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Massimo Masetti, Valerio Scali

Electrophoretic studies on gene-enzyme systems in Maniola jurtina (Lepidoptera Satyridae): the PGM polymorphism in central Italy

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Genetica di popolazione. — *Electrophoretic studies on gene-enzyme systems in* Maniola jurtina *(Lepidoptera Satyridae): the* PGM *polymorphism in central Italy* (*). Nota di MASSIMO MASETTI e VALERIO SCALI, presentata (**) dal Socio M. BENAZZI.

RIASSUNTO. — Sono state analizzate le varianti elettroforetiche della fosfoglucomutasi (PGM) di otto popolazioni, sia insulari che continentali, della farfalla satiride *M. jurtina*, scelte in base alle differenze riscontrate per alcune caratteristiche del ciclo vitale e dello spotting. Sono state individuate 6 varianti enzimatiche (A–F) ed è stato visto che ogni individuo ne possiede una o due; non esistono differenze nel pattern elettroforetico di maschi e femmine, né c'è associazione col fenotipo (= classe numerica) degli spots.

In ovature di femmine con fenotipo PGM noto è stata osservata la segregazione mendeliana delle forme enzimatiche e, per l'accertata monogamia delle femmine, è stato possibile risalire anche al genotipo del maschio. Il quadro dei patterns elettroforetici risulta quindi interpretabile in base alla esistenza di 6 alleli funzionali del locus PGM ciascuno dei quali produce una diversa forma enzimatica. In ognuno dei campioni analizzati, che sono risultati in equilibrio di Hardy–Weinberg, ci sono 5 o 6 alleli ed esiste lo stesso quadro di variabilità: l'allele D è il più frequente (56–74%), mentre l'allele C è il secondo (21–38%); tutti gli altri alleli hanno sempre frequenze assai più basse, ma in molti casi superano chiaramente l'1%, come ad esempio il B (6% a S. Marcello) e l'F (2,7% al Giglio). L'eterozigosi media per il locus PGM oscilla dallo 0,398 allo 0,551. Nonostante l'ordinato quadro di distribuzione delle frequenze alleliche esistono chiare differenze fra le varie popolazioni, in primo luogo fra quelle continentali e quelle insulari, ed anche fra quelle dell'Elba e del Giglio: è soprattutto l'aumento dell'allele D e la contemporanea diminuzione di C che diversifica le colonie.

Il quadro delle somiglianze e delle differenze fra le popolazioni per le varianti elettroforetiche della PGM è paragonabile a quello ottenuto attraverso l'analisi dello spotting e ne conferma così, in pieno, il controllo genetico.

L'esistenza dello stesso complesso di alleli e dello stesso pattern distributivo anche in popolazioni completamente isolate come quelle insulari e di consistenza numerica assai diversa, elimina la migrazione quale fattore importante nel mantenere le somiglianze alleliche ed al tempo stesso indica il ruolo decisivo della selezione naturale nel mantenimento dell'eterozigosi e della differenziazione genetica. La situazione da noi riscontrata per la PGM rappresenta quindi un chiaro esempio di polimorfismo genetico bilanciato.

INTRODUCTION

In *M. jurtina* the number of spots found at definite positions on the underside of the hindwings varies, generally in a similar way, on each side, from 0 to 5 depending on whether or not one is present at each position. Since first reported the spots have been thought to be under multifactorial control [6, 7, 8] and, although their formal genetics has not been fully worked out, their number shows a good heritability [22, 10].

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(**) Nella seduta del 13 dicembre 1975.

Population studies on the spot distribution have been conducted over the years by comparing the relative frequencies of specimens with the same spot-number in the same colony or in different populations; this has been done mainly in the British Isles and Central Italy [10, 24, 25]. One of the outstanding features of the " spotting " is the occurrence of orderly spot-patterns overriding great environmental differences over large areas, so that in each population a general condition of spotting stability has been clearly ascertained from year to year. Several shifts in spot-frequencies have, however been witnessed in certain years and some of them (transient or stable) have occurred in relation to obvious ecological or climatic changes [10, 24, 25]. Besides such interseasonal spotting shifts, changes within a single generation, of which there is one per year, have been observed; intraseasonal spotting shifts are especially frequent in the aestivating populations of Central Italy [10, 23, 24, 25, 27]. Inter- and intraseasonal spotting shifts show features that are most easily explained through natural selection. Selection pressures involved in maintaining and adjusting the populations at their characteristic spot distributions in each generation in some instances reach the values of 70-80 per cent. against specific spot-phenotypes [9, 5, 24, 25, 27]. In spite of sharp differences in the time of hatching, and of the presence-absence of the imaginal aestivation in females, continental populations have shown similar spot-distributions and intraseasonal spotting shifts [23, 24, 19, 27]; on the other hand Tuscan insular populations sharply differ from the continental ones in having a much more intense spotting and, a to lesser extent, from each other on each island by having their own pattern of spot-distribution (25 and our unpublished data).

