[Jpn. J. Parasitol., Vol. 36, No. 3,160–165, June, 1987]

Isozyme Genetics of a Japanese Lung Fluke, *Paragonimus ohirai*

TAKESHI AGATUMA[1] AND SHIGEHISA HABE[2]

(Received for publication; March 5, 1987)

Abstract

Four cross experiments were carried out using electrophoretic variants of three enzymes, glutamic-oxaloacetic transaminase (GOT), leucylglycylglycine aminopeptidase (LGG) and tetrazolium oxidase (TO) as markers between individuals of *Paragonimus ohirai* sampled from natural populations in Japan. Parents of the first cross (N5a x N5b) were characterized by FS and FS for GOT, FF and FF for LGG, and SS and SS for TO. Similarly, the second cross (K6a x K6b) revealed parents' phenotypes for the three enzymes as follows: FS and SS for GOT, FS and FF for LGG, and FF and FS for TO. In the third cross (K11a x K11b), only LGG phenotypes were surveyed and characterized as FS and FS. The last cross (K12a x K12b) showed the parents to be FS and FF for GOT, FS and FS for LGG, and SS and FS for TO, respectively. In the first cross, the segregation for GOT was SS:FS:FF=27:48:28, and for the other two enzymes, all of the F_1 individuals showed the respective parental patterns. In the second, two kinds of infection procedure to snails were performed, five miracidium-infection and one miracidium infection per snail. Firstly, in the former infection, 19 of SS and 42 of FS, and 20 FS and 41 FF, 16 FS and 45 FF, appeared in the progeny for GOT, LGG, and TO, respectively. In the second infection experiment only LGG segregation was observed, of which ratio was FS:FF=20:9. In the third cross, similarly only LGG was surveyed. Five of SS, 15 of FS and 14 FF appeared in the progeny. In the last cross, a total of 73 F_1 individuals were examined for each enzyme. The segregation ratios were as follows: FS:FF=45:28 for GOT, SS:FS:FF=9:46:18 for LGG, and SS:FS=35:38 for TO.

Except for several crossings, almost all the crosses, on the whole, showed that the observed number of the variants in the F_1 progeny were in good agreement with the expected values based on the Mendelian inheritance fashion. The deviation from the expected values was considered as results of accumulated sampling errors due to passages of three different hosts.

Key words: lung fluke, *Paragonimus ohirai*, isozyme, cross experiment, genetics

Introduction

Electrophoretic variants of enzymes (isozymes) have been reported for a variety of parasitic organisms (Taylor and Muller, 1979; Chance and Walton, 1982; Walliker, 1983). Although isozymes are known to be useful as genetic markers, it is worthwhile confirming whether the isozymes are inheritable or not, especially when new species and isozymes are involved in the studies. Because isozyme variants sometimes are non-genetic due to post-translational modification or random aggregation of the molecules (Markert, 1975).

We have found isozyme polymorphism in natural populations of Japanese lung flukes, *Paragonimus ohirai* (Agatsuma and Habe, 1986a), and have confirmed by crossing experiments that some of the isozyme variants are under genetic control (Agatsuma and Habe, 1985a, b, 1986b, c). In this study, we will report the results of another crossing experiments between variants of three enzymes, glutamic-oxaloacetic transaminase (GOT), leucylglycylglycine (LGG) and tetraoxidase (TO) in *P.*

[1]*Department of Parasitology, Kochi Medical School, Nankoku City, Kochi 781-51, Japan.*
[2]*Department of Parasitology, School of Medicine, Fukuoka University, Fukuoka 814-01, Japan.*
吾妻　健　（高知医科大学寄生虫学教室）
波部重久　（福岡大学医学部寄生虫学教室）

ohirai.

Materials and Methods

Four kinds of crossing experiments were carried out by pair matings of individuals sampled from two localities, Kinosaki (Hyogo Prefecture) and Nobeoka (Miyazaki Pref.), in Japan; cross 1: N5a x N5b, cross 2: K6a x K6b, cross 3: K11a x K11b, cross 4: K12a x K12b (The symbols show individual metacercariae sampled from Nobeoka (N) and from Kinosaki (K), where the a and b indicate parental metacercariae in each cross pair).

