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Allozyme Analyses of *Bithynia manchourica*, *B. misella* and *B. kiusiuensis* (Gastropoda: Prosobranchia)

Jae Jin Kim and Sei Chang Kim*

*Department of Biology, Yonsei University and Department of Biology, Pai-Chai University**

=국문초록=

***Bithynia manchourica, B. misella* 및 *B. kiusiuensis*(복족강: 전새아강) 3종의 Allozyme 연구**

김 재 진 · 김 세 창*

연세대학교 생물학과, 배재대학교 생물학과*

한국과 일본에서 채집한 *Bithynia manchourica, B. misella* and *B. kiusiuensis* 등 3종의 Bithyniidae과 패류의 allozyme을 분석한 결과 *B. manchourica*가 다른 2종에 비해 유전적 거리가 멀었고(0.246) *B. misella*와 *B. kiusiuensis*에서는 유전적 거리가 0.217로 나타났다. 아울러 이들 3종의 GPI 주행양상은 채집지에 따른 변이가 심하지 않았고 각 종에 따른 특이한 allele을 가지고 있었다.

INTRODUCTION

There are two or three species of bithyniids, *Bithynia manchourica (Parafossarulus manchouricus), B. misella* and *B. (Gabbia) kiusiuensis* in Korea(Chung, 1984). *B. misella* and *B. kiusiuensis* has been confused as a distinct species of same species due to lack of detailed morphological description. Various species names were reported since the first description of *B. (Gabbia) kiusiuensis*(Shiba, 1934).

Electrophoresis of protein has been used extensively in taxonomic research on mollusks for past 20 or more years(Burch *et al.*, 1989). More recent studies have used electrophoresis for genetic analyses, and these study have the potential for being a powerful tool when appied to systematics, although this method has some limitation(Davis, 1978).

The present allozyme analysis was undertaken to provide a systematic data with Korean and Japanese population of bithyniids.

MATERIALS AND METHODS

Snails were collected from various location

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in their natural habitats in Korea and Japan (Table 1). All of the snails were placed in the glass bottles and starved for 4 hours to clear their alimentary tracts and trematode infected snail were discarded. Soft part of each snail was isolated from its shell and frozen at −60°C. Individual animals were thawed and minced with a knife chamber and then grinded with a glass homogenizer after addition of 500 ml of distilled water. Each animal was sonicated for a few seconds with a sonicator (Lab-Line). Above procedures were performed in ice chamber. The samples were centrifuged at 7,000 × g for 30 min. at −4°C. Supernatant was placed in 1 ml of cryotube and stored at −60°C until used for electrophoresis. Storage of these tissue extracts did not exceed a month. Total protein concentra-

Table 1. Polpulations of *Bithynia manchourica, B. misella* and *B. kiusiuensis* for the allozyme study

Species Population	Locality* collected	Date of collection	Collector
Bithynia manchourica			
1) Daejeo (대저동)	Pusan, Korea 35.06N, 129.03E	July 26, '88	Kim, JJ.
2) Kaejong (개정면)	Kunsan, Korea 35.58N, 126.41E	July 18'88	Kim, JJ.
3) Yangsoori (양수리)	Yangp' yong, Korea 37.30N, 127.29E	Sep. 27, '87	Kim, JJ.
4) Chunpo (춘포면)	Iri, Korea 35.56N, 126.57E	July 1, '80	Kim, JJ.
5) Daeya (대야면)	Imp'a, Korea 36.27N, 127.07E	July 1, '87	Kim, JJ.
6) Chori (조리면)	Munsan, Korea 37.51N, 126.48E	Aug. 3, '88	Kim, JJ.
Bithynia misella			
7) Woosong (우성면)	Konju, Korea 36.27N, 127.07E	July 19, '88	Kim, JJ.
8) Osan (오산면)	Imp'a, Korea 36.27N, 127.07E	July 18, '88	Kim, JJ.
9) Chori (조리면)	Munsan, Korea 37.51N, 126.48E	Aug. 3, '88	Kim, JJ.
Bithynia kiusiuensis			
10) Saga	Saga-Ken, Korea 38.22N, 140.52E	May 27, '87	Terasaki

Remarks;

*: The nearest city in Britannic Atlas from the collection site, and the longitude and latitude of the city are in parentheses.

tion of each sample were adjusted to about 3 mg/ml.

1. Starch Gel Electrophoresis

Twelve percent starch gel was prepared using potato starch(Sigma, S-4501) by the method of Divall(1984). The gel plates were kept overnight at 4°C. Fifty microliter of tissue extract was applied to Whatman No. 1 filter paper and inserted into a position previously marked 3 cm from cathodal end of the slab. After loading of samples electrophoresis was carried out for 5-6 hrs. at 150 volts in the cold room(−4°C). Following electrophoresis the gels were sliced in 2.0 mm thick. Each sliced gel was transferred separately into a staining dish. A gel slice was incubated with specific staining solution. Stained gels were rinsed and fixed with fixative(2 parts of water, 1 part of ethyl alcohol, 1 part of acetic acid, and 1 part of glycerin). The fixative was discarded and washed with tap water. The gels were kept in the moist chamber in the refrigerator after taking photograph of recording. The gel and electrode buffer system of Poulik(1957) was employed. The gel buffer contained 0.076 M Tris-(hydroxymethyl)-aminomethane and 0.05 M citric acid(pH 8.65).