The spotting analysis has therefore given many indications, besides others, about the genetic similarities and diversities existing among different populations, and in order to get a more precise picture of their genetic differentiation we started an electrophoretic analysis of the phosphoglucomutase (PGM) isozymes, which appear to be of primary importance in carbohydrate metabolism catalysing the transfer of glucose-1-phosphate to glucose-6-phosphate.

Preliminary results on PGM phenotypes have already been published [21]; this note deals with the final picture of PGM morphs for the 1975 samples, the genetic interpretation of this enzyme system and the comparative analysis of the PGM gene sets of the different populations.

MATERIALS AND METHODS

Adults of *M. jurtina* have been collected from colonies which, with the exception of Il Volterraio, are well known for spotting and main life-cycle characteristics [23, 24, 25, 19, 20, 26, 27].

The map, fig. 1, shows the position of the collecting sites: S. Marcello and Prunetta are mountain colonies (700 and 1,000 m a.s.l. respectively) and Il Boschetto is a continental colony on the coast at sea level; the others

are insular populations, S. Giovanni, Il Perone and Il Volterraio on Elba, and Le Porte and L'Appiata on Giglio. Owing to their markedly different habitats it is to be expected that these populations should give satisfactory information about PGM isozyme variability.

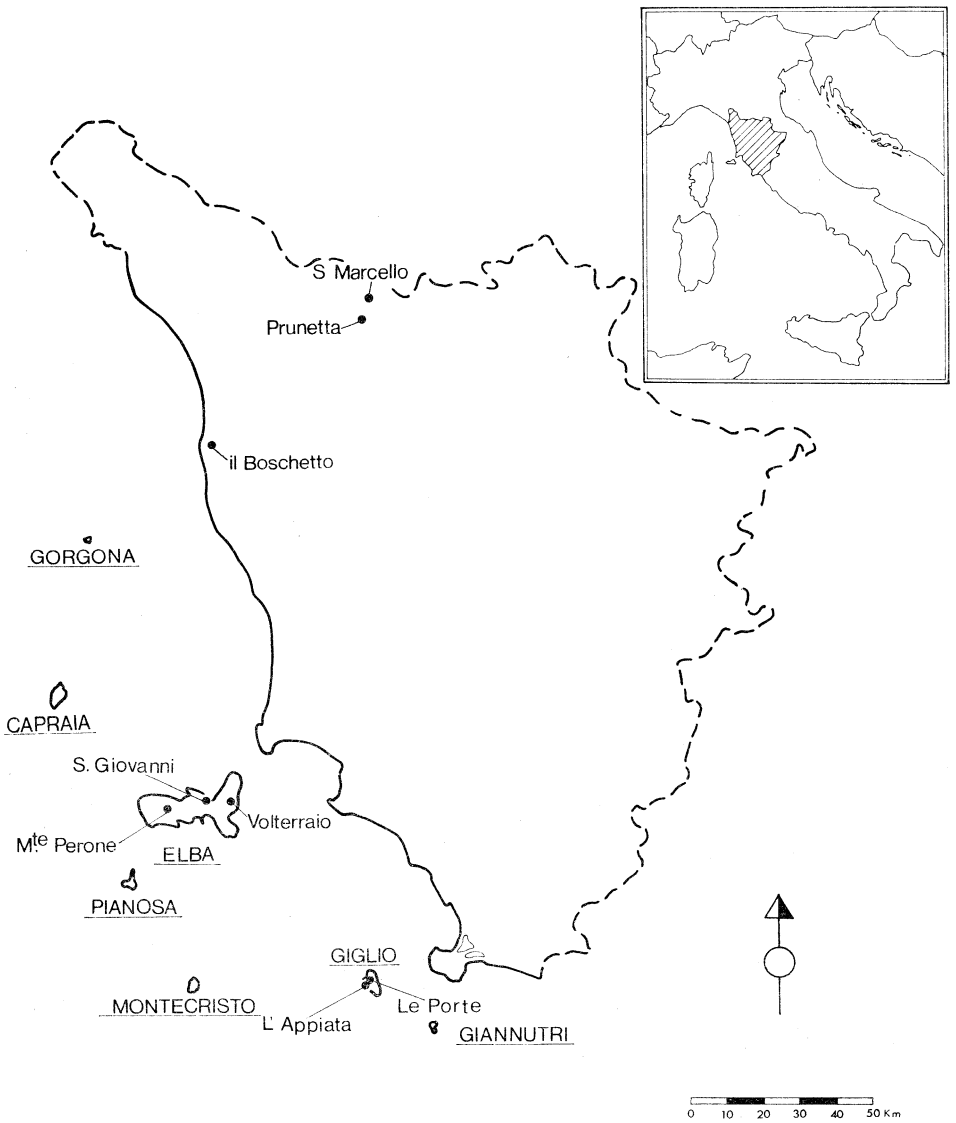

Fig. 1. – Map of Tuscany showing the collecting sites. The inset shows the position of Tuscany.

For electrophoresis the thorax of each specimen is ground up in 0.2 ml of distilled water; after centrifugation at 8,000 g for 4 minutes, 0.01 ml of the homogenate is put onto a small square (0.5 × 0.5 cm) of filter paper (Whatman n. 3). The squares are then lined up in a perpendicular cut of the horizontal starch-gel plate; media for the run and the staining reagents are those described

