

## Ecological and Genetic Biodiversity of *Corbicula leana* in the Nakdong River and the Nam River

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**ABSTRACT:** The eleven local samples of six species including *Corbicula leana* examined in this study were collected from the Nam River and the Nakdong River in Korea. Buzas's new formula was used to evaluate the ecological biodiversity among eleven populations and six species. In addition, enzyme electrophoresis was used to genetic diversity within and among populations of *C. leana*. The upper populations of the river have fewer species than the middle and low populations of this river. The genetic diversity trends to increase from the source of the main river to the mouth. It suggests that the population of downstream might be expanded toward upstream.

**Key words:** *Corbicula leana*, Ecological biodiversity, Nakdong River

### INTRODUCTION

Processes that accurate population differentiation such as geographic isolation, natural selection, and genetic drift are often inferred from analyses of morphological and genetic variation across a species' geographic distribution (Stabile *et al.* 1996). Observed patterns of variation among allopathic populations led to the theory that adaptation to local environmental conditions is maintained by barriers to gene flow. The extent and direct of gene flow among distinct populations can act as a strong force either in maintaining or homogenizing genetic differences (Huh 1998).

Recent investigations of the spatial distribution of molecular markers such as allozyme or DNA within populations of animal or plant species have indicated that individuals are not likely to be randomly distributed owing to the effects of factors such as limited gene flow and microhabitat selection (Poudevigne and Baudry 2003).

Species can be divided into groups of individuals that share kinship, as indicated by genetic or physical similarity. In some causes a large part of biodiversity between individuals in a species is distributed in this way such as between local populations. Therefore, a genetic population can be usefully defined as a group of conspecific organisms that share greater kinship with each other than the members of other similar groups. The forces that can genetically differentiate populations of this type are genetic drift, natural selection, and DNA turnover mechanisms. Most often populations of this type are regional. That is, the isolating mechanism that has reduced the movement of breeding individuals is usually distance, or some

geographic barrier (Wright 1965, Hoelzel and Dover 1991).

In this paper, we investigated biodiversity as the biological response to an artificial environment. The pattern of biodiversity as well sees it today concerns the total biological adaptation to the ever-changing environment variation. These measurement may be useful this pattern of biodiversity, both to maintain it as such, and to allow the process to proceed in future. Only if these measurements contain information on at least the pattern of biodiversity reduction, and preferably on that of the generating process as well as, can we properly take counters measures that work.

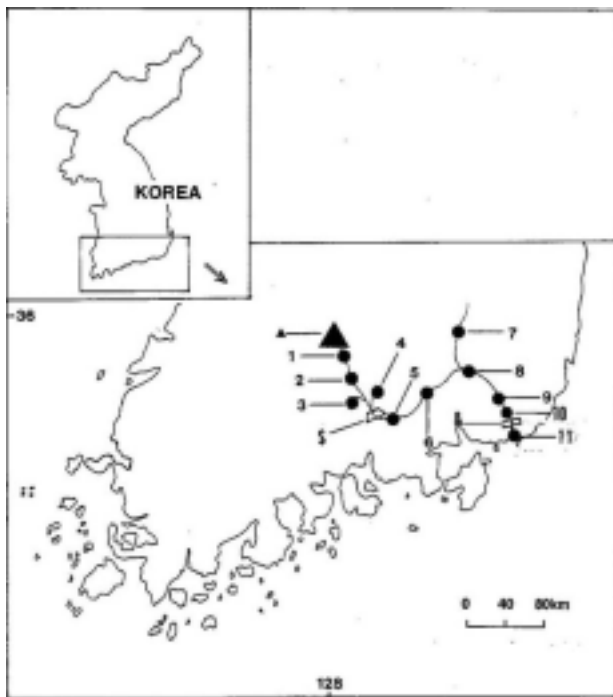
The purpose of this paper was to describe a statistical analysis for detecting a ecological biodiversity which is valid even though the assumption of two artificial constructions (the Namkang Dam and Nakdong Tide Embankment) is not violated spatial randomness of family Curbiculidae. In addition, two questions are addressed: 1) is there a spatial genetic structure within only one species, *Corbicula leana* which is distributed all populations? and 2) if so, what is the spatial pattern of variation and is it the same for all loci?

### MATERIALS AND METHODS

#### Ecological biodiversity

The eleven local samples of six species including *Corbicula leana* examined in this study were collected either by hand-picking or using a scoop from the Nakdong River in Korea (Fig. 1 and Table 1). The samples were collected in May 2002~September 2004 per a monthly interval. The six species are *Corbicula leana*, *C. felnouilliana*, *C. fluminea*, *Nuttallia oivacea*, *Anodonta woodina*,

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▲: Mt. Giri; □: The Namkang Dam; ○: The Nakdong Tide Embankment.  
 Fig. 1. Localities of six species in order *Palaeoheterodonta* as source for measures of biodiversity.