Two metacercariae were introduced intraperitoneally into each albino rat. Fifty three days after inoculation, adult worms were recovered from the lung cysts of the rats, eggs were collected, and adults' isozyme patterns of three enzymes, GOT, LGG and TO, were determined by starch gel electrophoresis. The eggs from each worm were incubated separately at 28°C for 28 days. Miracidia hatched from the eggs were exposed to uninfected brackish water snails. *Angustassiminea parasitologica.* In the present study, the snails were exposed to one miracidium (the cross experiment 2) or five (all the cross experiments) for about 5 hr in small test tubes, separately. Cercariae were observed in snail hosts 110 days after infection, and introduced into the uninfected brackish crabs, *Sesarma dehaani.* Metacercariae were recovered from the crab host 90 days later. Metacercariae derived from each crab were inoculated separately into rats. The adult worms were obtained from the rat 53 days after inoculation and examined, electrophoretically. Preparation of extracts for electrophoresis, electrode buffers and enzyme staining methods have been described previously (Agatsuma and Habe, 1986a, b).

Results

I. *Cross experiment 1 (N5a x N5b)*

The first cross was carried out between N5a and N5b derived from Nobeoka. By starch gel electrophoresis, parents obtained were characterized by FS and FS for GOT, FF and FF for LGG, and SS and SS for TO, where FF, SS and FS means a fast-migrating band, a slow migrating band, and double or triple banded patterns, respectively. The FS pattern of GOT showed triplet band, and the patterns of FF and SS for the three enzymes showed single band. Table 1 gives the results of segregation ratios of isozyme phenotypes in the progeny produced by one parent, N5a. A total of 103 progeny derived from 16 snails, each of which was infected with 5 miracidia, were surveyed for the two enzyme patterns of the GOT and TO, while 55 individuals from 6 snails were surveyed for the LGG pattern. In the GOT segregation, some of the snail groups showed only one phenotype, while others showed two or three phenotypes as shown in Table 1. The segregation number of the GOT phenotypes in the F_1 progeny was SS:FS:FF=27:48:28, which is not significantly different from the expected ratio of Mendelian inheritance. In the LGG and TO isozyme patterns, both of all the F_1 individuals showed parental patterns for each enzyme.

II. *Cross experiment 2 (K6a x K6b)*

1) Five miracidia-infection per snail

Sixty-one individuals were surveyed in the progeny of the second cross, K6a x K6b, of which phenotypes were characterized as FS and SS for GOT, FS and FF for LGG, and FF and FS for TO. The phenotype number in each snail group was shown in Table 2. Similarly, some snail groups showed only one phenotype, while others showed two phenotypes. In the GOT phenotypes, 19 of SS and 42 of FS appeared in the progeny produced by one parent, K6a, which was characterized as FS. Twenty FS and 41 FF for the LGG segregation were obtained from the progeny of the same parent (K6a) characterized as FS. On the other hand, in the TO segregation, FS and FF appeared in the ratio of 16:45 in the progeny of the parent K16a

Table 1 Segregation of the isozyme phenotypes of three enzymes (GOT, LGG, TO) in the progeny produced by one parent (N5a) in a cross N5a x N5b

N*	n†	GOT (FS‡×FS)			LGG (FF‡×FF)			TO (SS‡×SS)		
		S S	F S	F F	S S	F S	F F	S S	F S	F F
1	9	0	9	0	0	0	9	9	0	0
2	13	4	0	9	0	0	13	13	0	0
3	8	5	3	0	0	0	8	8	0	0
4	8	5	3	0	0	0	8	8	0	0
5	11	0	4	7	0	0	8	11	0	0
6	9	0	9	0	0	0	9	9	0	0
7	5	0	3	2	−§	−	−	5	0	0
8	5	3	2	0	−	−	−	5	0	0
9	5	1	3	1	−	−	−	5	0	0
10	4	2	2	0	−	−	−	4	0	0
11	4	0	3	1	−	−	−	4	0	0
12	3	3	0	0	−	−	−	3	0	0
13	5	1	2	2	−	−	−	5	0	0
14	5	1	0	4	−	−	−	5	0	0
15	5	0	3	2	−	−	−	5	0	0
16	4	2	2	0	−	−	−	4	0	0
Total	103	27	48	28	0	0	55	103	0	0

*: The number of snails, each of which was infected with 5 miracidia.
†: The number of examined adult offspring from each snail.
‡: The parent (N5a) of offspring examined in the present study.
§: Not done.