by [28] and have been used with the minor modifications introduced by [3]. Each run was at 5 ± 1 °C, for 3 h 30′ at 10 v/cm.

To get information on the genetics of the PGM morphs, 2nd instar larvae hatched from eggs laid by single post-aestivation females collected at Il Boschetto, have been analyzed. Twelve fertilized females were put separately into cylindrical plastic cages having transparent walls lined with green muslin and a perforated metal lid for good ventilation; each cage was provided with a small cup of diluted honey. The cages were kept in the open air in a medium-shaded courtyard all day. In these conditions females survived several days and nine of them laid eggs. The high mortality of embryos just before hatching from the egg and of larvae when they should have started feeding greatly reduced the size of families. Whole second instar larvae were ground in 0.04 ml of distilled water which was then used to damp the square of filter paper. Electrophoretic runs of such specimens were then carried out in just the same way as for the adults; some adult' homogenates of known pattern were always put into the same plate to act as a control.

RESULTS

In our starch-gel plates it is invariably apparent that each specimen possesses either one or two of six enzyme variants (from A to F) (fig. 2 shows their positions in relation to the D band. That the bands obtained are really due

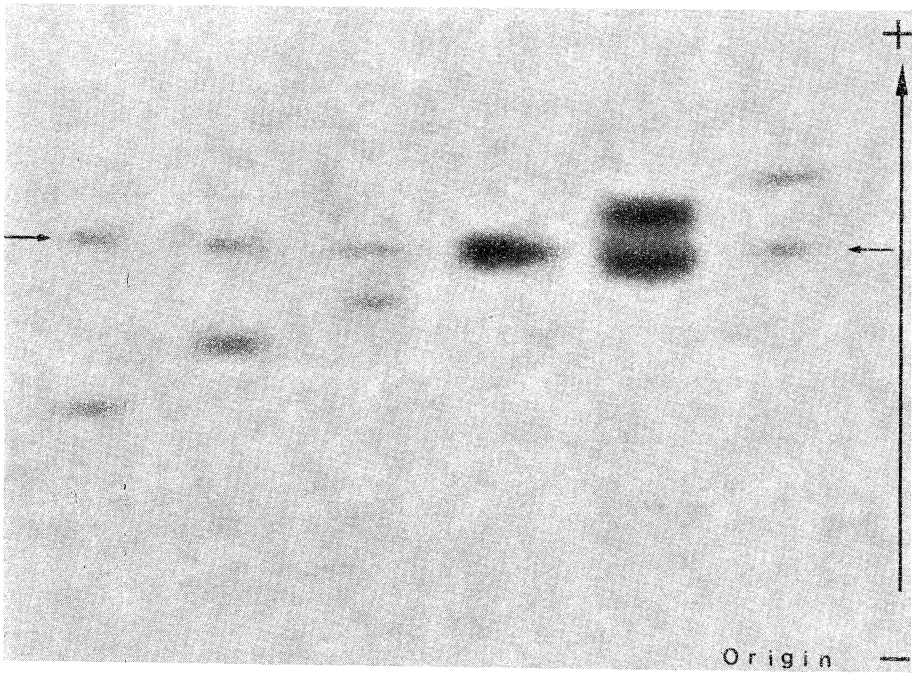

Fig. 2. – Starch-gel electrophoresis showing the six PGM variants found in *M. jurtina*. Horizontal arrows point out the D band position; the six phenotypes shown are, from the left, AD, BD, CD, D, DE, DF. Individual differences in the amount of enzymes are quite common (see the CD and D specimens).

