The relations between G-6-PD deficiency, thalassemia and malaria. Further analysis of data from Sardinia and the Po Valley

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Summary. Discriminant analysis carried out on a set of environmental and sociocultural variables in the Sardinian population suggests that G-6-PD deficiency and thalassemia move along 2 dimensions partially independent of each other. Partial correlation analysis also suggests that malaria, by itself, may exert opposite effects on thalassemia and G-6-PD selection. The present results support the hypothesis that thalassemia may be the primary genetic factor selected by malaria, whereas G-6-PD selection may be a secondary adaptation phenomenon strongly dependent on other genetic and environmental variables.

The problem of the relation between G-6-PD deficiency, thalassemia and malaria is still largely unsettled[1,2]. We have recently suggested that the primary effect of malaria may be a positive selection on thalassemia, whereas G-6-PD deficiency might undergo a secondary selection strongly dependent on other environmental and genetic factors[3,4].

In the present study we have carried out some new analyses on the data previously reported from Sardinia and the Po Valley[5-11]. The results indicate that G-6-PD and thalassemia move along two dimensions partially independent of each other. The results also suggest that malaria, by itself, may exert opposite effects on thalassemia and G-6-PD deficiency.

Material and methods. Data from 43 Sardinian villages were analysed. The following variables were included: thalassemia, G-6-PD deficiency, past malarial morbidity, altitude, population size, inbreeding.

Data from 9 villages located in the area of the Po Delta were also considered. Only data on thalassemia, G-6-PD deficiency and past malarial morbidity were available for these villages.

Discriminant analysis and partial correlation analysis were performed according to SPSS programs[12] using an IBM 370/158 computer.

Results. A 4-group discriminant analysis was carried out on altitude, malarial morbidity, population size and inbreeding in order to evaluate their contribution to thalassemia and G-6-PD differentiation. The first group (gt) includes villages with low prevalence of both G-6-PD deficiency and thalassemia (i.e. both frequencies below the mean value of the sample); the second group (gT) includes villages with low prevalence of G-6-PD deficiency and high prevalence of thalassemia; the third group (Gt) includes villages with high prevalence of G-6-PD deficiency and low prevalence of thalassemia; the fourth group (GT) includes villages with high prevalence of both traits. The results of this analysis are reported in table 1 and figure 1.

Table 1 shows that altitude is negatively associated with the prevalence of both G-6-PD deficiency and thalassemia. Malaria appears to be associated positively with thalassemia but negatively with G-6-PD deficiency. Inbreeding is associated (negatively) more strongly with G-6-PD deficiency than with thalassemia. The effect of population size appears less clear.

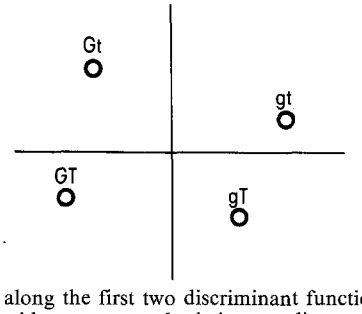
The results of the discriminant analysis reported in figure 1 give a clearer and more understandable picture of the pattern of relations among the variables. The first two discriminant functions show a considerable discriminant power. The 1st, corresponding to the horizontal axis, represents mainly inbreeding and altitude, while the 2nd function, corresponding to the vertical axis, represents mainly past malarial morbidity and population size. The general configuration of the 4 groups in figure 1 is tetrapolar indicating a 2-dimensional structure of the system. The prevalence of G-6-PD deficiency, discriminated along the horizontal dimension, is therefore dependent mainly on inbreeding and altitude. The prevalence of thalassemia which is discriminated along the vertical dimension, is therefore dependent mainly on past malarial morbidity and population size.

Fig. 1. Discriminant analysis on environmental and socio-cultural variables to evaluate their contribution to thalassemia and G-6-PD differentiation. Data from 43 Sardinian villages. Groups gt, gT, GT and Gt defined in the text.

Discriminant function	Eigenvalue	Relative percentage	Canonical correlation	Functions derived	Wilks' lambda	Chi square	DF	Significance
				0	0.3377	41.248	12	0.00
1	1.21177	79.06	0.740	1	0.7470	11.084	6	0.08
2	0.24983	16.30	0.447	2	0.9336	2.609	2	0.27
3	0.07108	4.64	0.258					

Standardized discriminant function coefficients

	Function 1 (Horizontal axis)	Function 2 (Vertical axis)
Altitude	0.28331	0.63262
Malarial morbidity	0.00305	−0.82095
Population size	−0.00158	−0.74709
Inbreeding	0.92373	−0.32176



Plot of the cases along the first two discriminant function continuum. Only group centroids are reported; their co-ordinates correspond to the mean discriminant scores for each group.