The electrode buffer contained 0.3 M boric acid and 0.05 M sodium hydroxide (pH 8.0). Total twelve isoenzymes were employed.

2. Staining Solutions

Staining solutions of the enzyme were made by the methods of Shaw and Koen(1968), Wurzinger(1979) and Vallejos(1983) with minor modifications.

1) Malate dehydrogenase(MDH)

1.0 M Na-L-malate, pH 7.0(5 ml), β-Nicotinamide adenine dinucleotide(NAD 10 mg), 1[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide(MTT 20 mg), Phenazine methosulfate(PMS 5 mg) and 0.2 M Tris-HCl buffer pH 8.0 (50 ml) were mixed.

2) Malic enzyme(ME)

2.0 M malic acid, pH 7.0(5 ml), β-nicotinamide adenine dinucleotide phosphate(NADP 20 mg), MTT(20 mg), PMS(5 mg) and 0.2 M Tris-HCl buffer, pH 8.0(40 ml) were mixed.

3) Glutamic oxaloacetic transaminase(GOT)

α-Ketoglutarate(100 mg), L-Aspartic acid(150 mg), Pyridoxal-5-phosphate(5 mg), Fast blue BB salt(100 mg) and 0.2 M Tris HCl buffer, pH 8.0(50 ml) were mixed. Fast blue BB salt was added just before staining.

4) Acid phosphatase(ACP)

Na-α-naphthyl acid phosphate (1% 3 ml), Fast black K salt(100 mg), 1.0 M $MgCl_2 \cdot 6H_2O$ (1 ml) and 0.2 M Acetate buffer, pH 5.0(100 ml) were mixed. Na-α-naphthyl acid phosphate was solved in 50% acetone solution. The sliced gel was preincubated in 0.2 M acetate buffer(pH 5.0) for one hour.

5) Alkaline phosphate(ALP)

Na-β-naphthyl acid phosphate(50 mg), Fast blue RR salt(50 mg) 1.0 M $MgCl_2 \cdot 6H_2O$(2 ml) and 0.2 M tris-HCl buffer, pH 9.5(50 ml) were mixed.

6) Esterase(EST)

1% substrate(W/V; 1 ml) was mixed with 35 mg of Fast blue RR salt and 50 ml of 0.2 M Tris-HCl buffer(pH 7.4). Substrate was α-naphthyl butyrate or α-naphthyl acetate.

7) Alanine aminopeptidase(AAP)

Solution A: D,L-alanyl-β-naphthylamide HCl (20 mg), 0.1 M $MgCl_2$(1 ml), 0.1 M phosphate buffer(pH 7.0) to make 50 ml.

Solution B: Fast black K salt(30 mg), 0.1 M Phosphate buffer(pH 7.0) to make 50 ml.

The gel slice was incubated in solution A for 30min. then the solution was decanted. The gel was incubated again in solution B for

30min.

8) Leucine aminopeptidase(LAP)

Solution A: D,L-leucine-β-naphthylamide HCl (20 mg), 0.1 M $MgCl_2$ (1 ml), 0.1 M phosphate buffer(pH 7.0) to make 50 ml.

Solution B: Fast black K salt (30 mg), 0.1 M phosphate buffer(pH 7.0) to make 50 ml.

The gel slice was incubated in solution A for 30 min. and then placed in solution B for 30 min.

9) Glucosephosphate isomerase(GPI)

$NADP^+$(8 mg), Fructose-6-phosphate(6 mg), G-6-Phosphate dehydro-genase(50units), $MgCl_2$ (40 mg), MTT(5 mg) and 0.2 M Tris-HCl buffer, pH 8.0(10 ml) were mixed with 10 ml of 1.2% melted agar and then poured on the sliced gel.

3. Allozyme Analysis

When no allele had a frequency greater than of equal to 0.95, the locus was considered polymorphic. Calculations of an allele frequency and heterozygosity followed Ferguson(1980). Nei's genetic diversity(1972) was calculated by using computed program of Green(1979) which was corrected by Hillis and Cannatella(1983). Dissimilarity between two operational taxonomic unit(OTU) was calculated with unweighted pair-group method using arithmetic averages(UPGMA). The values of this method employed Nei's coefficient of genetic identity matrix .

