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Phosphoglucomutase polymorphism in seven Lepidoptera species

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Genetica. — *Phosphoglucomutase polymorphism in seven Lepidoptera species*. Nota di LUCIANO BULLINI (*), ROSSELLA CIANCHI (*), GIUSEPPE NASCETTI (*) e LUIGI RENNA (*), presentata (**) dal Socio G. MONTALENTI.

RIASSUNTO. — Vengono descritti polimorfismi per la fosfoglucomutasi (PGM) in sette specie di Lepidotteri: *Brithys pancratii*, *Apopestes spectrum*, *Eilema caniola*, *Eilema lurideola*, *Phragmatobia fuliginosa*, *Dyxauses famula* e *Maniola jurtina*. La genetica formale dei varianti PGM è stata studiata in *Ph. fuliginosa* e in *M. jurtina*. I risultati dimostrano la presenza in entrambe le specie di vari alleli codominanti di un singolo gene *Pgm*. Anche nelle altre cinque specie, in cui i dati genetici mancano o sono solo parziali, le frequenze dei fenotipi elettroforetici osservati sono in buon accordo con l'ipotesi di un singolo gene *Pgm* con vari alleli codominanti. Ogni allele PGM determina un pattern elettroforetico monobanda; un pattern bibanda è presente, invece, negli etorozigoti. In tutte e sette le specie studiate le popolazioni sono risultate polimorfe per 2–6 alleli. In *M. jurtina* sono stati esaminati campioni aventi diversa origine geografica (Svizzera, Italia settentrionale e centrale). Benchè in tutte le popolazioni di questa specie da noi saggiate l'allele Pgm^D sia risultato sempre il più frequente, nell'area studiata le frequenze alleliche appaiono notevolmente differenziate. In particolare le frequenze alleliche nella popolazione di Promenthoux (Svizzera) differiscono significativamente da quelle delle popolazioni italiane saggiate. In base ai dati in nostro possesso il polimorfismo per la fosfoglucomutasi in *M. jurtina* non risulta geneticamente associato con il numero delle macchie ocellari delle ali posteriori.

INTRODUCTION

The marked improvement in recent years of techniques for protein separation has led to the discovery of numerous new polymorphisms in a large variety of organisms. Rather surprisingly very little is known about isozyme variation in butterflies and moths, in spite of the exceptional importance of this material in research fields as different as genetics, evolutionary biology, systematics, ecology, ethology and applied entomology.

In the present paper we present the evidence for polymorphisms involving phosphoglucomutase (PGM) in seven Lepidoptera species: *Brithys pancratii*, *Apopestes spectrum*, *Dyxauses famula*, *Phragmatobia fuliginosa*, *Eilema caniola*, *Eilema lurideola* and *Maniola jurtina*. This enzyme catalyzes the reversible transfer between glucose 1-phosphate and glucose 6-phosphate, and it has an important role in carbohydrate metabolism.

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(**) Nella seduta del 13 dicembre 1975.

ELECTROPHORETIC TECHNIQUES

The electrophoretic separation of the PGM variants was done in starch-gel utilizing, with minor modifications, the method described by Spencer *et al.* [1]. For each individual examined a small piece of filter paper (Whatman No. 3) of about 5×3 mm was impregnated with the insect homogenate (obtained by grinding the thorax in 0.01 ml of the gel buffer) and inserted in starch-gel. This was performed in plexiglas trays ($0.4 \times 10 \times 20$ cm) with a gel composed of 10 per cent starch dissolved in 1 : 10 dilution of the maleic acid-Tris-EDTA-$MgCl_2$ buffer at pH = 7.4 as described in the original technique. Horizontal starch-gel electrophoresis was carried out at + 5 °C at 4 V/cm for 14 hr or 9 V/cm for 4 hr. The gel was sliced and developed for PGM as described by Spencer *et al.* [1], with the modification that the developing solution was solidified by agar at a final concentration of 0.8 per cent. The PGM patterns appear after about 30–45 minutes of incubation at 37 °C in the dark.

The following evidences support the conclusion that the bands shown in Plates I and II really represent PGM activity:

1) if glucose 1-phosphate is omitted from the reaction mixture, none of the bands develops;

2) if glucose 6-phosphate dehydrogenase in omitted from the reaction mixture, only minor bands appear presumably due to endogenous G6PD present in the homogenate;

3) if NADP or PMS is omitted from the reaction mixture, no bands develop.

RESULTS AND CONCLUSIONS

The formal genetics of the PGM variants was studied in *Phragmatobia fuliginosa* and in *Maniola jurtina*. The results indicated the occurrence, in both species, of various codominant alleles of a single gene *Pgm*. In the other species, for which the formal genetics data were lacking or incomplete, the observed frequencies of the various electrophoretic phenotypes were in agreement with the hypothesis of a single gene *Pgm* with various codominant alleles.