to PGM activity is supported by their complete absence if glucose-1-phosphate or glucose-6-phosphate-dehydrogenase or both are omitted from the reaction mixture; furthermore individual patterns are quite stable, being constantly found in different runs. No difference exists for PGM variability between the two sexes ($P > 9$), and the distribution of the enzyme morphs is independent of the spot-phenotypes since no consistent association between these two variables has been found ($\chi^2_{(9)} = 12.14$ with $0.3 > P > 0.2$ for females; $\chi^2_{(4)} = 2.53$ with $0.7 > P > 0.5$, for males).

Table I gives the actual distribution of isozyme variants in adults of eight populations for 1975. Since growing evidence suggested that isozymes could be controlled by a series of six functional iso-alleles, we decided to test the offspring of single females.

At the time of copulation a female receives a big spermatophore which completely fills her "copulation pouch" [23]. During the past nine years we have dissected several hundred females for various purposes: in the 483 fertilized females there was always only one spermatophore contained in the "copulation pouch"; therefore each female has only one successful mating. Other evidence of the monogamy of the female can be derived from her behaviour: hundreds of times we observed in the field the characteristic vigorous wing-shaking with which many females drove away courting males. The dissection of 10 such females constantly showed that they had already been fertilized.

Table II reports the electrophoretic pattern obtained from 6 mothers and their offspring: in all instances the observed phenotypes of the progeny are compatible with that of the mother and in three families (1080, 1090, 1091) segregation has occurred indicating a mendelian transmission. These results, therefore, fully support the hypothesis of a multiple allelic series of six members and allow us to translate the electrophoretic patterns into genotypes and allele frequencies. Owing to monogamy the father's genotype in the family 1080 must be BD, while the most likely ones for males 1035, 1090 and 1091 are DD, CC and CD respectively.

Calculations show that every population is very close to the Hardy-Weinberg distribution of genotypes. Because of their homogeneity we grouped the rather small samples from different sites on each island and we report in Table III the percent allele frequencies for the five samples so obtained. In all of them the most frequent allele is D, while C is the second one. It will be noticed, however, that on Giglio and Elba D is markedly higher than in continental populations and that C is lowered accordingly. The "slowest" alleles (A and B) are more frequent on the continent while the "fastest" one (F), which has not been found in continental mountain colonies and it is very rare on the plains (Il Boschetto), reaches the frequency of A and B in the insular samples. A comparison of the homogeneous continental samples ($\chi^2_{(4)} = 6.04$; $0.2 > P > 0.1$) with the two insular samples gives a high heterogeneity ($P \ll 0.001$). It is to be noticed that the same kind of comparison between the two islands also gives a significant diversity, though of lesser magnitude ($\chi^2_{(3)} = 8.03$; $0.05 > P > 02$).

TABLE I

Isozyme morph distributions in eight populations of Maniola jurtina.

	S. Marcello	Prunetta	Il Boschetto	L'Appiata	Le Porte	S. Giovanni	Perone	Volterraio
AB			1 (0.23)					
AC			1 (0.23)					1 (2.56)
AD	2 (1.07)	1 (0.79)	10 (2.32)	1 (1.61)		1 (3.70)		
AE	1 (0.53)							
B			1 (0.23)					
BC	5 (2.66)	10 (7.94)	12 (2.79)		1 (1.18)		1 (1.10)	
BD	7 (3.72)	7 (5.56)	22 (5.11)	1 (1.61)	4 (4.71)		3 (3.30)	1 (2.56)
C	26 (13.83)	19 (15.08)	42 (9.75)	3 (4.84)	5 (5.88)	1 (3.70)	4 (4.39)	1 (2.56)
CD	89 (47.34)	42 (33.33)	197 (45.71)	28 (45.16)	38 (44.70)	7 (25.93)	36 (39.56)	11 (28.21)
CE			1 (0.23)					
CF			1 (0.23)					
D	57 (30.32)	45 (35.71)	135 (31.32)	22 (35.48)	31 (36.47)	17 (62.97)	44 (48.35)	24 (61.55)
DE	1 (0.53)	2 (1.59)	6 (1.39)	3 (4.84)	2 (2.35)		1 (1.10)	1 (2.56)
DF			1 (0.23)	3 (4.84)	4 (4.71)	1 (3.70)	2 (2.20)	
E			1 (0.23)					
Totals ...	188	126	431	62	85	27	91	39

Percent values are given in brackets.