RESULTS

Twelve loci from 9 enzymes were detectable in this study. Six out of 12 loci were polymorphic(GOT, EST-2, ALP, ACP and GPI) and the number of alleles per polymorphic locus varied from two to five. Average heterozygosity in *Bithynia manchourica* ranged from 0.09 to 0.17 and from 0.09 to 0.14 in *B. misella* and 0.10 in *Bithynia kiusiuensis*.

1. Malate dehydrogenase(MDH) and Malic enzyme(ME)

Monomorphic double bands of MDH were detected in all samples examined. MDH-1 showed strong and fast band and MDH-2 was slow and the activity was poor(Fig. 1). Single band of ME were found in all populations (Fig. 2).

2. Glutamic oxaloacetic transaminase (GOT)

Four alleles of GOT were detected. In all populations of *B. manchourica* except the Chori population, single band(GOT-b) was found. The Chori population showed 4 bands. Osan population of *B. misella* had a fast moving monomorphic band(GOT-b) as like of *B. manchourica*. But Chori and Woosong populations of *B. misella* had two bands(GOT-b and GOT-c). A single slow band(GOT-c) was detected in all individuals of *B. kiusiuensis* examined(Fig. 3)

3. Acid phosphatase(ACP)

Two bands of ACP were occored. A fast moving band was common in all three bothyniids. A slow moving band was observed in Daejeo, Chunpo and Chori populations of *B. manchourica*, and Woosong and Osan populations of *B. misella*(Fig. 7).

4. Alkaline phosphate(ALP)

Three electromorphs for ALP were detected. The fast moving band(ALP-a) was common in *B. manchourica* and *B. kiusiuensis*, but *B. misella* didn't show this band. The intermediate band(ALP-b) was common in all populations of three bithyniids. The slow movng

Table 2. Allozyme frequencies and average heterozygosities of *Bithynia manchourica, B. misella* and *B. kiusiuensis*

Locus	Allozyme (allele)	*B. manchourica*						*B. misella*		*B. kiusiuensis*	
		1	2	3	4	5	6	7	8	9	10
MDH-1	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	H*	0	0	0	0	0	0	0	0	0	0
MDH-2	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	H*	0	0	0	0	0	0	0	0	0	0
ME	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	H*	0	0	0	0	0	0	0	0	0	0
GOT	a	–	–	–	–	–	0.36	–	–	–	–
	b	1.00	1.00	1.00	1.00	1.00	0.54	0.13	1.00	0.95	–
	c	–	–	–	–	–	0.05	0.87	–	0.05	1.00
	d	–	–	–	–	–	0.05	–	–	–	–
	H	0	0	0	0	0	0.58	0.23	0	0.09	0
EST-1	a	ND	–	0.12	0.05	–	–	–	ND	–	–
	b		1.00	0.88	0.95	1.00	1.00	1.00		1.00	1.00
	H	–	0	0.21	0.09	0	0	0	–	0	0
EST-2	a	0.25	0.50	0.50	0.50	0.50	0.50	0.18	–	0.05	0.06
	b	0.75	0.50	0.50	0.50	0.50	0.50	0.18	0.34	0.05	0.32
	c	–	–	–	–	–	–	0.32	0.33	0.45	0.31
	d	–	–	–	–	–	–	0.32	0.33	0.45	0.31
	H	0.38	0.50	0.50	0.50	0.50	0.50	0.73	0.67	0.59	0.70
EST-3	a	ND	1.00	ND	ND	1.00	1.00	1.00	1.00	1.00	ND
	H	–	0	–	–	0	0	0	0	0	–
ALP	a	0.61	0.35	0.95	0.62	0.69	0.45	–	–	–	0.61
	b	0.17	0.65	0.05	0.38	0.31	0.55	1.00	0.83	0.75	0.39
	c	0.22	–	–	–	–	–	–	0.17	0.25	–
	H	0.55	0.46	0.09	0.47	0.43	0.49	0	0.28	0.38	0.48
ACP	a	0.56	1.00	1.00	0.25	1.00	0.45	0.88	0.93	1.00	1.00
	b	0.44	–	–	0.75	–	0.55	0.12	0.07	–	–
	H	0.49	0.46	0	0.38	0	0.49	0.21	0.13	0	0
AAP	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	H	0	0	0	0	0	0	0	0	0	0
LAP	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	H	0	0	0	0	0	0	0	0	0	0
GPI	a	–	0.05	–	–	–	–	0.06	–	–	–
	b	–	–	–	–	–	–	0.36	–	–	–
	c	–	0.05	–	0.19	0.13	–	0.58	1.00	1.00	–
	d	–	–	–	–	–	–	–	–	–	1.00
	e	1.00	0.90	1.00	0.81	0.87	1.00	–	–	–	–
	H	0	0.19	0	0.31	0.23	0	0.53	0	0	0
No. examined		9	10	13	13	13	11	12	8	10	16
Average heterozygosity		0.12	0.10	0.07	0.15	0.10	0.17	0.14	0.09	0.09	0.10

