

# Allozyme Variation of 6-Phosphogluconate Dehydrogenase in the Freshwater Snail Genus *Gyraulus* (Pulmonata: Planorbidae)

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## ABSTRACT

The electrophoretic banding patterns of 6-phosphogluconate dehydrogenase (PGD) in the two different chromosomal ploidy groups of *Gyraulus* were compared. The monomeric or dimeric banding patterns or allelic variations in a locus of PGD were observed in four diploid populations (Osan, Sohre, Kimpo and Kangwha) of *G. convexiusculus* occurring in Korea, whereas the isozyme banding patterns encoded by at least 3 different loci were shown in the tetraploid populations of *G. (Torquis)* groups collected from Michigan, the U.S.A. Of 3 different tetraploid groups, *G. (T.) circumstriatus* group showed 3 monomorphic isozyme banding patterns, and the other 2 populations showed some allelic variations. Such results provided good evidence to differentiate tetraploid subgenus *Torquis* group from the diploid *Gyraulus* populations.

**Keywords:** Allozyme electrophoresis, *Gyraulus*, 6-Phosphogluconate dehydrogenase, Polyploidy of chromosome

## INTRODUCTION

The genus *Gyraulus* is very small freshwater snail species belonging to the pulmonates in

family Planorbidae. Although various species have each been circumscribed and often limited distributions, the genus itself is worldwide in distribution, occurring abundantly and commonly in diverse aquatic habitats. Many of the species act as the molluscan intermediate hosts of trematode parasites (Malek, 1962).

Anatomical details on genus *Gyraulus* were published previously by Baker (1928, 1945). Later, the morphological and anatomical studies of *Gyraulus* group have been made by Meier-Brook (1983).

A number of groups of species (*i.e.*, subgenera) comprise the genus *Gyraulus*. One of these groups, the subgenus *Torquis*, contains some of the smaller members of the genus, and includes *G. (T.) parvus*, *G. (T.) circumstriatus* and *G. (T.) huronensis* in North America. An unusual characteristic of the subgenus *Torquis* is the occurrence of polyploid (tetraploid) species, which have exactly twice the number of chromosomes found in most other members of the family Planorbidae. Polyploidy is rare not only in mollusks, but also in animals in general. The morphological and anatomical studies of North American *G. (Torquis)* species have been reported (Burch and Jung, 1991; Jung and Burch, 1990). The chromosome number of *G. (T.) circumstriatus* has also been reported as tetraploid ( $n=36$ ,  $2n=72$ ) (Burch, 1960). Furthermore, the same chromosome numbers for the other two species of subgenus, *G. (T.) parvus* and *G. (T.) huronensis* in North America, have later been discovered (Burch and Jung, 1993).

The allozyme analyses to study genetic similarities within and between populations and

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species has been utilized with a number of molluscan taxa. Also, a comparative enzyme electrophoresis of African *Bulinus* species each with different chromosomal set have been studied by Wurzinger (1980). On the other hand, the chromosome number of *G. convexiusculus* in Korea has been reported as diploid ( $n=18$ ,  $2n=36$ ) (Kim and Song, 1983). The characteristics of morphology, anatomy, enzyme and mitochondrial DNA have also been studied on *G. convexiusculus* in Korea (Chung *et al.*, 1993, 1994, 1995).

This study was carried out to compare the allozyme polymorphisms of 6-phosphogluconate dehydrogenase in the *Gyraulus* groups with diploid and tetraploid chromosome numbers.

## MATERIALS AND METHODS

### 1. Snail specimens employed

*Gyraulus convexiusculus* was collected in the several localities of Korea, and three species of *Gyraulus* (*Torquis*); *G. (T.) circumstriatus*, *G. (T.) parvus*, and *G. (T.) huronensis* were collected in Michigan, USA (Table 1). The snail species were identified on the basis of taxonomic keys according to Burch *et al.* (1987), Burch (1989) and Kwon (1990). Prior to the electrophoretic analyses, the snails of each population were

placed in plastic trays and starved for 48 hrs to clear their digestive tracts. All of the snails also were checked for the shedding of trematode cercariae. Only parasite-free snails were employed in this study. The snails in each population were cleansed of dirt and debris, and then stored at  $-70^{\circ}\text{C}$  until use.

