The inheritance of enzyme variants of glutamic-oxaloacetic transaminase in *Paragonimus ohirai*

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SUMMARY

Lung flukes, *Paragonimus ohirai*, from Kinosaki, Japan, characterized by 3 electrophoretic variants, FF, FS and SS, of glutamic-oxaloacetic transaminase (GOT: EC 2.6.1.1) were crossed in the laboratory. In the case of a cross between FS and SS, a parent, FS, produced 9 SS and 16 FS clones in the offspring, numbers not significantly different from that expected (1:1) from Mendelian inheritance. From the other cross FS × FF, 20 clones originating from the respective 20 miracidia were obtained from a parent FS. The phenotype segregation ratio was 6 FF and 14 FS, which is not significantly different from Mendelian inheritance expectation. Only 3 clones were obtained from the other parent FF, but these segregated in a ratio of 2 FS:1 FF. These breeding data indicate that the GOT isozymes of *P. ohirai* are controlled by 2 codominant alleles, Got^F and Got^S at a single locus whose products aggregate randomly, forming a dimer.

INTRODUCTION

There are many electrophoretic studies on enzymes in parasitic protozoa (see for example Taylor & Müller, 1979; Chance & Walton, 1982). Genetic cross experiments have been carried out using such electrophoretic variants (Walliker, 1983).

Among parasitic helminths, few studies have been reported as regards cross experiments for genetic analysis. The reasons seem to be that the intermediate or definitive host is difficult to rear, that a suitable animal host model is not available, or that no individual marker such as an enzyme variant has been found so far. Recently, however, electrophoretic studies have begun in parasitic helminths and the effectiveness of employing enzyme markers to analyse genetic variability has been well established (Vrijenhoek, 1978; Agatsuma, 1981*a*, *b*, *c*; Fletcher, LoVerde & Woodruff, 1981), although there has been no attempt to cross parasites differing by these enzyme markers.

The Japanese lung fluke, *Paragonimus ohirai*, which is a hermaphrodite, appears to offer new possibilities for genetic work, since it is relatively easy to maintain in the laboratory using two intermediate hosts, snails and crabs, and a definitive host, the rat, all of which can be reared with little difficulty. Moreover, it has recently been shown that this parasite species exhibits wide enzyme variation in natural populations (Agatsuma, 1981*c*; Agatsuma & Habe, unpublished observations).