* Heterozygosity per locus

Populations: 1; Kimhae, 2; Kaejong, 3; Yangsoori, 4; Chunpo, 5; Daeya 6; Chori, 7; Woosong 8; Osan 9; Chori, 10; Saga

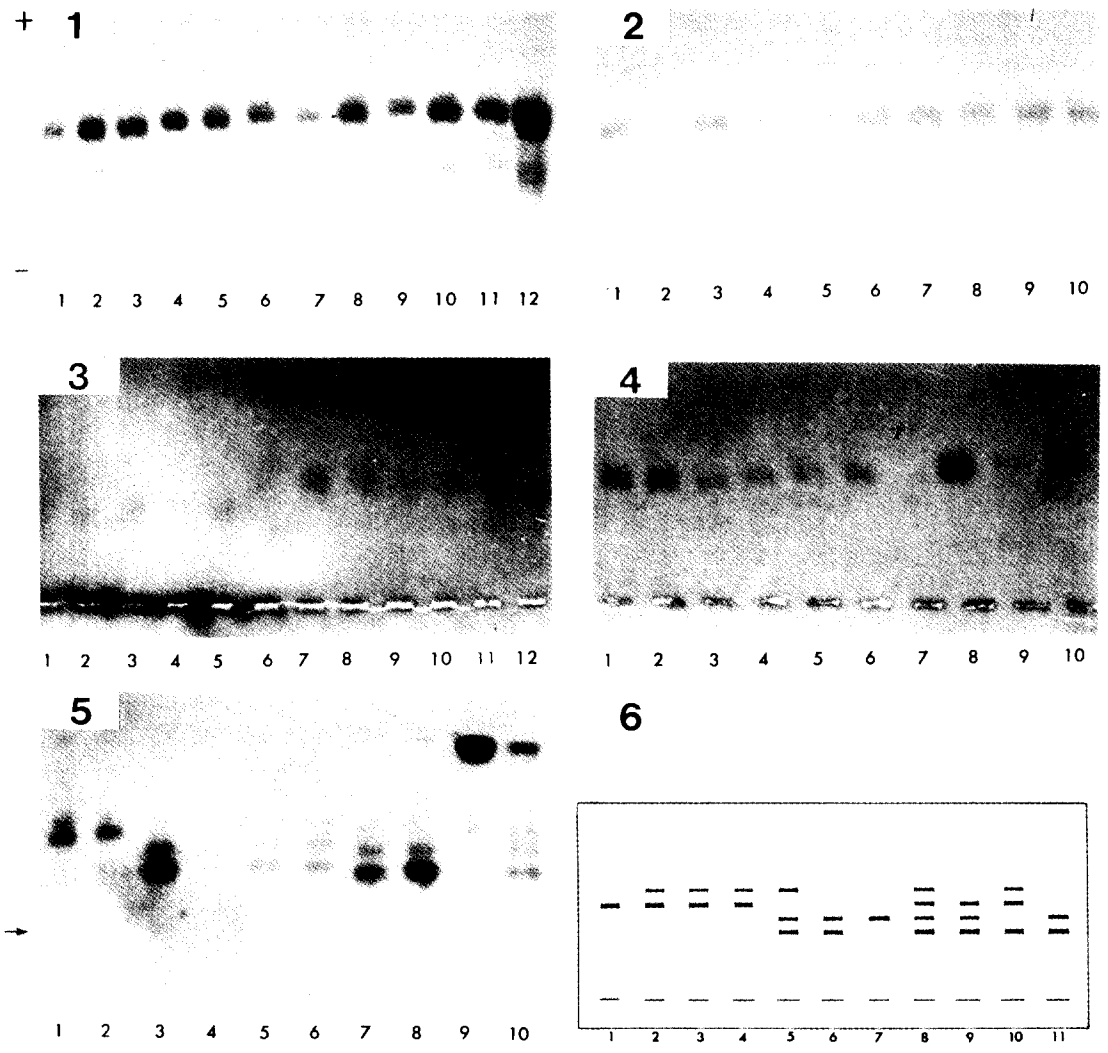

Fig. 1. Malate dehydrogenase patterns of *Bithynia manchourica* (1-4), B. *misella* (5-8) and *B. kiusiuensis* (9-12). All populations showed monomorphic electromorphs.

Fig. 2. Malic enzyme patterns of *B. manchourica* (1-4), *B. misella* (5-8) and *Bithynia kiusiuensis* (9-12). Only a monomeric band was detectable in the all three species.

Fig. 3. The enzymatic activities of glutarmate-oxaloacetate transaminase of *B. misella*, Osan population (6-12) and *B. kiusiuensis* (1-5).
Homozygotes showing the fast-moving band occurred in *B. misella* and the slow-moving band was found in *B. kiusiuensis*.

Fig. 4. Akaline phosphatase activities of female snails of *B. manchourica* (1-2), *B. misella* (2-7) and *B. kiusiuensis* (8-10).