### 2. Sample preparation

Each snail was thawed, and then excess surface water was removed using filter paper. The snails were then individually placed in a separate depression on a Coors porcelain spot plate. The porcelain spot plate was then placed on crushed ice in a pan. Ten  $\mu\text{l}$  of grinding buffer was placed in each well. This grinding buffer consisted of Tris (1.2 g), EDTA (0.04 g), NADP (0.01 g), NAD (0.01 g), and  $\beta$ -mercaptoethanol (0.25 ml) in 100 ml of deionized water and the buffer was adjusted to pH 7.0 with HCl. Each snail was ground by a rough-ended glass rod. A small filter paper, 2x10 mm, was used to absorb the fluid from each well, and the wicks were placed at regular intervals on the cut edge of one of the halves of the gel plate. One of the wicks with sample fluid was saturated with a trace dye (0.1% naphthol blue black, Sigma Co.), to be able to track the leading edge of the electrophoretic run.

Table 1. Snail species of the genus *Gyraulus* collected from different localities in the U.S.A. and Korea.

Snail species	Local area collected	Date collected	Habitats
<i>G. convexiusculus</i>	Osan, Kyunggi, Korea	July 13, 1998	rice field
	Sohre, Inchon, Korea	Aug. 10, 1999	rice field
	Kimpo, Kyunggi, Korea	July 13, 2000	rice field
	Kangwha, Inchon, Korea	July 13, 2000	rice field
<i>G. (T.) circumstriatus</i>	Shore of Burt Lake, Maple Bay, Burt Township, Cheboygan County, Michigan, USA	July 20, 1988	wood pool
<i>G. (T.) parvus</i>	Hammond Bay, Bearinger Township, Presque Isle County, Michigan, USA	July 20, 1988	swamp
<i>G. (T.) huronensis</i>	Lake Huron, north of Hammond Bay, Bearinger Township, Presque Isle County, Michigan, USA	July 20, 1988	lake shore

3. Starch gel electrophoresis

A 13% starch gel was prepared using Electrostarch (lot #392, Sigma Co.). Morpholine citric acid buffer system, pH 6.0 (Clayton and Tretiak, 1972) was routinely used to detect 6-phosphogluconate dehydrogenase (PGD) activities. Samples were applied in the middle of the gel. Electrophoresis was terminated when the trace dye, naphthol blue black had migrated 6 cm from the origin. It took about 6 hrs at 25 mA/gel in morpholine citric acid buffer system. The assay solution composed of 0.1 M MgCl<sub>2</sub> (10 mg), 6-phosphogluconic acid (25 mg), NADP (10 mg), MTT (10 mg), PMS (5 mg) in 50 ml of 0.2 M

Tris-HCl buffer, pH 8.0 (Shaw and Prasad, 1970; Siciliano and Shaw, 1976; Wurzinger, 1980).

RESULTS

Electrophoretic data of 6-phosphogluconate dehydrogenase in the *Gyraulus* groups using morpholine citric acid buffer system (pH 6.0), showed genetically distinguishable zymodemes between geographically or chromosomally two different groups of *Gyraulus* (Figs. 1 and 2). Allozyme variations encoded by a single locus were observed in the snail specimens from four diploid populations in Korea, whereas the isozyme banding patterns with some allelic variations were

