

Polymorphic and sex-limited phosphoglucomutase in *Parastrongylus cantonensis* (Nematoda: Angiostrongylidae)

Praphathip Eamsobhana¹, Naowarat Dechkum² and Yong Hoi-Sen³

¹Department of Parasitology, Faculty of Medicine Siriraj Hospital, ²Department of Parasitology, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand;

³Institute of Biological Sciences, University of Malaya, Kuala Lumpur 50603, Malaysia

Abstract. Phosphoglucomutase was studied by polyacrylamide gel electrophoresis in the Thailand and Hawaii isolates of *Parastrongylus cantonensis* (also known as *Angiostrongylus cantonensis*). Two loci were present. The faster-moving locus (PGM-1) was polymorphic in the Hawaii isolate, represented by two alleles – the faster-moving, less common *Pgm-1^A* and the slower-moving, more common *Pgm-1^B*. It was monomorphic for the faster-moving allele *Pgm-1^A* in the Thailand isolate. The slower-moving locus (PGM-2) was invariant, with a single band of enzyme activity, in the female worms of both the Thailand and Hawaii isolates. There was no detectable enzyme activity at this PGM-2 locus in the male worms of both isolates. The non-expression or 'null' PGM-2 phenotype in the male worms was presumed to be sex-limited. The present findings differ significantly in several aspects (polymorphic locus, proportion of polymorphic loci, heterozygosity, deviations from Hardy-Weinberg expectations, sex-limited expression) from the Japan isolate of *P. cantonensis* reported in the literature.

INTRODUCTION

Parastrongylus cantonensis (earlier known as *Angiostrongylus cantonensis*) is a metastrongyloid nematode that inhabits the lungs of rats, and is commonly known as the rat lungworm. It is widely distributed in Southeast Asia, the Pacific Islands and many tropical and subtropical regions of the world (Cross, 1987; Kliks & Palumbo, 1992). In humans, an accidental host, it causes a form of eosinophilic meningitis or eosinophilic meningo-encephalitis (Alicata, 1991).

Human infection is due to ingestion of infective larvae in uncooked or undercooked food – such as snails and slugs (the intermediate hosts), crabs and shrimps (paratenic hosts that have ingested infected intermediate hosts), and leafy vegetables harbouring infected mollusks or contaminated by mollusk slime – and through handling infected mollusks. The incubation period in humans is 1-3 weeks.

Many aspects of the biology of *P. cantonensis* have been well documented (Eamsobhana & Tungtrongchitr, 2005; Eamsobhana *et al.*, 1998, 2000, 2004). There is however limited studies on the genetics of this nematode parasite. We report here polymorphism and sex-limited expression of the gene-enzyme system phosphoglucomutase (PGM, EC 5.4.2.2, formerly EC 2.7.5.1) in two isolates (Thailand and Hawaii) of the rat lungworm *P. cantonensis*.

MATERIALS AND METHODS

The nematode of *P. cantonensis* (Thailand strain/isolate) used in this study was provided to us by Dr. Chalit Komalamisra, Faculty of Medicine, Mahidol University. Life cycle of the worm has been maintained in our laboratory. The Hawaii strain/isolate of *P. cantonensis* was kindly provided to us by Dr. Akira Ishii and Dr. Hideto Kino, Hamamatsu University

School of Medicine, Japan and has been maintained in our laboratory.

For both the Thailand and Hawaii isolates, 48 male and 48 female adult worms, recovered from an infected laboratory white rat *Rattus norvegicus*, were used for electrophoresis following the method of Takai (1984) with slight modification. Vertical polyacrylamide slab gel with 5% stacking gel and 7% separating gel layers was prepared in 0.5M Tris-HCl, pH 6.8 and 1.5M Tris-HCl, pH 8.9, respectively. Electrophoresis was carried out in 0.005M Tris-0.038M glycine, pH 8.3. Adult male and female worms were individually homogenized in 100 μ l and 200 μ l of 20% sucrose solution, respectively, using a Teflon homogenizer in an ice-bath. Homogenates were centrifuged at 10,000 g for 10 min at 4°C. 30 μ l of sample supernatant was loaded into a sample slot of the stacking gel. To avoid the possibility of ingested blood from the adult worms, serum and red blood cells of the rat were also included in all gels as controls. The

controls were prepared as described by Sawabe & Makiya (1994).

Electrophoresis was done for three hours at a constant voltage of 300 volts at 4°C and stopped when the indicator dye (bromophenol blue) had migrated about 10 cm into the separating gel. Detection of PGM was according to the method of Steiner & Joslyn (1979), and Tsukamoto (1984, 1989), with slight modification.