In all the species examined every PGM allele determines a single-band electrophoretic pattern. A double-band phenotype is shown by the heterozygotes. A similar pattern can be also produced by an artificial mixture of approximately equal amounts of homogenates from two specimens, respectively homozygotes for two different alleles.

The distribution of the PGM alleles in the populations studied are reported below [1].

(1) The various PGM alleles are named with capital letters in order of increasing electrophoretic mobility (e.g. Pgm^A , Pgm^B , Pgm^C , etc.).

Brithys pancratii Cyr. (*Noctuidae, Hadeninae*).

The material examined consisted of samples collected during 1975 at Marina di Pescia Romana, s.l. (Viterbo). This population was polymorphic for two PGM alleles, showing the following frequencies: $Pgm^A = 0.0759$; $Pgm^B = 0.9241$ (Table I).

TABLE I

Distribution of the PGM *phenotypes in a sample of 158* Brithys pancratii *from Marina di Pescia Romana, s.l. (Viterbo).*

Phenotypes	Prevalence %	Absolute frequencies	
		Observed	Expected (*)
A	0.63	1	0.91
AB	13.92	22	22.18
B	85.44	135	134.91
Totals	99.99	158	158.00

(*) Expected from a Hardy-Weinberg equilibrium involving two codominant alleles.

Estimates of gene frequencies $\begin{cases} Pgm^A = 7.59 \pm 1.49 \\ Pgm^B = 92.41 \\ \hline 100.00 \end{cases}$

Apopestes spectrum Esp. (*Noctuidae, Amphipyrinae*).

The electrophoretic study was carried out on a population sample from Canino, m. 230 (Viterbo), collected in February 1974. This material was polymorphic for five PGM alleles, the most frequent (Pgm^C) showing intermediate electrophoretic mobility. The distribution of the PGM alleles in this population is reported in Table II. The phenotypes AB, AC, BC, C, CD and CE are represented in Pl. I, fig. 1.

Eilema caniola Hbn. (*Arctiidae, Lithosiinae*).

The population sample tested, collected at Olgiata, m 100 (Rome) in June 1975, was polymorphic for three PGM alleles showing the following frequencies: $Pgm^A = 0.0294$; $Pgm^B = 0.9228$; $Pgm^C = 0.0478$ (Table III). The phenotypes AB, B and BC are represented in Pl. I, fig. 2.

TABLE II

Distribution of the PGM *phenotypes in a sample of* 151 Apopestes spectrum *from Canino, m 230 (Viterbo).*

Phenotypes	Prevalence %	Absolute frequencies	
		Observed	Expected (*)
A	—	—	0.37
AB	1.32	2	0.40
AC	8.61	13	13.66
AD	—	—	0.15
AE	—	—	0.05
B	—	—	0.11
BC	3.97	6	7.28
BD	—	—	0.08
BE	—	—	0.03
C	83.44	126	125.21
CD	1.99	3	2.73
CE	0.66	1	0.91
D	—	—	0.01
DE	—	—	0.01
E	—	—	0.002
Totals	99.99	151	151.00

(*) Expected from a Hardy-Weinberg equilibrium involving five codominant alleles.

Estimates of gene frequencies

$Pgm^A = 4.97 \pm 1.25$
$Pgm^B = 2.65 \pm 0.92$
$Pgm^C = 91.06 \pm 1.64$
$Pgm^D = 0.99 \pm 0.57$
$Pgm^E = 0.33 \pm 0.33$
100.00

TABLE III

Distribution of the PGM *phenotypes in a sample of 136* Eilema caniola *from Olgiata, m 100 (Rome).*

Phenotypes	Prevalence %	Absolute frequencies	
		Observed	Expected (*)
A	—	—	0.12
AB	5.88	8	7.38
AC	—	—	0.38
B	84.56	115	115.81
BC	9.56	13	12.00
C	—	—	0.31
Totals	100.00	136	136.00

(*) Expected from a Hardy-Weinberg equilibrium involving three codominant alleles.

Estimates of gene frequencies $\begin{cases} Pgm^A = 2.94 \pm 1.02 \\ Pgm^B = 92.28 \pm 1.62 \\ Pgm^C = 4.78 \pm 1.29. \\ \hline 100.00 \end{cases}$

Eilema lurideola Zincken (*Arctiidae, Lithosiinae*).