Table 2 Segregation of the isozyme phenotypes of three enzymes (GOT, LGG, TO) in the progeny produced by one parent (K6a) in a cross K6a x K6b

N	n	GOT (FS*×SS)			LGG (FS*×FF)			TO (FF*×FS)		
		S S	F S	F F	S S	F S	F F	S S	F S	F F
1	5	0	5	0	0	0	5	0	0	5
2	4	3	1	0	0	0	4	0	4	0
3	5	0	5	0	0	3	2	0	1	4
4	5	0	5	0	0	1	4	0	0	5
5	5	0	5	0	0	3	2	0	0	5
6	5	3	2	0	0	2	3	0	1	4
7	5	5	0	0	0	0	5	0	0	5
8	5	3	2	0	0	2	3	0	3	2
9	5	1	4	0	0	0	5	0	3	2
10	4	1	3	0	0	3	1	0	0	4
11	5	1	4	0	0	2	3	0	0	5
12	5	0	5	0	0	3	2	0	2	3
13	3	2	1	0	0	1	2	0	2	1
Total	61	19	42	0	0	20	41	0	16	45

*: The parent of offspring. (K6a)
For abbreviation see Table 1.

showing FF phenotype. All the ratios obtained here deviated from the expected ones, but any unexpected phenotype was not found.

2) One miracidium-infection per snail

Only the LGG phenotypes were examined in this experiment (Table 3). Twenty of FS and 9 FF appeared in the progeny of the parent, K6a (FS), and the ratio was in agreement with the expected one from the Mendelian inheritance (Table 6).

Table 3 Segregation of the isozyme phenotypes of LGG in the progeny produced by one parent (K6a) in a cross K6a x K6b

N	n	LGG (FS* × FF)		
		S S	F S	F F
1	5	0	5	0
2	4	0	0	4
3	5	0	0	5
4	5	0	5	0
5	5	0	5	0
6	5	0	5	0
Total	29	0	20	9

*: The parent of offspring (K6a)
For abbreviation see Table 1.

III. *Cross experiment 3 (K11a × K11b)*

The third cross was performed between K11a and K11b. In this cross, only the LGG phenotypes were surveyed and were characterized.

Table 4 Segregation of the isozyme phenotypes of LGG in the progeny produced by one parent (K11a) in a cross K11a x K11b

N	n	LGG (FS* × FS)		
		S S	F S	F F
1	5	0	2	3
2	5	0	3	2
3	5	4	1	0
4	5	0	5	0
5	4	1	3	0
6	6	0	0	6
7	4	0	1	3
Total	34	5	15	14

*: The parent of offspring (K11a).
For abbreviation see Table 1.

Table 5 Segregation of the isozyme phenotypes of three enzymes (GOT, LGG, TO) in the progeny produced by one parent (K12a) in a cross K12a x K12b

N	n	GOT (FS* × FF)			LGG (FS* × FS)			TO (SS* × FS)		
		S S	F S	F F	S S	F S	F F	S S	F S	F F
1	5	0	2	3	2	2	1	0	5	0
2	5	0	5	0	0	0	5	0	5	0
3	5	0	0	5	0	5	0	0	5	0
4	5	0	0	5	0	0	5	0	5	0
5	5	0	4	1	1	3	1	3	2	0
6	5	0	5	0	0	5	0	0	5	0
7	5	0	0	5	0	3	2	4	1	0
8	5	0	5	0	0	5	0	4	1	0
9	5	0	5	0	0	5	0	0	5	0
10	5	0	4	1	0	3	2	5	0	0
11	5	0	5	0	0	5	0	5	0	0
12	5	0	4	1	1	2	2	3	2	0
13	5	0	0	5	5	0	0	5	0	0
14	3	0	1	2	0	3	0	2	1	0
15	5	0	5	0	0	5	0	4	1	0
Total	73	0	45	28	9	46	18	35	38	0

*: The parent of offspring (K12a).
For abbreviation see Table 1.