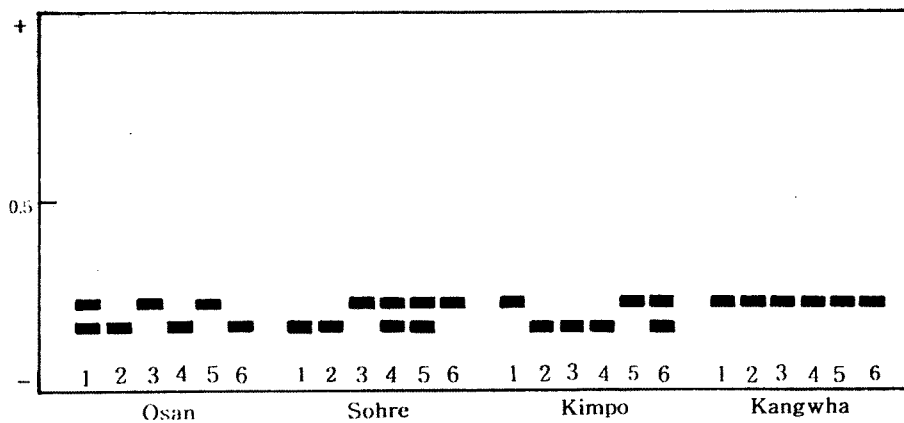


Fig. 1. Allozyme variations of 6-phosphogluconate dehydrogenase in four populations of *Gyraulus convexiusculus* from Korea.

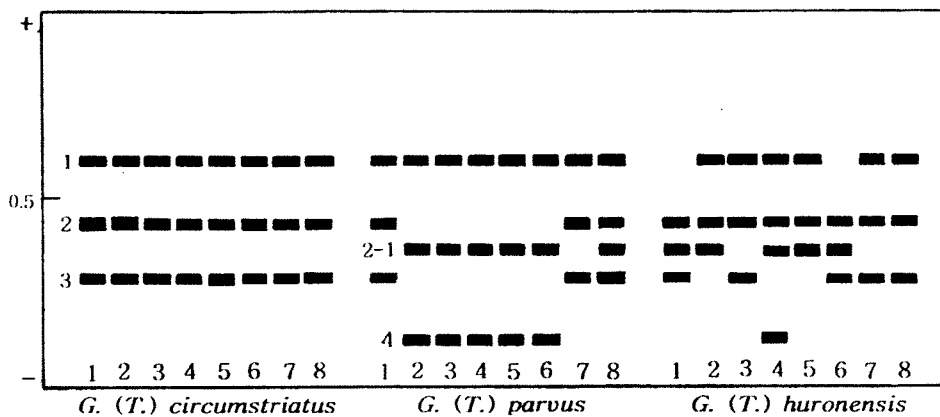


Fig. 2. Allozyme variations of 6-phosphogluconate dehydrogenase in three species of *Gyraulus (Torquis)* from Michigan, the U.S.A.

shown in those of the polyploid populations from Michigan, U.S.A.

Among the four local populations of *G. convexiusculus* in Korea, monomeric hybrid bands were shown in Osan, Sohre and Kimpo populations, whereas Kangwha population showed monomorphic homozygous banding patterns which were matched with fast bands observed in the other local populations.

On the other hand, three species of *G. (Torquis)* generally showed isozyme banding patterns with some complicated variations. *Gyraulus (T.) circumstriatus* group showed the 3 monomorphic isozyme banding patterns in which exactly three loci (locus-1, -2 and -3) were involved. However, *G. (T.) parvus* and *G. (T.) huronensis* groups showed at least 5 different loci involved (locus-1, -2, -2-1, -3 and -4). Occurrence of locus-2-1 between locus-1 and locus-2 in *G. (T.) circumstriatus* group, and of locus-4 was indicative in *G. (T.) parvus* and *G. (T.) huronensis* groups. Allelic variations among locus-2, locus-2-1, and locus-3 were different from those of *G. (T.) circumstriatus* group which showed no polymorphic allelic variation.

Allozyme banding patterns of *G. (Torquis)* groups with tetraploid chromosome sets were quite different from those of *G. convexiusculus* group which had diploid chromosome sets and showed only monomeric isozyme patterns.