The material examined consisted of samples collected in July 1975 at Camerata Nuova, m 900 (Rome). At least four PGM alleles were observed (some of which with a very similar electrophoretic mobility), showing the following frequencies: $Pgm^A = 0.0215$; $Pgm^B = 0.1129$; $Pgm^C = 0.0269$; $Pgm^D = 0.8387$ (Table IV). The phenotypes AD, BD, CD and D are represented in Pl. II, fig. 1.

Phragmatobia fuliginosa L. (*Arctiidae, Arctiinae*).

The population sample tested was collected at Olgiata, m 100 (Rome) in June 1975. The distribution of the PGM alleles in this material is reported in Table V.

Dyxauses famula Frr. (*Ctenuchidae=Amatidae=Syntomidae*).

Also this species was collected at Olgiata, m 100 (Rome) in June 1975. The sample tested was found polymorphic for two PGM alleles, showing the following frequencies: $Pgm^A = 0.9612$; $Pgm^B = 0.0388$ (Table VI).

TABLE IV

Distribution of the PGM *phenotypes in a sample of 93* Eilema lurideola *from Camerata Nuova, m 900 (Rome).*

Phenotypes	Prevalence %	Absolute frequencies	
		Observed	Expected (*)
A	—	—	0.04
AB	2.15	2	0.45
AC	—	—	0.11
AD	2.15	2	3.35
B	2.15	2	1.19
BC	—	—	0.56
BD	16.13	15	17.61
C	—	—	0.07
CD	5.38	5	4.19
D	72.04	67	65.42
Totals	100.00	93	92.99

(*) Expected from a Hardy-Weinberg equilibrium involving four codominant alleles.

Estimates of gene frequencies:
$Pgm^A = 2.15 \pm 1.06$
$Pgm^B = 11.29 \pm 2.32$
$Pgm^C = 2.69 \pm 1.18$
$Pgm^D = 83.87 \pm 2.70$
100.00

TABLE V

Distribution of the PGM *phenotypes in a sample of 92* Phragmatobia fuliginosa *from Olgiata, m 100 (Rome).*

Phenotypes	Prevalence %	Absolute frequencies	
		Observed	Expected (*)
A	2.17	2	1.09
AB	16.30	15	17.39
AC	1.09	1	0.43
B	77.17	71	69.57
BC	3.26	3	3.48
C	—	—	0.04
Totals	99.99	92	92.00

(*) Expected from a Hardy-Weinberg equilibrium involving three codominant alleles.

Estimates of gene frequencies:
$Pgm^A = 10.87 \pm 2.29$
$Pgm^B = 86.96 \pm 2.48$
$Pgm^C = 2.17 \pm 1.07$
100.00

TABLE VI

Distribution of the PGM *phenotypes in a sample of 116* Dixauses famula *from Olgiata, m 100 (Rome).*

Phenotypes	Prevalence %	Absolute frequencies	
		Observed	Expected (*)
A	93.10	108	107.17
AB	6.03	7	8.65
B	0.86	1	0.17
Totals	99.99	116	115.99

(*) Expected from a Hardy-Weinberg equilibrium involving two codominant alleles.

Estimates of gene frequencies $\begin{cases} Pgm^A = 96.12 \\ Pgm^B = \underline{3.88} \pm 1.27 \\ \quad\quad 100.00 \end{cases}$

TABLE VII

Distribution of PGM *alleles in population samples of* Maniola jurtina *from Italy and Switzerland.*

Geographical Origin	Number tested	PGM ALLELE FREQUENCIES					
		Pgm^A	Pgm^B	Pgm^C	Pgm^D	Pgm^E	Pgm^F
Campagnano, m 300 (Rome)	301	0.0100	0.0631	0.3156	0.6013	0.0066	0.0033
San Polo dei Cavalieri, m 800 (Rome) . . .	18	0.0278	0.0556	0.2500	0.6667	—	—
Cellère, m 350 (Viterbo)	69	0.0072	0.0725	0.3478	0.5580	0.0145	—
Amatrice, m 900 (Rieti)	24	—	0.0625	0.3750	0.5208	0.0417	—
Ville di Fano, m 900 (L'Aquila)	371	0.0081	0.0606	0.3693	0.5580	0.0027	—
Selva di Progno, m 600 (Verona)	76	0.0132	0.0592	0.3289	0.5855	—	0.0132
Promenthoux, m 400 (Lake of Geneva) .	51	—	—	0.1275	0.8431	0.0294	—