The PGM loci were designated numerically according to decreasing electrophoretic mobility, as adopted for the Japan isolate (Sawabe & Makiya, 1994). Alleles (electromorphs) were designated alphabetically according to decreasing electrophoretic mobility. Uppercase was used to denote dominance. The designation of alleles by numerals as employed by Sawabe & Makiya (1994) was not followed so as to avoid confusion, particularly the identity of the alleles (electromorphs) in different studies. The G-test (log likelihood χ^2 test) was used for determining Hardy-Weinberg equilibrium.

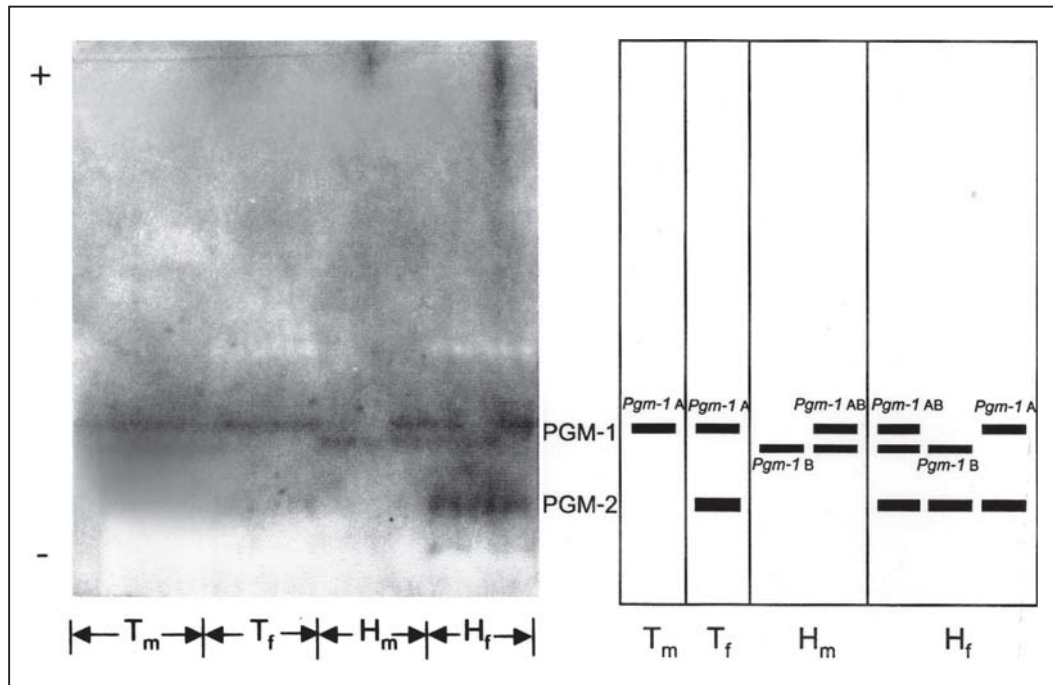


Figure 1. Electrophoretic phenotypes of phosphoglucosmutase in the Thailand and Hawaii isolates of *Parastrongylus cantonensis*. Tm = male; Thailand isolate, Tf = female; Thailand isolate, Hm = male; Hawaii isolate, Hf = female; Hawaii isolate.

Table 1. Distribution of phosphoglucosmutase electrophoretic phenotypes, frequencies of *Pgm* alleles, heterozygosities, 95% confidence limit values, and *G*-values for the log likelihood χ^2 test for Hardy-Weinberg expectations in the Thailand and Hawaii isolates of *Parastrongylus cantonensis*

	Thailand isolate		Hawaii isolate	
	Male	Female	Male	Female
PGM-2 phenotype				
A	0	48	0	48
'null'	48	0	48	0
PGM-1 phenotype				
A	48	48	0	14
AB	0	0	23	18
B	0	0	25	16
<i>Pgm-1</i> allele frequency				
A \pm SD	1.00	1.00	0.24 \pm 0.04	0.48 \pm 0.05
B	0	0	0.76	0.52
PGM-1 heterozygosity				
Observed	0	0	0.48	0.38
Expected	0	0	0.36	0.50
95% C.L. for <i>Pgm-1</i> ^A frequency				
	–	–	0.16–0.32	0.38–0.58
<i>G</i> -value				
	–	–	7.38	3.00
P-value				
	–	–	0.10>P>0.05	0.01>P>0.005

RESULTS

Two zones of PGM activity were present in the electropherogram, the faster-moving PGM-1 and the slower-moving PGM-2 (Fig. 1). PGM-2 was invariant in both the Thailand and Hawaii isolates (Fig. 1; Table 1). Enzyme activity for PGM-2 was observed only in the female worms. In the absence of enzyme activity the males were designated as having 'null' phenotype.