Fig. 5. Non-specific esterase activities of *B. manchourica*, Daeya population (1-2), Woosong population (4-8) and *B. kiusiuensis* (9-10).
The slowest thin bands(arrow) were found in *B. manchourica*.

Fig. 6. Schematic representation of the non-specific esterase activities for the EST-2 locus in *B. manchourica* (1-4), *B. misella* (5-9) and *B. kiusiuensis* (10-11).
Slow-moving monomeric bands were not detectable in *B. manchourica*.

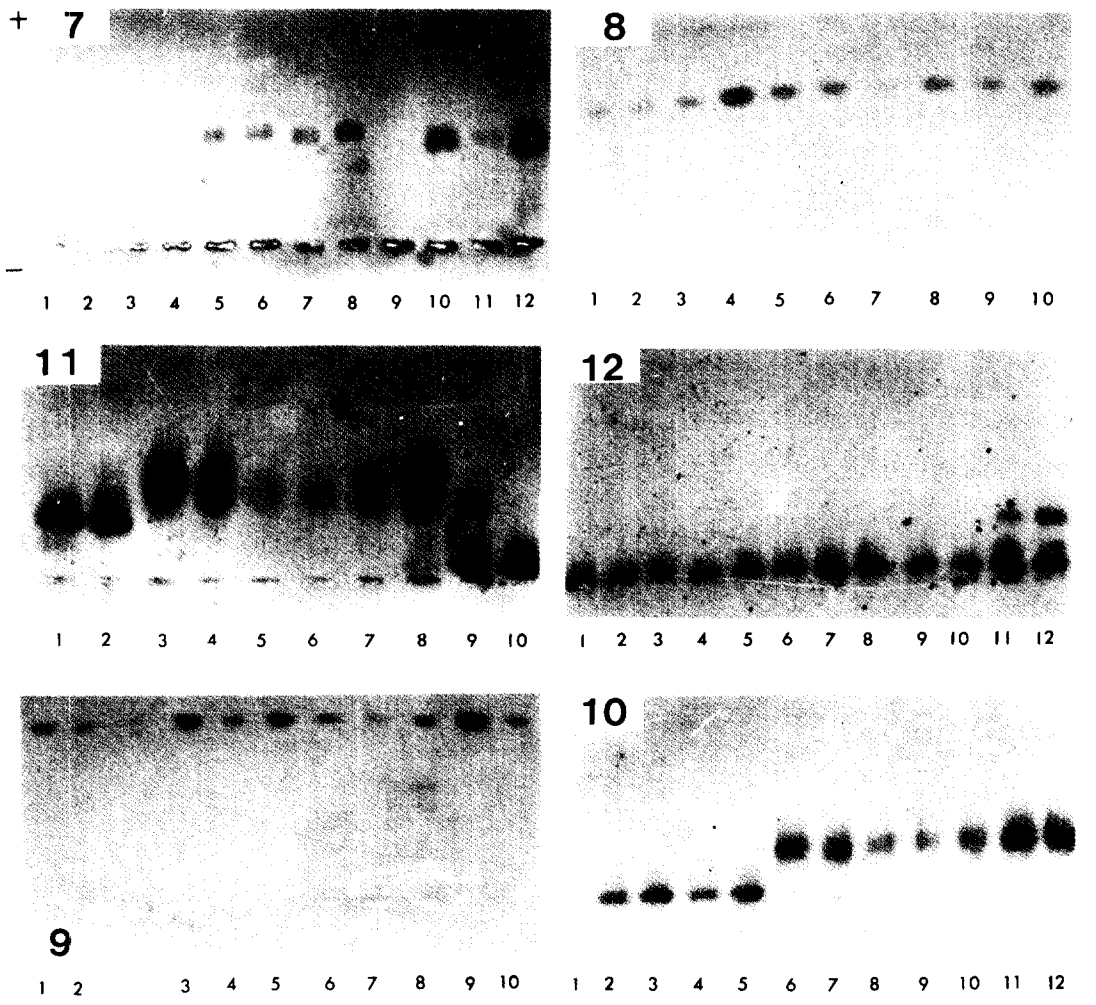

Fig. 7. Acid phosphatase patterns of *B. manchourica* (1-8), *B. misella* (9-10) and *B. kiusiuensis* (11-12). The fast-moving band was common in all three bithyniids. The slow-moving band occurred in Kimhae population of *B. manchourica* (2) and Osan population of *B. misella* (10).

Fig. 8. The enzymatic activities of alanine aminopeptidase of *B. manchourica* (1-4), *B. misella* (5-7) and *B. kiusiuensis* (8-10). All populations of three bithyniids showed the monomorphic band.

Fig. 9. Leucine aminopeptidase patterns of *B. manchourica* (6-10), *B. misella* (3-5) and *B. kiusiuensis* (1-2). Only a single monomorphic band was detectable in the all three species.