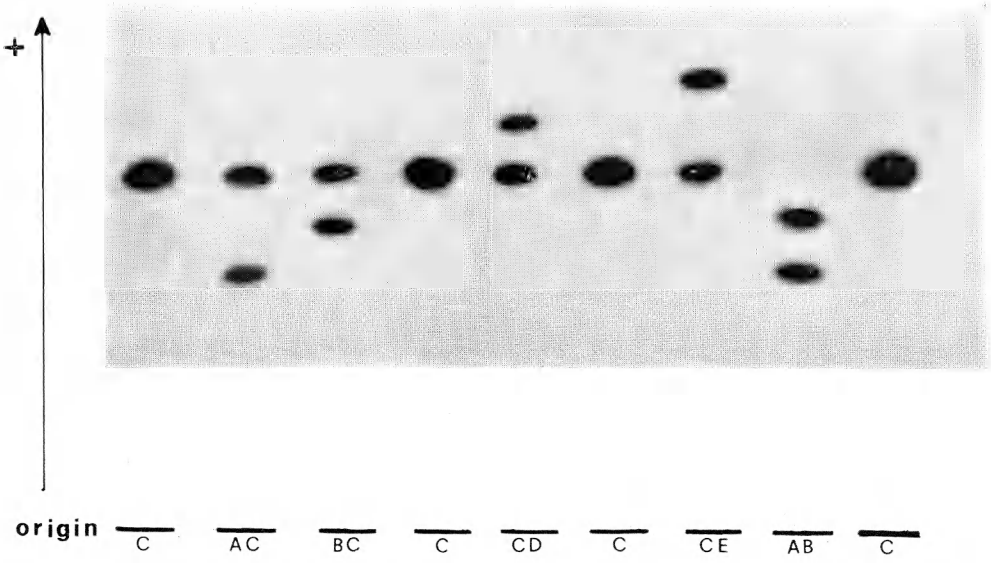

Fig. 1. – Photograph of the phosphoglucomutase zymograms of *Apopestes spectrum* showing the following electrophoretic phenotypes: AB, AC, BC, C, CD and CE.

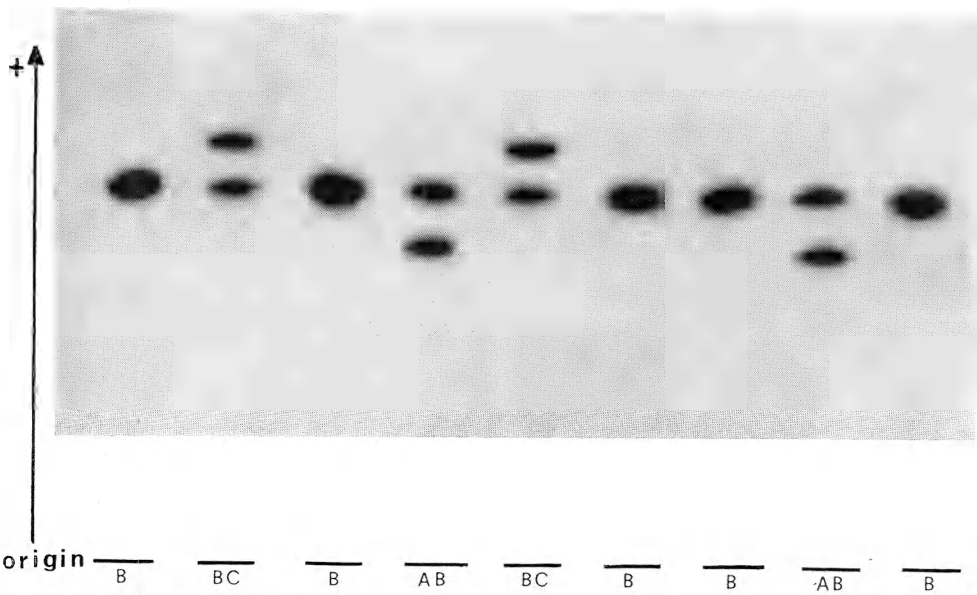

Fig. 2. – Photograph of the phosphoglucomutase zymograms of *Eilema caniola* showing the following electrophoretic phenotypes: AB, B and BC.