PGM-1 was monomorphic in the Thailand isolate, represented by the allele *Pgm-1*^A (the faster-moving electromorph or allele in the samples). On the other hand, it was polymorphic in both the male and female worms of the Hawaii isolate (Fig 1; Table 1). The commonest allele in the Hawaii sample was *Pgm-1*^B, the slower-moving electromorph. The heterozygote (PGM-1 AB) was represented by two bands of enzyme activity, indicating a monomeric enzyme and codominance.

The distribution of the PGM-1 phenotypes in the male sample of Hawaii

isolate differed significantly from Hardy-Weinberg expectations ($G = 7.38$; $0.01 > P > 0.005$), due to the absence of the homozygote phenotype (PGM-1 A). In contrast, for the PGM-1 phenotypes in the female sample the distribution was in agreement with Hardy-Weinberg expectations ($G = 3.00$; $0.10 > P > 0.05$). The PGM-1 allele frequencies were significantly different between the male and female samples, as indicated by 95% confidence limit values (Table 1). The male and female samples also differed significantly in heterozygosity – more than expected for the male sample, but deficit for the female sample.

DISCUSSION

Isoenzymes (or isozymes) have been used for the investigation of many kinds of biological problems, such as genetics, systematics and developmental biology. In the study of nematode parasites,

isoenzymes have been used, among others, for (1) species discrimination, and (2) intraspecific variation – interpopulation and intrapopulation genetic variation (Yong & Mak, 1988).

Phosphoglucumutase is a key enzyme in the glycolytic pathway, catalyzing the interconversion of glucose-1-phosphate and glucose-6-phosphate. It was one of the first enzymes that were shown to function by formation of a protein seryl-phosphate intermediate. It has been studied in many organisms, for example the mosquito *Aedes albopictus* (Yong *et al.*, 1981).

Six gene-enzyme systems (glucose phosphate isomerase, hexokinase, lactate dehydrogenase, malate dehydrogenase, malic enzyme, and phosphoglucumutase) have been reported for *Parastrongylus cantonensis* in Japan (Sawabe & Makiya, 1994). The proportion of polymorphic loci was 6/10 ($P = 0.6$) and the average heterozygosity was 0.151. Two loci were present for phosphoglucumutase – one was monomorphic (faster-moving locus – PGM-1), while the other (slower-moving locus – PGM-2) was polymorphic with two alleles. In the polymorphic locus, the frequency for the slower-moving allele (designated *Pgm-2*²⁴) was 0.766, and that for the faster-moving allele (*Pgm-2*³³) was 0.234, with heterozygosity of 0.358.

In the study of the Japan *P. cantonensis*, two other species – *Parastrongylus malaysiensis* and *Parastrongylus costaricensis* – were included for comparison. *Parastrongylus cantonensis* and *P. malaysiensis* were reported to possess the same invariant *Pgm-1*⁴⁴ allele. *Parastrongylus malaysiensis* was represented by a slower-moving, PGM-1 electromorph than that in *P. cantonensis* and *P. costaricensis* (Sawabe & Makiya, 1994).

It is noteworthy that the present findings, employing Thailand and Hawaii isolates of *P. cantonensis*, differ greatly from the results reported for the Japan isolate (Sawabe & Makiya, 1994). In the present study, the slower-moving locus (PGM-2) was invariant for the male or female worms respectively, while the

faster-moving locus (PGM-1) was polymorphic (Table 1, Fig. 1). The opposite was reported for the Japan *P. cantonensis* – the faster-moving locus monomorphic and the slower-moving locus polymorphic. A possible explanation for this difference is geographical variation. For this reason, the present study designated the alleles alphabetically in order to avoid conveying the impression of genetic identity or difference between different studies. In the numerical designation of alleles, the commonest allele is conventionally assigned the number 100, with the other alleles showing relative mobility – either faster (more than 100) or slower (less than 100).

In the present study of the Thailand and Hawaii isolates of *P. cantonensis*, five other gene-enzyme systems (glucose phosphate dehydrogenase, glucose phosphate isomerase, lactate dehydrogenase, malate dehydrogenase and malic enzyme – each represented by two presumptive loci) were studied in addition to phosphoglucumutase. All were monomorphic. Superoxide dismutase, which appeared on gels stained for certain enzymes, was also invariant. The results indicated a much lower proportion of polymorphic loci and average heterozygosity in the Thailand and Hawaii isolates compared to the Japan isolate.