In this paper we report the genetic analysis of electrophoretic variants (isozymes) of glutamic-oxaloacetic transaminase (GOT:EC 2.6.1.1) in *P. ohirai* and demonstrate that these variants are controlled by genes which are inherited in simple Mendelian fashion.
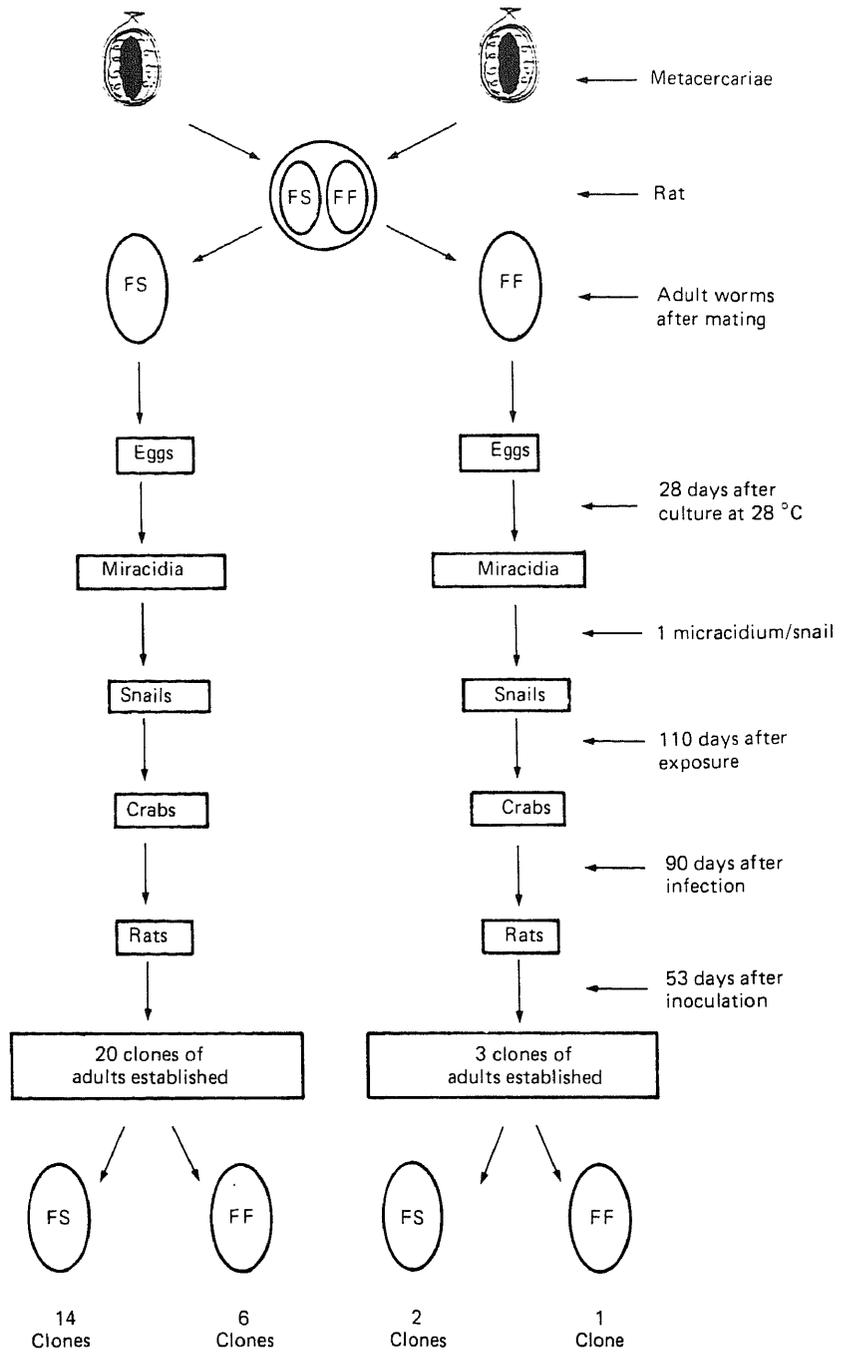
Fig. 1. Diagram of the cross procedure and the result of inheritance of glutamic-oxaloacetic transaminase (EC 2.6.1.1) isozymes, as an example, in a cross between FS and FF phenotypes of adult worms in *Paragonimus ohirai*.

MATERIALS AND METHODS

Parasites, experimental hosts and crossing procedure

The experimental procedure used is outlined in Fig. 1.

Metacercariae of *P. ohirai* were harvested from the crab, *Sesarma dehaani* collected in Kinosaki, Japan, since we had already found that the parasites from this area possessed various GOT variants. Two of the metacercariae were introduced *per os* into each albino rat (Wistar). At 53 days post-inoculation adult worms were recovered from the lung cyst of the rat and their GOT isozymes were examined by starch gel electrophoresis after they had laid eggs. If the two adult worms were found not to coexist in the cyst, or if only one was detected there, the parasites were not examined further. The eggs from each worm were incubated separately at 28 °C for 28 days. Then, miracidia which hatched from the eggs were exposed to brackish water snails. *Angustassiminea parasitologica*, which were collected from Fukuoka, Japan, where no infected crab has so far been found. In order to reconfirm this fact, more than 500 individuals of the snail population from the same locality were checked before the infection experiment; no infected snails were found. In this study, 1 snail was exposed to 1 miracidium. At 110 days post-infection cercariae were observed in their snail hosts. The cercariae were then introduced into the brackish crabs, *Sesarma dehaani*. These were also taken from Fukuoka, Japan, where no infected crab has yet been found. As with the snail, we were unable to detect any naturally infected crabs in this locality. After 90 days metacercariae were recovered from the crab host. Each group of metacercariae, derived from only 1 miracidium, was separately inoculated into a rat. The adult worms then were obtained from the rat 53 days after inoculation and were examined electrophoretically.