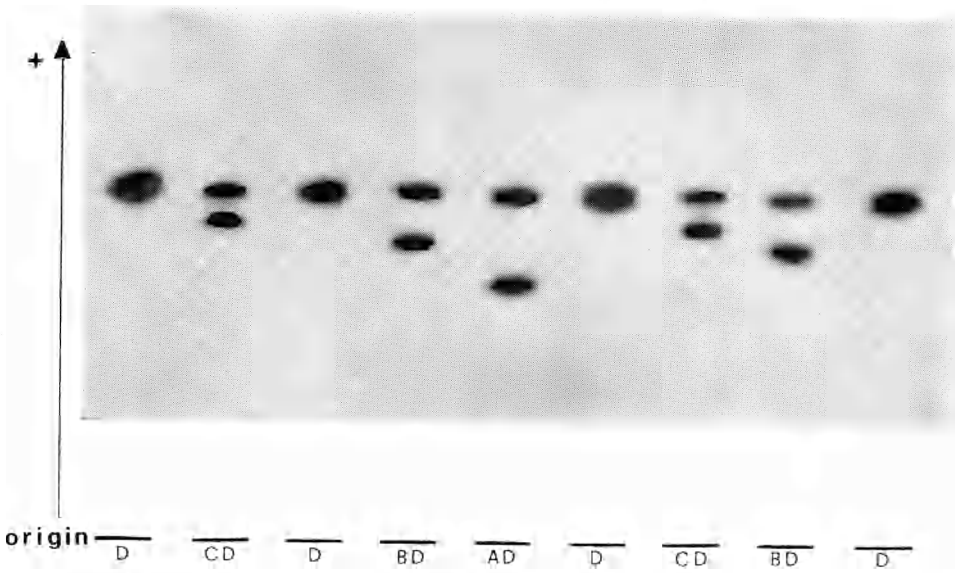

Fig. 1. – Photograph of the phosphoglucomutase zymograms of *Eilema lurideola* showing the following electrophoretic phenotypes: AD, BD, CD and D.

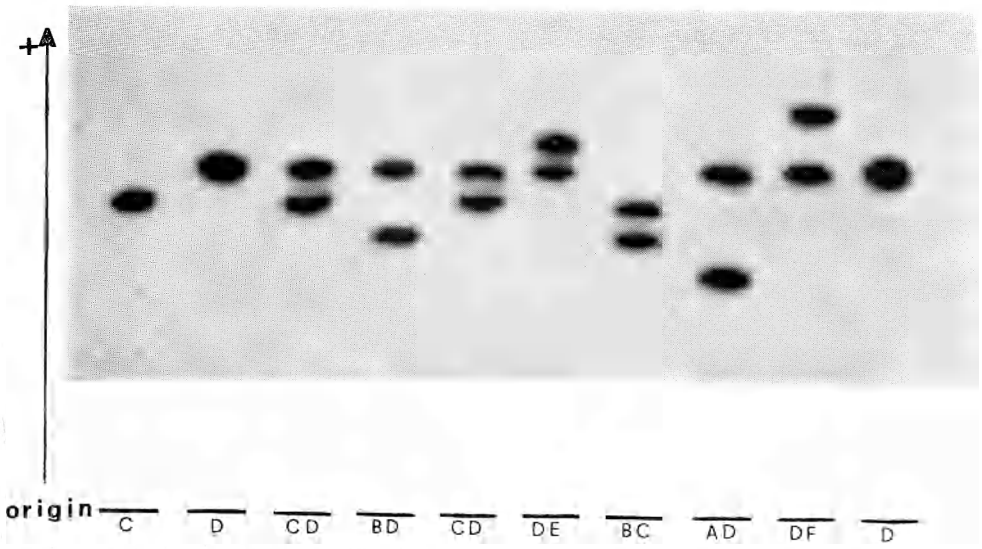

Fig. 2. – Photograph of the phosphoglucomutase zymograms of *Maniola jurtina* showing the following electrophoretic phenotypes: AD, BC, BD, C, CD, D, DE and DF.

Maniola jurtina L. [2] (*Satyridae*, *Satyrinae*).

The material examined consisted of samples from seven populations having different geographical origins (Switzerland, northern and central Italy). All the seven populations were polymorphic for three to six alleles, the most frequent being in all samples Pgm^D. The phenotypes AD, BC, BD, C, CD, D, DE and DF are represented in Pl. II, fig. 2. The distribution of the PGM alleles in the seven population samples tested is reported in Table VII. The allele frequencies appear to be fairly differentiated over the area considered (minor differences between some localities can be attributed to sampling errors). It is to be noted that remarkable differences in the PGM allele frequencies seem to exist between *Maniola jurtina* populations living respectively north and south of the Alps. This appears confirmed by preliminary data obtained on small samples of this species from Austria and Germany.

According to our data phosphoglucomutase polymorphism is not genetically associated with the hind-wing spotting.

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[3] P. T. HANDFORD (1973) – *Patterns of variation in a number of genetic systems in* Maniola jurtina: *The Isles of Scilly*, « Proc. R. Soc. Lond. » B, *183*, 285–300.

(2) Two polymorphic esterase systems were described in this species by Handford [2], [3]. Some more data on phosphoglucomutase polymorphism in *M. jurtina* for Central Italy are reported in a paper by Masetti and Scali in the same volume.