Table 6 Summary of isozyme segregation of three enzyme (GOT, LGG and PGM) in the progeny produced by four crossing experiments in *Paragonimus ohirai*

Cross	Parent	offspring			N	X^2	P (df = 1)
1) GOT		S S	F S	F F			
a) FS×FS	FS N5a	27 (27.75)*	48 (51.50)	28 (27.75)	103	0.261	0.7 > P > 0.5
b) FS×SS	FS K6a	19 (30.50)	42 (30.50)	0 (0.00)	61	8.672	0.01 > P > 0.001
c) FS×FF	FS K12a	0 (0.00)	45 (36.50)	28 (36.50)	73	3.959	0.05 > P > 0.02
2) LGG							
a) FF×FF	FF N5a	0 (0.00)	0 (0.00)	55 (55.00)	55	—	—
b) FS×FF	FS K6a	0 (0.00)	20 (30.50)	41 (30.50)	61	7.230	0.01 > P > 0.001
c) FS×FS	FS K12a	9 (18.25)	46 (36.50)	18 (18.25)	73	7.421	0.01 > P > 0.001
d) FS×FS	FS K11a	5 (8.50)	15 (17.00)	14 (8.50)	34	5.245	0.05 > P > 0.02
e) FS×FF†	FS K6a	0 (0.00)	20 (14.50)	9 (14.50)	29	4.172	0.05 > P > 0.02
3) TO							
a) SS×SS	SS N5a	103 (103.00)	0 (0.00)	0 (0.00)	103	—	—
b) FF×FS	FF K6a	0 (0.00)	16 (30.50)	45 (30.50)	61	13.787	P < 0.001
c) SS×FS	SS K12a	35 (36.50)	38 (36.50)	0 (0.00)	73	0.123	0.8 > P > 0.7

*: Figures in brackets give the number expected under Mendelian inheritance.
†: Only this cross was performed using one miracidium.

terized as FS and FS. Table 4 showed the result of the segregation ratio; 5 of SS, 15 of FS and 14 FF appeared in the progeny of parent (K11a). The ratio was in good agreement with the Mendelian law in the significant level of 0.01 (Table 6).

IV. *Cross experiment 4 (K12a × K12b)*

In the fourth cross, parents were characterized as FS and FF for the GOT phenotypes, FS and FS for LGG, and SS and FS for TO, respectively. A total of 73 F_1 individuals were surveyed in the progeny derived from one parent (K12a). The segregation number in the offsprings was shown in Table 5. From the parent with FS for the GOT, 45 FS and 28 of FF were produced. Three kinds of phenotypes, SS, FS and FF, were detected in the ratio of 9:46:18 for the LGG segregation. In the TO phenotype, 35 of SS and 38 FS appeared in the F_1 progeny. All the ratios obtained were in accord with the Mendelian inheritance (Table 6).

Discussion

Genetic studies of the parasitic organisms have mainly been carried out on Protozoa so far. Using isozyme variants, Walliker (1983) made crossing experiments between strains of malaria parasites and Coccidia to investigate the linkage of isozyme gene with drug resistant genes, and proved that there are several linkage group between certain isozymes and the drug resistant genes. On the other hand, in helminths, only a few cross experiments between closely related species have been reported, showing interspecific hybridization (Wright and Southgate, 1976; Agatsuma and Habe, 1985c). Recently, Agatsuma and Habe (1985a) performed intraspecific crossing experiments using GOT isozymes as markers in *Paragonimus ohirai* and found the variation to be under genetic control. Afterwards, they attempted similar experiments and found that LGG and TO isozyme variants are also controlled by the simple Mendelian inheritance (Agatsuma and Habe, 1985b, 1986b, c).