Fig. 10. Glucose phosphate isomerase patterns of *B. kiusiuensis* (1-5) and *B. misella,* Osan population (6-12).
Slow-moving bands(GPI-d, GPI-e) were not found in *B. misella.*

Fig. 11. Glucose phosphate isomerase patterns of *B. kiusiuensis* (1-2), *B. misella* (3-8) and *B. manchourica* (9-10).
The slowest band(GPI-e) was found only in *B. manchourica* (9, 10). Faster- and intermediate-bands (GPI-b, c) occurred in *B. misella* (3, 4, 8), and a monomeric slow-moving band(GPI-d) was found in *B. kiusiuensis* (1, 2).

Fig. 12. Glucose phosphate isomerase patterns of *B. manchourica,* Chori(1-4) and Daeya(5-12) populations. The slowest band(GPI-e) was common in all populations of *B. manchourica.*

band(ALP-c) was occed in Daejeo population of *B. manchourica*, and Woosong and Osan populations of *B. misella*(Fig. 4).

5. Esterase(EST)

Three regions of esterase activity were detected. There were two alleles in EST-1 locus. EST-1 was composed of two bands. A fast band(EST-1-a) was observed only in *B. manchourica*(Yangsoori and Chunpo populations). Locus lof EST-2 was composed of four alles. Monomorphic faster two bands(EST-2-a, EST-2-b) were detected in *B. manchourica*. Slower bands(EST-2-c, EST-2-d) were common in *B. misella* and *B. kiusiuensis*. Heterozygosity of *B. manchourica* in locus EST-2 was less than or equal to 0.50. and that of the other two species was more than 0.50(Table 3). EST-3 contained single band, but no band was detected in Daejeo, Yangsoori and Chunpo populations of *B. manchourica* and *B. kiusiuensis*(Fig. 5, 6).

6. Alanine aminopeptidase(AAP) and Leucine aminopeptidase(LAP)

A single monomorphic band of AAP or LAP was observed in all bithyniids employed (Fig. 8, 9).

7. Glucosephosphate isomerase(GPI)

Glucosephosphate isomerase was composed of 5 bands. This locus was species specific. The slowset band(GPI-e) was demonstrated only in all populations of *B. manchourica*, a slow band(GPI-d) was observed in *B. kiusiuensis* only, and intermediate band(GPI-c) was detected in *B. misella*(Fig. 10~12).

8. Genetic distance

Genetic distances, average homozygosities

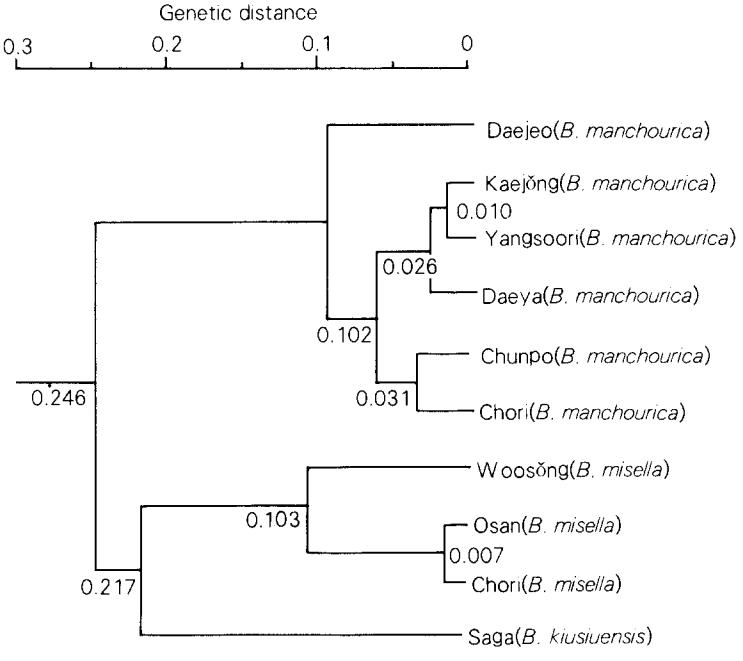

Fig. 13. Phenogram based on the genetic identities, shown in Table 3, of *B. manchourica*, *B. misella* and *B. kiusiuensis*.

Table 3. Genetic distances(above diagonal), the average homozygosities(on the diagonal) and the genetic identities(below the diagonal) of *Bithynia manchourica, B. misella* and *B. kiusiuensis*