Preparation of extracts for electrophoresis

All samples of adults were washed with 0·8 % physiological saline, and then subsequently stocked in a deep freezer at −80 °C until required for electrophoresis. The extracts were prepared by homogenizing individually in 100 μl of 0·1 M phosphate buffer solution (pH 7·5) with a Teflon homogenizer in an ice water bath. The homogenized worms were centrifuged at 3000 r.p.m. for 3 min at room temperature, which does not affect the electrophoretic patterns of the GOT isozymes. The supernatant fractions obtained were used in this study. The amount of starch used was usually 12·0 g/100 ml of gel buffer.

Electrophoretic buffer and enzyme staining method

The electrophoretic buffer (pH 8·2) was 0·3 M boric acid and 0·06 M NaOH. The gel buffer (pH 8·7) was 0·076 M Tris and 0·005 M citric acid (pH 8·7). Electrophoresis was carried out at constant voltage of 250 V for 5 h. Staining for glutamic-oxaloacetic transaminase was done at 37 °C for 30 min with a reaction mixture containing 200 mg L-aspartic acid, 100 mg α-ketoglutaric acid and 100 mg Fast blue BB in 100 ml of 0·1 M phosphate buffer, pH 7·5 (Shaw & Prasad, 1970).
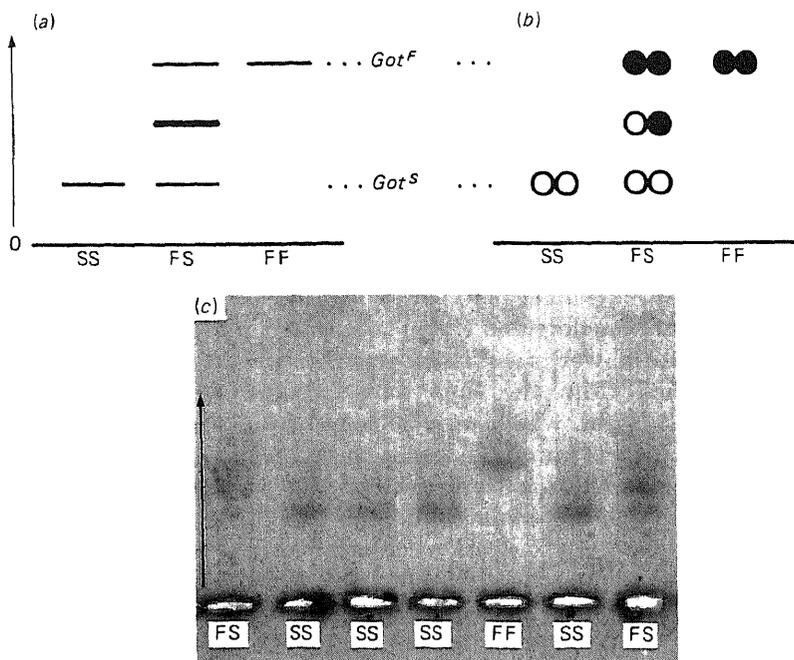
Fig. 2 (a) Schematic presentation of the glutamic-oxaloacetic transaminase isozyme pheno-types observed in a natural population of *Paragonimus ohirai* in Kinosaki, Japan. SS, FS and FF are electrophoretic variants, showing slow-migrating band, triplet pattern and fast-migrating band, respectively. O; origin. (b) Diagrammatic representation of molecular combination in the GOT isozymes of *P. ohirai*. (○), Subunit produced by the *Got^S* allele; (●), subunit produced by the *Got^F* allele. (c) Photograph of electrophoretic variant pattern of glutamic-oxaloacetic transaminase obtained by starch gel electrophoresis of *P. ohirai*.

Table 1. *Inheritance of GOT isozymes in* Paragonimus ohirai

		Offspring					
Cross	Parent	S	FS	F	Total	χ^2	P (D.F. = 1)
FS × S	FS	9 (12·5)*	16 (12·5)	0 (0·0)	25	1·96	0·2 > P > 0·1
FS × F	FS	0 (0·0)	14 (10·0)	6 (10·0)	20	3·20	0·1 > P > 0·05
	F	0	2	1	3	—	—

* Figures in parentheses give number expected from Mendelian inheritance.