Table 1. Populations of species and the number of individuals (*N*) of order *Palaeoheterodonta* sampled

Codes	<i>N</i>	Collection sites
1	148	Naedae-ri, Sancheong-gun, Gyeongsangnam-do
2	120	Seongnae-ri, Sancheong-gun, Gyeongsangnam-do
3	136	Shinhung-ri, Sachen-ci, Gyeongsangnam-do
4	147	Danke-ri, Sancheong-gun, Gyeongsangnam-do
5	170	Deokkok-ri, Jinju-ci, Gyeongsangnam-do
6	176	Backsan-ri, Haman-gun, Gyeongsangnam-do
7	216	Samhak-ri, Habcheon-gun, Gyeongsangnam-do
8	248	Namgi-ri, Changreong-gun, Gyeongsangnam-do
9	259	Doyo-ri, Kimhae-ci, Gyeongsangnam-do
10	332	Mulkum-ri, Yangsan-ci, Gyeongsangnam-do
11	373	Eulsookdo, Saha-gu, Busan-ci

and *Unio douglasiae*. We used the biodiversity formula from Buzas and Hayek (1996). The species diversity of six species was determined by Shannon function.

$$H = -\sum p_i(\ln p_i)$$

The  $p_i$  is the frequency of species proportion (Shannon 1948, Margaref 1957). Buzas's new formulae were used to evaluate the

ecological biodiversity among 11 populations and six species (Buzas and Hayek 1996).

$$H = \ln S + \ln E$$

$S$  is the number of species in a population and  $E$  is species evenness.

#### Genetic biodiversity

Enzyme electrophoresis was used to genetic diversity within and among populations of *C. leana* because other species can not collected from all populations. Buffer enzyme staining procedures from Soltis *et al.* (1983) were used to analyze the following enzyme systems; Samples were homogenized by mechanical grinding to release enzymes from cell and organellar membranes, using a Tris-HCl grinding buffer-PVP solution. Electrophoresis was performed with an 11% starch gel. Ten enzyme systems were assayed: diaphorase (DIA), fluorescent esterase (EST), leucine aminopeptidase (LAP), and peroxidase (PER) were resolved on System 9 of Soltis *et al.* (1983); glucose phosphate isomerase (PGI), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (PGD), phosphoglucomutase (PGM), and shikimate dehydrogenase (SKD) on Soltis *et al.*'s System 10.

Statistics of enzyme data was based on allele and genotype frequencies in each population. The following genetic parameters were calculated using a POPGENE computer program (version 1.31) developed by Yeh *et al.* (1999): the percentage polymorphic loci ( $P_p$  for population level and  $P_s$  for species level), mean number of alleles per locus ( $A$ ), effective number of alleles per locus ( $A_e$ ), and gene diversity ( $H_e$ ) (Hamrick *et al.* 1992). Species (indicated with the subscript  $s$ ) and mean population (indicated with the subscript  $p$ ) levels of genetic diversity were calculated as in Hartl and Clark (1989). Observed heterozygosity ( $H_o$ ) was compared with Hardy-Weinberg expected values using Wright's fixation index ( $F$ ) or inbreeding coefficients (Wright 1965). Deviations from genotype frequencies expected under the Hardy-Weinberg equilibrium were tested using the GENEPOP ver. 3.1 program (Raymond and Rousset 1995). Multiple tests were performed using the sequential Bonferroni procedure (Lessios 1992).

## RESULTS

#### Ecological biodiversity

When we examine only  $S$  at any level of  $N$ , we conclude that the upper populations (populations 1~4) of the Nakdong River has fewer species (less species richness) than the middle and low populations of this river (Table 2). When we examine the  $H$  values as a function of  $N$ ,  $H$  is nearly constant with increasing the flow of water. Namely,  $H$  values trend to increase from the source of the

Table 2. Ecological analysis on species (*S*) from complete surveys of *Palaeoheterodonta* on 100 m × 100 m plots in eleven populations

Locality	<i>S</i>	ln <i>S</i>	<i>H</i>	ln <i>E</i>	ln <i>E</i> /ln <i>S</i>
1	4	1.386	1.172	-0.214	-0.154
2	4	1.386	1.752	-0.234	-0.169
3	6	1.722	1.211	-0.511	-0.297
4	4	1.386	1.317	-0.069	-0.050
5	5	1.609	1.463	-0.227	-0.141
6	6	1.792	1.482	-0.310	-0.173
7	6	1.792	1.572	-0.220	-0.123
8	6	1.792	1.502	-0.290	-0.162
9	6	1.792	1.569	-0.223	-0.124
10	6	1.524	1.172	-0.268	-0.150
11	6	1.792	1.568	-0.224	-0.125

river to the mouth. Population 4 exhibit the lowest evenness value (*E*) in the eleven populations.