OTU	1	2	3	4	5	6	7	8	9	10
1	(0.737)	0.105	0.078	0.080	0.090	0.103	0.395	0.278	0.315	0.384
2	0.901	(0.822)	0.039	0.073	0.012	0.053	0.198	0.129	0.148	0.251
3	0.925	0.962	(0.849)	0.075	0.010	0.078	0.316	0.226	0.240	0.266
4	0.923	0.932	0.928	(0.771)	0.061	0.031	0.276	0.192	0.223	0.321
5	0.914	0.988	0.990	0.940	(0.820)	0.059	0.236	0.152	0.168	0.241
6	0.902	0.949	0.925	0.969	0.943	(0.745)	0.224	0.211	0.238	0.266
7	0.674	0.820	0.729	0.759	0.790	0.799	(0.774)	0.105	0.101	0.128
8	0.757	0.879	0.798	0.826	0.859	0.810	0.900	(0.827)	0.007	0.266
9	0.730	0.862	0.787	0.800	0.845	0.788	0.904	0.993	(0.828)	0.257
10	0.681	0.778	0.766	0.726	0.786	0.766	0.880	0.767	0.773	(0.819)

OTU(Operational Taxonomic Unit) 1; Kimhae, 2; Kaejong, 3; Yangsoori, 4; Chunpo, 5; Daeya, 6; Chori 7; Woosong 8; Kaejong 9; Osan 10; Saga

and the genetcic identities were calculated (Table 2). The average homozygosities for the six populations of *B. manchourica* were ranged from 0.737(in Daejeo population) to 0.849 (in Yangsoori population). The average homozygosities for the the three populations of *B. misella* were ranged from 0.774(Woosong population) to 0.828(Chori population), and the homozygosity of the Japanese(Saga) populations of *B. kiusiuensis* was 0.819.

It can be seen in a phenogram(Fig. 13) based on the basis of the genetic distance which was shown in Table 3. The genetic distance value between six populations of *B. manchourica* and the other two species was 0.246. The genetic distance between conspecific populations was very low(less than 0.103 in *B. misella*, less than 0.062 in *B. manchourica*). The genetic distance value between of *B. misella* and *B. kiusiuensis* was 0.217.

DISCUSSION

Isozyme electrophoresis and restriction site analysis, are applicable to most studies of intraspecific variation. These methods remain the useful tools of choice, although DNA sequencing may be needed to resolve particuraly difficult problems(Hillis and Moritz, 1990). Electrophoresis has been widely used in molluscan systematics in recent(Wright and Ross, 1963, 1965, 1966; Davis, 1967; Davis and Lindsay, 1968; Wu and Burch, 1975; Wurzinger, 1979; Kitikoon, 1982; Chung and Burch, 1983, Chung, 1984; Viyanant *et al.*, 1985). Genotypic information form this method is useful especially in systematics between lower taxa.

Korean populations of *B. misella* and Japanese populations of *B. kiusiuensis* are still on the debate especially in systematics(Kim, 1989). The present study aimed to futher investigation of genetic distance between three bithyniids which were collected in Korea and Japan, using starch gel electrophoresis.

For GOT activity, Chung(1983) observed three presumptive alleles in bithyniids. In this study, there were four presumptive alleles in a single presumptive genetic locus. In present study, four bands occured in Chori

population of *B. manchourica*. Chori area is located in norther part of Seoul, Korea and other collecting sites are located southern part of Korea. These sites are recognized as endemic area of clonorchiasis, but Chori is not included in endemic area. It is obvious that there is no gene flow between the Chori and the other areas from the lack of allele encoding three bands which only found in Chori population.

There are a number of sex-limited proteins restricted to the gonads(Ferguson, 1980). Chung(1984) reported that he could not observe the some alleles of ALP locus in males of *B. manchourica* and no activity at all even in female of *B. tentaculata* and *B. misella*. The fast moving band of ALP was common in *B. manchourica* and *B. kiusiuensis*. There was no evidence that encoding this isozyme was related with sex in this study.

Another interesting isozyme in present study is GPI. All populations of three species showed monomorphic bands. The slowest band(GPI-e) occured only in all populations of *B. manchourica*, and a slow band(GPI-d) was observed in *B. kiusiuensis*. Intermediate band between above two species, was detected in *B. misella*. These results indicate that gene flow among these three species.

Genetic distance value between *B. manchourica* and the other species was 0.246. This value is above conspecific level of Avise's suggestion(1974). Kim(1989) observed that there was no diffence in shell shape between Korean populations of *B. misella* and Japanese population of *B. kiusiuensis*, and he suggested that these species are synonym or would be ranked in subspecies level.

Morphologically distinct species show very low levels of divergence even when many loci are screened(Murphy *et al.*, 1983), and allozyme divergence may have proceeded to the point where too few electromorphs are shared, and many of those that shared are convergent(Derr *et al.*, 1987). However, genetic variability could be high in environmental condition(Levins, 1968; Gooch and Schopt, 1973), tropic resources(Valentine, 1976; Ayala *et al.*, 1975; Mileman, 1987). In this point of view, electromorphs would be varied even in identical species. Chung(1984) reported that *B. misella* should be stand as a subgenus of *Parafossarulus* rather than *Bithynia*, or perhaps should stand alone as a genus. This study employed only Korean populations of *B. misella* and *Parafossarulus*, and compared with American and European *B. tentaculata*. Also present study was limited in collection. So, such a systematic change should await broader, more inclusive study of the family Bithyniidae, with widley collected samples from Africa, Southeast Asia and Australia.