In the present study, further several cross combinations were made among the isozyme variants of three enzymes of *P. ohirai*, and the inheritance of the varients was analyzed in the F_1 progeny. As a result, the whole segregation ratios showed a good agreement

with the expected one from the Mendelian's law. But, several cross combinations gave significant deviations; FS x FS for GOT, FS x FF and FS x FS for LGG, and FS x FF for TO. These deviations seem to be caused by five miracidium-infection procedure which we adopted here, in addition to accumulated sampling errors resulting from the three host-passages for completion of the life cycle of this parasite. Namely, in all the experiments except one experiment, miracidium infection to snail host was made with five miracidia per snail. One exception was with only one miracidium. Ideally, one miracidium infection should be made to give exact segregation ratios in the progeny of the next generation. However, this procedure in general provides very low infection rate to snails. Since we just had very limited number of uninfected snails available, we adopted 5 miracidia infection system in the present study, and final number of F_1 progeny examined was approximately 5 per snail. Consequently, it is inevitable that the deviation between the isozyme gene frequencies of the 5 miracidia used for infection and the 5 or so F_1 progeny examined for their enzyme patterns arise for each snail infection, because not all the 5 miracidia complete the infection to the snail host, and also only 5 individuals were taken up for the examination from among more than one hundred progeny produced. On the other hand, in the one miracidium infection experiment, all the F_1 individuals which emerged from each snail gave the same phenotype of enzymes, that is, there is no difference in isozyme gene frequencies between the miracidia population used for infection and the F_1 adult population examined in the experiment. In fact, one miracidium infection experiment gave no significant deviation from the expected one in the present study (Table 3 and 6) as well as in our previous studies (Agatsuma and Habe, 1985a, b, 1986b). From the above, however, it can be said that the present results do not contradict the Mendelian inheritance, essentially, and this lung fluke is perhaps an outbreeder.

Acknowledgments

We wish to thank Professor Noriji Suzuki and Associate Professor Yoshihisa Hashiguchi, Kochi Medical School, for their invaluable comments and reviews of this manuscript. We also thank Mrs. S. Yamamoto for her technical assistance and Mrs. K. Imamura for her typing of the manuscript.

References

1) Agatsuma, T. and Habe, S. (1985a): The inheritance of enzyme variants of glutamic-oxaloacetic transaminase in *Paragonimus ohirai*. Parasitology, 91, 483–488.
2) Agatsuma, T. and Habe, S. (1985b): *Paragonimus ohirai*: Genetic control of tetrazolium oxidase isozymes. Exp. Parasit., 60, 309-313.
3) Agatsuma, T. and Habe, S. (1985c): Interspecific hybridization in three species, *Paragonimus ohirai, P. iloktsuenensis* and *P. sadoensis*, with special reference to isozyme patterns in F_1 hybrids. Jpn. J. Parasitol., 34, 389–394.
4) Agatsuma, T. and Habe, S. (1986a): Genetic variability and differentiation of natural populations in three lung flukes, *Paragonimus ohirai, P. iloktsuenensis* and *P. sadoensis*. J. Parasit., 72, 417–433.
5) Agatsuma, T. and Habe, S. (1986b): The mode of inheritance of the isozymes of three enzymes (GOT, LGG, PGM) in *Paragonimus ohirai*. Jpn. J. Parasitol., 35. 127–134. (in Japanese).
6) Agatsuma, T. and Habe, S. (1986c): Genetic analysis of electrophoretic variants of leucylglycylglycine aminopeptidase in *Paragonimus ohirai*. Z. Parasitenkd, 72, 693–696.
7) Chance, M. L. and Walton, B. C. (1982): *Biochemical characterization of Leishmania*. Proceedings of a Workshop held at the Pan American Health Organization, Washington, D. C.: UNDP/WORLD BANK/WHO.
8) Markert, C. L. (1975): *Isozyme IV. Genetics and Evolution*. Academic Press, New York.
9) Taylor, A. E. R. and Muller, R. (1979): *Problems in Identification of Parasites and their vectors*. Blackwell Scientific Publications, Oxford.
10) Walliker, D. (1983): *The Contribution of Genetics to the study of Parasitic Protozoa*. England, Research Studies Press Ltd.
11) Wright, C. A. and Southgate, V. (1976): Hybridization of schistosomes and some of its implications. In *Symposium of the British Society for Parasitology*, vol. 14, (Ed. A. E. R. Taylor and R. Muller), pp. 55–86. Blackwell Scientific Publications, Oxford.