RESULTS

Phenotypic patterns of GOT isozymes by starch gel electrophoresis

Three phenotypes were recognized by electrophoresis in the lung fluke, *P. ohirai*, derived from Kinosaki, Japan. These were a slow-migrating band, a fast-migrating band and a triplet pattern, which possessed the same bands as the slow and fast, together with an intermediate band, as shown in Fig. 2. We designate them SS, FF and FS, respectively.

Cross experiments

Experiment 1

The parentals in the first cross were characterized by FS and SS. Each snail was infected with only 1 miracidium and 2–5 individual adults derived from each miracidium were examined for their enzyme type. Only the clonal offspring produced by the parent, FS, were examined. The 25 clones of the adult originating from the respective 25 miracidia produced by this parent were surveyed electrophoretically. All the individuals from each clone showed only 1 of 2 phenotypes, FS or SS. The phenotype segregation ratios are shown in Table 1. Nine of SS and 16 of FS were obtained, numbers not significantly different from those expected under the assumption of Mendelian inheritance (at the 0·01 level).

Experiment 2

In the second cross, the parents were characterized by FS × FF and 20 and 3 clones in total were obtained from the parents, FS and FF, respectively. In the 20 clones produced by the parent FS, 14 of FS and 6 FF were observed, and the ratio was not different from that expected (1:1) at the significance level of 0·01. In the offspring of the other parent, FF, only 3 clones were obtained because of the extremely low level of hatchability and infectivity of miracidium, but the phenotypes separated into 2 FS and 1 FF (Table 1).

DISCUSSION

In this study, an attempt was made to carry out a genetic cross in a parasitic helminth, *P. ohirai*. The genetics of the parasite has been paid little attention so far, mainly because unlike many free-living organisms mating experiments seem to be extremely difficult. In addition, the mode of reproduction is ambiguous and has been believed to be by inbreeding or parthenogenesis; however, in almost all cases this has still to be determined. While genetic studies have been made on the parasitic protozoan species using isoenzymes (Walliker, Carter & Sanderson, 1975; Knowles, Sanderson & Walliker, 1981; Padua, 1981), few studies have been reported on helminth isozyme genetics (Wright & Southgate, 1976; Wright & Ross, 1980).

In trematodes, a miracidium multiplies in the snail host, resulting in the production of large numbers of cercariae, all of which are considered to have the same genotype. This phenomenon is called paedogenesis, and it is possible that this affects final segregation ratios of the phenotypes in the offspring when the sample size for investigation is small. Therefore, each snail was infected with only 1 miracidium. In the process of the paedogenesis in the snail, there is a possibility that somatic segregation, such as mitotic cross-over, may occur. This will be influential in understanding the genetic system in the lung fluke. However, in both of the present experiments, only one phenotype appeared in each clone. This indicates that the somatic segregation had not occurred.

From the present investigation, it was concluded that the GOT isozymes were controlled by 2 codominant alleles, *Got^F* and *Got^S* at a single locus, and that the molecular configuration may be a dimer, as judged from the band pattern of a heterozygote. Thus, it is clear that clones showing FF or SS are homozygous (*Got^F/Got^F* or *Got^S/Got^S*) and those showing FS are heterozygous (*Got^F/Got^S*) at the GOT locus.

In bisexual parasites like the present fluke, there are another two possible ways of

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reproduction, cross-fertilization and self-fertilization, in addition to parthenogenesis. In the case of self-fertilization, if it is heterozygous at a given locus, a parent could produce offspring of 3 genotypes, as a result of random combinations of the gene from sperm and eggs derived from the same individuals. In fact, in a single infection of a metacercaria in a rat, the resulting adult worm sometimes produces eggs, which can develop into miracidia (Hashiguchi, 1973). This suggests that self-fertilization can occur, although there is no evidence that these miracidia can produce fertile adults. In the present study, in contrast, there was no evidence of reproduction by self-fertilization; that is, only 2 kinds of phenotypes were observed in the offspring of both of 2 experiments. Therefore, we conclude that the manner of reproduction in the lung fluke, *P. ohirai*, is cross-fertilization in the case of infection with 2 metacercariae, and the mode of inheritance of the GOT variants accords with simple Mendelian fashion.

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