Table 2 shows a partial correlation analysis among malaria, G-6-PD deficiency and thalassemia. The positive correlation between malaria and thalassemia increases when controlling for G-6-PD deficiency. The negative correlation between malaria and G-6-PD deficiency observed in Sardinia increases in absolute value when controlling for thalassemia. In the population from the Po Valley the slightly positive correlation between malaria and G-6-PD deficiency becomes negative when controlling for thalassemia. The positive correlation between thalassemia and G-6-PD deficiency increases slightly when controlling for malaria. It seems interesting to note that the correlation between malaria and thalassemia is much stronger in the Po Valley (where there is a low prevalence of G-6-PD deficiency) than in Sardinia (where this prevalence is very high).

The likely relations among malaria, G-6-PD and thalassemia are summarized in figure 2.

Discussion. On the basis of the relationship, which is generally observed in many areas of the world, between malarial endemicity and the presence of HbS, thalassemia and G-6-PD deficiency, it has been suggested that these polymorphic traits might confer some resistance against malaria[1,13,14].

Concerning G-6-PD, however, a local positive correlation between malarial endemicity and enzyme deficiency has not been generally found[15,16].

Direct tests have shown with reasonable confidence that carriers of HbS are indeed more resistant to malaria[14]. Concerning G-6-PD deficiency, similar investigations have given contrasting results, which do not even exclude the possibility of an increased susceptibility of carriers of malaria[17,18]. The observations of Luzzatto and co-workers[19,18] are generally considered to constitute the most valid argument on clinical grounds in favour of a protective action of G-6-PD deficiency against malaria[2]. They reported[19] that in female children heterozygous for G-6-PD deficiency with acute *Plasmodium falciparum* malaria, the parasite rate was 2–80 times higher in normal than in deficient erythrocytes. Later they observed[18] that females of Gd^{A-}/Gd^{B} genotype had a significantly lower parasite count than any other group of females or males. However, very recent studies carried out in the same population studied by Luzzatto and co-workers have failed to show any evidence of protection of G-6-PD deficient children against malaria[20]. Furthermore their data have been re-evaluated and their conclusions challenged[20].

Very little attention has been given in the literature to the fact that as a rule, in malarial areas, G-6-PD deficiency is associated either with thalassemia or with HbS and it has not been selected alone. Moreover, at least in the Mediterranean area, there are regions like Sardinia which show a high prevalence of both thalassemia and G-6-PD deficiency and regions like the Po Valley which show a high prevalence of thalassemia but a very low prevalence of G-6-PD deficiency. It has been suggested that malaria was introduced in the Po Delta Valley much more recently than in Sardinia[5,6].

Several investigations carried out at the biochemical, the clinical and the population levels suggest that the G-6-PD system interacts with various factors, both genetical (thalassemia, erythrocyte acid phosphatase, adenosine deaminase) and environmental (*Vicia faba,* altitude, viral and protozoal diseases). In a malarial environment, therefore, the fitness of the different G-6-PD genotypes depends on numerous variables[4,21–24].

The results of the present analysis seem to offer some explanation for most of the facts considered above and support our suggestion that thalassemia may be the primary genetic factor selected by malaria, whereas G-6-PD selection may be a secondary adaptation phenomenon heavily dependent on other variables. Confirmation of the pattern in other populations would be very important. Unfortunately, in our country, reliable and accurate information on malarial endemia during the decades preceding eradication is available only for Sardinians.

Further investigations to test directly the resistence of thalassemia and/or G-6-PD deficiency carriers towards malaria should take into account the present observations. It may well be that carriers of both traits are the most resistant to malaria and in general the most fit, followed by carriers of the thalassemia trait alone, whereas subjects carrying only the gene for G-6-PD deficiency may not show any particular advantage in a malarial environment.

A G-6-PD variant which decreased even slightly the stress imposed on the bone marrow by the thalassemia gene, could be positively selected since it would increase the fitness of thalassemia carrier. Such a variant may be advantageous only in a malarial environment and in the

Table 1. Mean values of altitude, malarial morbidity, population size, and inbreeding in relation to the prevalence of G-6-PD deficiency and thalassemia in 43 Sardinian villages

	Group 1* (gt)	Group 2 (gT)	Group 3 (Gt)	Group 4 (GT)
Altitude (m above sea level)	457	232	237	146
Malarial morbidity	50%	67%	42%	56%
Population size**	2915	2658	2407	3467
Inbreeding***	9.2	7.5	4.6	3.7
Number of villages	12	10	10	11

* See text for definition of groups. ** Number of inhabitants from the 1938 census. *** Percent of consanguineous marriages in the period 1940–1949.