## DISCUSSION

Allozyme variations of 6-phosphogluconate dehydrogenase in the diploid Korean populations of *G. convexiusculus* were generally monomeric, even though monomorphic bands without variation were detected in Kangwha population. In the tetraploid *G. (Torquis)* groups collected from North American areas, at least more than 3 loci were involved to express this enzyme. The present study provided the evidence of genetic differences between two different geographical and chromosomal groups of genus *Gyraulus*, which would be noteworthy for the differentiation of tetraploid, subgenus *Torquis* group from diploid *Gyraulus*.

However, allozyme studies in the diploid and

polyploid chromosomal groups of the same *Gyraulus* species should have been done in this study. Unfortunately, the detailed chromosomal karyology on the *Gyraulus* species in Korea has not been performed, since Kim and Song (1983) studied once diploid chromosome numbers of *G. convexiusculus*.

Similar allozyme studies with the polyploid African *Bulinus* species have been done by Wurzinger (1980). Even though the allozyme banding patterns of these polyploid snail species were expressed with complications in his study, 6-phosphogluconate dehydrogenase locus of all diploid and tetraploid *Bulinus (Isidora)*, as well as *B. (Physopsis)* populations showed monomorphic single bands. This conservative banding patterns of PGD in *Bulinus* snails were applicable in *Gyraulus* groups for their taxonomic classification.

In many hermaphroditic animals, self-fertilization or inbreeding within lines usually makes the low amount of heterozygosity, and outbreeding between lines occurs only rarely. McLeod *et al.* (1981) collected individuals of a sphaeriid clam *Musculium partumeium* from two locations in west-central Ohio, and ten enzyme systems were analyzed employing a horizontal starch gel electrophoresis. As a result, five loci were polymorphic in samples from the ephemeral pond, and genotypic frequencies for only one polymorphic locus (PGI-1) were in Hardy-Weinberg equilibrium. On the other hand, the collection from the permanent pond was completely monomorphic at every locus. In the hermaphroditic slugs, no detectable heterozygosity was observed in self-fertilizing species; however, some species were in strongly polymorphic at their structural gene loci. These polymorphic taxa were considered to be either predominantly or obligatorily outcrossing species (McCracken and Selander, 1980). The above reporters agreed that the homozygous, self-fertilizing species actually occupy a wider range of habitats and have more successfully invaded natural areas than have the heterozygous, outcrossing species.

The fact that the freshwater hermaphroditic snails can reproduce without mating has been known for a long time, especially since the studies of Colton (1912) and Colton and Pennypacker

(1934). The latter authors bred *Lymnaea (Pseudosuccinea) collumella* for 93 successive generations without the snails ever mating, and without the snail offspring exhibiting any loss in viability up to the time the studies were discontinued. Mulvey and Vrijenhoek (1981) found little evidence for self-fertilization in *Biomphalaria obstructa*.

However, Wu (1972) found cross-fertilization in *Bulinus* snails; *i.e.*, mating snails use the allosperms from the mating partner before resorting to self-fertilization. Pulmonate snails have been regarded to be amphimictic in spite of being hermaphroditic, resorting to automixis only when isolated. *Gyraulus* species have not been studied in regard to mode of reproduction, but it has been assumed that they normally reproduce by out-crossing. However, Richards (1962) reported the retardation of development of the male reproductive system in Florida *Gyraulus*. These electrophoretic results with the Michigan populations of *Torquis* indicate that they are not reproducing by cross-fertilization, at least not by *panmixis*, because most of allozyme patterns of all individual *Torquis* snails were homozygous except for 6-phosphogluconate dehydrogenase and a few loci. Moreover, *G. (T.) circumstriatus* group which showed completely monomorphic banding patterns at every locus even in PGD locus was collected an ephemeral wood pool habitat, differently from *G. (T.) parvus* and *G. (T.) huronensis* groups collected from the permanent ponds and a big lake, respectively.

It should be pursued further to determine the actual mode of reproduction in populations that would produce these electrophoretic results, and to compare the electrophoretic polymorphisms of *Gyraulus* groups with environmental stresses.

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