REFERENCES

Avise, J.C. (1974) Systematic value of electrophoretic data. *Syst. Zool.*, **23**: 465-481

Ayala, F.J., Valentine, J.W., DeLaca, T.E. and Zumwalt, G.S. (1975) Genetic variability of the Antarctic brachiopod *Liothyrella notorcadensis* and its bearing on mass extinction hypothesis. *J. Paleontol.*, **49**: 1-9

Chung, P.R. (1984) A comparative study of three species of Bithyniidae (Mollusca: Prosobranchia): *Parafossarulus manchouricus, Gabbia misella* and *Bithynia tentaculata*. *Malacological Review*, **17**: 1-66

Davis, G.M. (1967) The systematic relationship of *Pomatiopsis lapidaria* and *Oncomelania hupensis formosana* (Prosobranchia: Hydrobiidae). *Malacologia*, **6**: 1-143

Davis, G.M. (1978) Experimental methods in molluscan systematics. *In:* Pulmonates. Vol. 2A. Systematics, evolution and ecology. (ed. by Fretter, V. and Peake, J.), pp. 99-169, *Academic*

Press, London

Derr, J.M., J.W. Bickham, Greenbaum, I.F., Rhodin, A.G.J. and Mittermeier, R.A. (1987) Biochemical systematics and evolution in the South American turtle genus *Platemys* (Pleurodira: Chelidae). *Copeia*, **1987**: 370-375

Divall, G.B. (1984) Starch gel electrophoresis of proteins. *In:* Methods in molecular biology. Vol. 1. Proteins. (ed. by Walker, J.M.), pp. 97-104, *Humana Press Inc., Clifton, N.J.*

Feguson, A. (1980) Biochemical systematics and evolution. 193 pp. *Blakie, Glasgow*

Gooch, J.L., Schopt, T.J.M. (1973) Genetic variability in the deep sea: Relation to environmental variability. *Evolution*, **26**: 545-552

Green, D.M. (1979) A BASIC computer program for calculating indices of genetic distance and similarity. *J. Heredity*, **70**: 429-430

Hillis, D.M. and Cannatella, D.C. (1983) Calculation of indices of genetic distance, genetic similarity, and average homozygosity: correction of Green's computer program. *J. Heredity*, **74**: 115

Hillis, D.M. and Moritz, C. (1990) An overview of applications of molecular systematics. *In:* Molecular systematics. (ed. by Hillis, D.M. and Moritz, C.), pp. 502-515, *Sinauer Associates Inc., Sunderland*

Kitikoon, V., 1982. Studies on Tricula aperta and related taxa. The snail intermediate hosts of *Schistosoma mekongi*. V. Electrophoretic studies. *Malacological Review*, **15**: 43-57

Levins, R. (1968) Evolution in changing environments. 120 pp., *Princeton Univ. Press Monogr. Pop. Biol.*

Mileman, S. (1987) Effect of culture conditions and bacterial associates on the zymodems of *Entamoeba histolytica*. *Parasitology Today*, **3**: 37-40

Murphy, R.W., Cooper, W.E. Jr. and Richardson, W. S. (1987) Phylogenetic relationships of the North American five-lined skinks, genus *Eumeces* (Sauria: Scincidae). *Herpetologica*, **39**: 200-211

Nei, M. (1972) Genetic distance between populations. *American Naturalist*, **106**(949): 283-292

Poulik, M.D. (1957) Starch gel electrophoresis in a discontinuous system of buffers. *Nature*, **180**: 1477-1479

Shaw, C.R. and Koen, A.L. (1968) Starch gel zone electrophoresis of enzymes. *In:* Chromatographic and electrophoretic techniques. Vol. 2, Zone electrophoresis(2nd ed.) (ed. by Smith, I.), pp. 325-364, *John Wiley & Sons Inc., N.Y.*

Shiba, N. (1934) Catalogue of the mollusca of Chosen(Corea). *J. Chosun Nat. Hist.*, **18**: 6-31

Valantine, J.W. (1976) Genetic strategies of adaptation. *In:* Molecular evolution (ed. by Ayala, F.J.), pp. 78-94, *Sinauer Associates Inc., Massachusetts*

Vallejos, C.E. (1983) Enzyme activity staining. *In:* Isozymes in plant genetics and breeding, Part A. (ed. by Tanksley, S.D. and Orton, T.J.), pp. 469-516. *Elsevier, Amsterdam*

Viyanant, V.S., Upatham, E.S. and Siriteramonkol, S. (1985) Enzyme analyses of *Bithynia* (Mollusca: Bithyniidae) by isoelectrofocusing. *Malacological Review*, **18**: 15-20

Wurzinger, K.H. (1979) Allozymes of Ethiopian *Bulinus sericinus* and Egyptian *Bulinus truncatus*. *Malacological Review*, **12**: 51-58