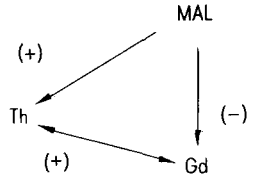


Fig. 2. A scheme describing the relationships between malaria (MAL), G-6-PD deficiency (Gd) and thalassemia (Th). The sign of the correlation is indicated near the arrow.

Table 2. The relationships between malaria, G-6-PD deficiency and thalassemia. A partial correlation analysis

Correlation		Sardinia Coefficient	Significance	Po valley Coefficient	Significance
Malaria V.S. Thalassemia	Zero order partial	0.16	0.15	0.472	NS
	Controlling for G-6-PD	0.25	0.06	0.477	NS
Malaria V.S. G-6-PD deficiency	Zero order partial	−0.12	0.23	0.063	NS
	Controlling for thalassemia	−0.22	0.08	−0.102	NS
Thalassemia V.S. G-6-PD deficiency	Zero order partial	0.47	0.001	0.315	NS
	Controlling for malaria	0.50	0.001	0.324	NS

presence of the thalassemic gene. Evidence in favour of such a mechanism could be gathered by studying appropriate erythropoietic parameters in subjects carrying only thalassemia and in carriers of both traits.

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AB0 agglutinins from *Biomphalaria straminea* snails

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Summary. Extracts from *B. straminea* spawn are active against A and B normal red cells. A_1 and A_2 subgroups may be differentiated with trypsin-, papain- and pronase-treated cells. 0 cells treated with papain, pronase and neuraminidase react weakly to the extracts.

Analysis of agglutinins in the Basommatophora (water snails) have revealed differences between this order and the Stylommatophora (land snails), since few members of the Basommatophora (4 out of 16 species) contain agglutinins whereas these are frequent in the Stylommatophora[2]. The Basommatophora snails are significant in public health considerations in Brazil, since 3 species of *Biomphalaria, B. glabrata, B. straminea* and *B. tenagophila* are the principal intermediary hosts of *Schistosoma mansoni.* This communication describes the AB0 agglutinins for 1 of those species, *Biomphalaria (Australorbis) straminea.*

Material and methods. The samples consisted of 2 pools of 275 and 296 individual specimens of fresh spawn from pigmented animals reared in the laboratory over 4 years. The spawn were washed and suspended in saline solution (1 g/1 ml) and after 15 min of sonication were centrifuged for 10 min at 800 × g. Methods for papain- and trypsin-treatment of red cells are described elsewhere[3]. Pronase: 1 part of 2% red cell suspension in saline and 2 parts of the working solution of pronase (protease type VI Sigma, 6.5 units/mg) were incubated for 15 min at 37 °C and afterwards centrifuged for 5 min at 925 × g. The cells were washed again, and resuspended at a 2% final concentration. The working solution of the enzyme was made by mixing 1 part of 1% pronase concentration in saline and 9 parts of buffered saline (pH = 7.3). Neuraminidase: 1 part of the enzyme (from *Virus influenzae,* A_2 Hong Kong/68 strain, 400 units/ml, kindly supplied by Prof. Raimundo D. Machado, Institute of Microbiology, UFRJ) was diluted with 11 parts of saline. 1 vol. of cell suspension and 2 vol. of the working solution of the enzyme were incubated for 30 min at 37 °C and afterwards centrifuged for 5 min at 925 × g. The packed cells were resuspended at a 2% final concentration after 3 washings with saline. A control of this enzyme included normal, papain- and neuraminidase-treated 0 cells which yielded 0, 0 and 256 end-point titers, respectively, against an *Arachis hypogaea* extract. Titrations were made incubating normal, trypsin-, papain- and pronase-treated cells with the spawn extract for 30 min at 37 °C; neuraminidase-treated cells, for 15 min at room temperature. The cells were centrifuged for 15 sec at 1000 rpm (Adams Sero-Fuge) and the results were read macroscopically.

Results and discussion. The results in the table show an anti-A, B agglutinin against normal cells. The degree of reactivity with the A antigens was higher than with B group cells. Differences between A_1 and A_2 were not significant. A low titer with 0 cells when they are treated with papain, neuraminidase and pronase, was also found. The last enzyme yielded the highest titrations with all the AB0 groups. Neuraminidase-treatment does not allow a distinc-

Average end-point titers of *B. straminea* spawn

Treatment	A_1	A_2	B	O
Normal	109	40	5	0
Papain	7332	272	137	4
Trypsin	1067	130	21	0
Pronase	20171	4096	193	4
Neuraminidase	4360	2048	49	2