TABLE II.

Distribution of PGM *phenotypes in the offspring from six females collected in the colony of Il Boschetto 1975 (S. Rossore–Pisa).*

Family number	Maternal phenotypes	Offspring phenotypes				Total
		BD	C	CD	D	
1034	D				1	1
1035	D				16	16
1080	CD	1		1	2	4
1090	CD		2	2		4
1091	CD			7	1	8
1092	D				2	2

TABLE III

Frequencies of PGM *alleles and of heterozygous individuals in five samples of* Maniola jurtina.

LOCALITIES / ALLELES	S. Marcello	Prunetta	Il Boschetto	Giglio	Elba
A	0.4 ± 0.4	0.8 ± 0.5	1.4 ± 0.0	0.3 ± 0.3	0.6 ± 0.4
B	6.7 ± 1.6	3.3 ± 0.9	4.3 ± 0.7	2.1 ± 0.8	1.6 ± 0.7
C	35.7 ± 3.0	38.9 ± 2.5	34.3 ± 1.6	28.6 ± 2.6	21.7 ± 2.3
D	56.4 ± 3.1	56.5 ± 2.5	58.7 ± 1.7	64.6 ± 2.8	74.5 ± 2.5
E	0.8 ± 0.5	0.5 ± 0.4	1.1 ± 0.3	1.7 ± 0.7	0.6 ± 0.4
F	—	—	0.2 ± 0.1	2.7 ± 0.9	1.0 ± 0.6
	100.0	100.0	100.0	100.0	100.0
Frequencies of heterozygous individuals	55.1	52.9	53.5	50.0	39.8

In the five samples, assuming a perfect Hardy-Weinberg equilibrium, the mean heterozygosity is similar and amounts to 0.398–0.551.

DISCUSSION

A comparable picture of similarities to and differences from that shown for the spotting is obtained from the PGM allele distributions and it is remarkable how such different characters as spotting and PGM morphs (which apparently do not show any causal relationship) can lead to the same conclusions about the genetic differentiation of the populations under study. The parallel pattern of variation discerned through the PGM alleles clearly confirms, after all, the genetic control of spotting. This result is also in line with those obtained by Handford with two esterase systems at the " boundary region " in South-Western England and in a number of the Isles of Scilly [11, 12]. It must be noticed, however, that for esterases a sharp difference between males and females seems to occur, while for PGM isozymes both sexes show the same kind and degree of variation.

Many features of our data fully support the balancing selection hypothesis of the maintenance of genetic variation, while they are at variance with the theory of evolution by " random walk " [14, 13, 16, 15, 4]. According to the neutral theory, we could predict that in different populations different sets of alleles should be found, and that, whenever the same alleles are present, their frequencies should be uncorrelated. On the contrary it is quite clear that in our samples the same set of alleles is found, and that the quantity and the pattern of variation remains remarkably constant from locality to locality. Nevertheless, orderly differences between localities occur, as already pointed out in the analysis of results [1].

Insular populations give further support to the selective hypothesis of genetic variation, since as a result of their complete isolation from continental populations (cfr. [10], and our unpublished data) migration as a possible factor for the maintenance of allelic similarities among populations can be ruled out [1, 2].

We do not know the effective population numbers of *M. jurtina* at the sites sampled, but it is quite obvious from our collecting experience that the continental colonies are much larger than the insular ones; this is particularly true for those of Giglio where the grassy areas, the only one suitable for the butterfly, are reduced in size and the population density is rather low [25]; nevertheless we find here the same number of alleles and about the same amount of heterozygosity: these facts are again at complete variance with the state of affairs expected according to the neutrality theory. We can therefore conclude that the variability shown by the PGM locus is a real instance of balanced polymorphism because in each population allele frequencies cannot be explained by recurrent mutation alone [10].

The lack of the F allele in mountain colonies together with its marked increase in frequency on Giglio where it is the third most common, could indicate that the corresponding enzyme variant is at an advantage in hot and dry places [17, 18, 19].

(1) BULLINI *et al.*, have analysed the same polymorphism in 7 more Italian populations of *M. jurtina* and reached very similar results; their data too appear in this volume.

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