 for the Cametá population and 0.262 ± 0.061 for the Paredão population as well as for the isolated Venezuelan Curiepe population (*H* = 0.243 ± 0.052) studied by Bortolini *et al.* (1992).

Harris and Hopkinson (1972) evaluated the extent of polymorphisms in humans by studying 71 genetic enzymatic systems. They observed that 28 of these systems were polymorphic when analyzed by electrophoresis and calculated an average heterozygosity (*H*) of 0.067. Neel (1984) estimated a value of *H* of between 0.120 and 0.130 for human protein *loci*. The *H* values for Valongo and for Trombetas, Cametá, Paredão and Curiepe were higher than expected. The reason for this might be the choice of the systems studied, probably based on the knowledge that they were polymorphic in other populations.

The *BCHE*A* allele, idiomorphic in sub-Saharan African populations (Whittaker, 1968) was polymorphic in Valongo and presented the highest frequency yet registered for any population (Szeinberg *et al.,* 1972; Roychoudhury and Nei, 1988; Cavalli-Sforza *et al.,* 1994). With regard to the *ABO, HBB, HP* and *ESD loci* and the *RHD-RHCE* and *GPA-GPB (MNSs)* haplotypes, genetic drift was involved in the absence of certain alleles and haplotypes considered polymorphic (*ABO*B, HBB*S, HP*2M, ESD*2, RHD-RHCE*DCe, GPA-GPB*NS* and *GPA-GPB*NS*^{U}) in sub-Saharan African populations (Roychoudhury and Nei, 1988; Cavalli-Sforza *et al.*, 1994). The *HBB* and *ESD loci* were monomorphic in this isolate.

The frequency of certain alleles at the origin of the Valongo population (founder effect) as well as the variation in the frequency of some other alleles in successive generations of this isolated community was determined by the small size of the effective population. In spite of the high inbreeding coefficient and the important role of genetic drift, average heterozygosity and the level of polymorphism was still high.

Finally, considering the allele frequencies of the Sub-Saharan African (**A**) and European (**E**) ancestral populations (Table 2), the studied population can be quantified as follows: 97.33% ± 10.41 of **A** alleles and 2.67% ± 10.41 of **E** alleles, with MSE = 27.07%. The values of admixture

Table 2 - Distribution of allele frequencies of the analyzed *loci* in the community of Valongo, in Sub-Saharan Africans and in Europeans.

Alleles	Valongo (1)	General Sub-Saharan African (2,3)	General European (2,4)	Alleles	Valongo (1)	General Sub-Saharan African (2,3)	General European (2,4)
*ABO*A*	0.042	0.176	0.309	*HBB*A*	1.000	0.959	0.999
*ABO*B*	0	0.129	0.059	*HBB*S*	0	0.040	0.001
*ABO*O*	0.958	0.695	0.632	*HBB*C*	0	0.001	0
*FY*A*	0.128	0.110	0.421	*HP*1*	0.653	0.658	0.378
*FY*B*	0.013	0.000	0.549	*HP*2*	0.347	0.320	0.622
*FY*0*	0.859	0.890	0.030	*HP*2M*	0	0.022	0
*P*1*	0.401	0.678	0.515	*TF*C*	0.865	0.961	0.991
*P*2* + *p	0.599	0.334	0.485	*TF*D*	0.135	0.035	0.004
*RHD-RHCE*DCE*	0	0.002	0.003	*TF*B*	0	0.004	0.005
*RHD-RHCE*DCe*	0	0.121	0.398	*ESD*1*	1.000	0.912	0.830
*RHD-RHCE*DcE*	0.158	0.069	0.107	*ESD*2*	0	0.088	0.170
*RHD-RHCE*Dce*+ *RHD-RHCE*D^{U}ce*	0.383	0.592	0.047	*CA2*1*	0.833	0.906	0.995
*RHD-RHCE*dCe*	0	0.012	0.009	*CA2*2*	0.167	0.094	0.005
*RHD-RHCE*dcE*	0	0.003	0.004	*BCHE*U*	0.729	0.985	0.981
*RHD-RHCE*dce*	0.459	0.201	0.432	*BCHE*A*	0.271	0.015	0.019
*GPA-GPB*MS*	0.091	0.105	0.255	*CHE2*C5-*	1.000	0.982	0.960
*GPA-GPB*Ms*	0.650	0.394	0.300	*CHE2*C5+*	0	0.018	0.040
*GPA-GPB*MS^{U}* + *GPA-GPB*NS^{U}*	0.164	0.092	0				
*GPA-GPB*NS*	0	0.040	0.071				
*GPA-GPB*Ns*	0.095	0.369	0.374				
*KEL*K*	0	0.023	0.039				
*KEL*k*	1.000	0.977	0.961				

The *RHD-RHCE*dCE* haplotype is very rare, so it was not considered.
(1) This study; (2) Cavalli-Sforza *et al.*, 1994; (3) Bortolini *et al.*, 1995; (4) Roychoudhury and Nei, 1988.

reflect, in part, the subjective classification based on physical appearance. Individuals sampled in Valongo were mostly identified as Negro or Mulatto with only one Caucasian individual existing in the population. The mean squared error (MSE) represents the proportion of allele frequency variation unexplained by the admixture model.

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