The very low proportion of polymorphic loci in the Hawaii isolate, and its absence in the Thailand isolate, is not totally unexpected. In the present materials of the Thailand and Hawaii isolates of *P. cantonensis*, the founder effect (a special type of genetic drift) may be operative, i.e. the founder individuals were few in number and might not represent the natural population.

For the polymorphic PGM-1 locus in the Hawaii isolate, there was an excess of heterozygotes in the male sample but a deficit in the female worms. When the male and female samples were combined, there was a slight deficit of heterozygotes (Table 2). Likewise, the distribution of the PGM-1 phenotypes in the male sample of Hawaii isolate differed significantly from

Table 2. Distribution of polymorphic phosphoglucosmutase electrophoretic phenotypes, allele frequencies, heterozygosities, 95% confidence limit values, and *G*-value for Hardy-Weinberg expectation in the combined male and female samples of the Hawaii isolate of *Parastrongylus cantonensis*

	Observed	Expected
PGM-1 phenotype		
A	14	12.44
AB	41	44.24
B	41	39.32
<i>Pgm-1</i> allele frequency		
A	0.36 ± 0.03	
B	0.64	
Heterozygosity	0.43	0.46

C.L. for *Pgm-1*^A frequency = 0.30-0.42; *G* = 0.25

Hardy-Weinberg expectations, while the female sample was in reasonable agreement (Table 1). When the male and female samples were combined, the distribution of the PGM-1 phenotypes agreed with Hardy-Weinberg expectations (*G* = 0.25; *P* > 0.5). In the Japan isolate, there was an excess of heterozygotes for the polymorphic PGM-2 locus, and the distribution of the phenotypes showed significant deviation from Hardy-Weinberg expectations (Sawabe & Makiya, 1994). Of the five forces – mating choice, mutation, migration, genetic drift, and natural selection – that may cause deviations from Hardy-Weinberg equilibrium, the most probable is genetic drift which operates especially in small populations. The causes and significance of the differences could not be meaningfully explored with the present data.

In both the Hawaii and the Japan isolates, certain phenotypes (or genotypes) in the polymorphic PGM locus were not observed, e.g. absence of PGM-1 A in the male worms although six individuals were expected according to Hardy-Weinberg Law. This condition is common in studies using worms recovered from experimental animals, for example glucose phosphate

isomerase in the human filarial parasite *Brugia malayi* (Yong & Mak, 1984). It is also encountered in many other organisms, e.g. phosphoglucosmutase in the tephritid fruit fly *Dacus dorsalis*, now known as *Bactrocera carambolae* (Yong, 1984).

Another significant difference was the absence of detectable PGM-2 enzyme activity in the male worms of both the Thailand and Hawaii isolates of *P. cantonensis* (Fig. 1). This was not reported in the Japan isolate – the data however did not separate the two sexes (Sawabe & Makiya, 1994). The number of specimens used in the present study was reasonably large (48 for each isolate). It is therefore not likely to be due to sampling or stochastic errors. As male worms were electrophoresed together with female worms on the same gel, the absence of detectable enzyme activity could not be possibly due to experimental conditions (buffer, electrophoresis conditions, staining and the like).

Assuming the results to be valid, PGM-2 in the Thailand and Hawaii isolates of *P. cantonensis* may be reasonably presumed to be sex-limited. Morgan (1910) used the term ‘sex-limited’ for *Drosophila* eye colour that occurred only in males – the condition was however subsequently shown to be sex-related, not ‘sex-limited’. Sex-limited traits, in contrast to sex-linked and sex-influenced traits, are traits that are expressed or visible only in one sex but are determined by genes present in both sexes.

There are many textbook examples of sex-limited traits, many of which are due to hormonal controls. As far as we could ascertain, there seem to be no report on sex-limited trait in nematode parasites. The gene governing phosphoglucosmutase is codominant. Its non-expression or inactivation in the male worms is therefore not easy to explain. It is unlikely to be due to the occurrence of a null allele, as PGM-2 activity was not detected in all the male worms in the present study involving two geographical isolates. The mechanism and significance therefore remain to be elucidated.

If subsequently confirmed to be valid, this sex-limited PGM-2 can be used as a marker for sex determination of the larval stages of *P. cantonensis*. Studies will be carried out to understand various aspects of the genetics of this nematode parasite.

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