**Genetic biodiversity**

We examined only one species, *C. leana* because this species is widespread and found in all populations. An average of 63.2% of the loci was polymorphic within populations, with individual population values ranging from 40.9% to 72.7% (Table 3). A considerable waterway differentiation was observed in the distribution of individual enzyme variants. The average number of alleles per locus (*A*) was 1.78 across populations, varying from 1.55 for the population with the lowest number of alleles and 1.91 for the population with the highest number with the lowest number of alleles. The population 11 had the highest expected diversity (0.342), while population 1 had the lowest (0.191). Genetic diversity at both species and population levels was high (*Hes* = 0.315; *Hep* = 0.275, respectively). The 4 upper populations of the river showed relatively low value of genetic diversity (mean *Hep* = 0.229), whereas the value in the two moth populations were distinctly high (mean *Hep* = 0.336). The genetic diversity trends to increase from the source of this main river to the mouth (Fig. 2).

**DISCUSSION**

The current up decedent loss of species is of worldwide concern (Buzas and Hayek 1996). Fundamental to effects to preserve such biodiversity is an understanding of its quantitative properties. A

Table 3. Genetic diversity for eleven populations of *Corbicula leana*

Locality	<i>Pp</i>	<i>Ap</i>	<i>A</i>	<i>Ae</i>	<i>Hop</i> (SD)	<i>Hep</i> (SD)
1	40.91	2.33	1.55	1.35	0.187(0.017)	0.191(0.047)
2	50.00	2.18	1.59	1.36	0.187(0.017)	0.208(0.045)
3	59.09	2.23	1.73	1.47	0.177(0.015)	0.257(0.049)
4	59.09	2.15	1.68	1.48	0.182(0.016)	0.259(0.049)
5	68.18	2.20	1.82	1.46	0.206(0.017)	0.261(0.044)
6	68.18	2.20	1.82	1.49	0.229(0.017)	0.280(0.044)
7	63.64	2.21	1.77	1.48	0.210(0.016)	0.271(0.045)
8	68.18	2.27	1.86	1.55	0.245(0.017)	0.300(0.047)
9	72.73	2.25	1.91	1.63	0.274(0.017)	0.329(0.046)
10	72.73	2.25	1.91	1.65	0.277(0.017)	0.330(0.048)
11	72.73	2.25	1.91	1.69	0.284(0.017)	0.342(0.048)
Mean	63.22	2.23	1.78	1.51	0.223(0.004)	0.275(0.012)

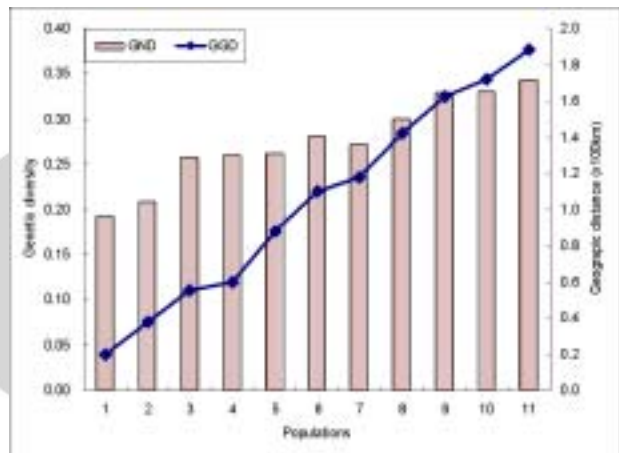


Fig. 2. Changes of genetic diversity from the source of the main river to the mouth in *C. leana*. GND: Genetic diversity, GGD: Geographic distance from Mt. Giri to each population.

core measure of biodiversity is the number of species (species richness, *S*) inhabiting communities. Another, less obvious but equally important, is the distribution of species abundance within these communities (species equitability or evenness, *E*). In this paper, we have extended biodiversity examples from ecological biodiversity to genetic diversity. The Namkang Dam is constructed the upper part (Nam River) of Nakdong River in 1968 and the Nakdong Tide Embankment is constructed in the mouth of the Nakdong River in 1988. Ecological relevant properties, such as salt tolerance, seasonality, migration distance, etc (Hengeveld 1996). Habitat destruction can affect species with high or low values for these properties differently, implying a systematic reduction in adaptive variation

among species. This means a greater biological loss than if this variation were random. For conservation purposes, it means that one can infer the probable cause of the reduction of biodiversity and prevent any further destruction of the habitat concerned.

In this paper, we can distinguish between responses to the abiotic part of the environment of physical factors. *Pelecypoda* including family *Cubiculidae* was evolved from marine to freshwater successfully. *Cubiculidae* is the one of edible fresh water and marine bivalvia. The Nakdong Tide Embankment has been control of a tidal current. We can suggest that gene flow and migration of individuals between populations of two artificial edifices (the Namkang Dam and the Nakdong Tide Embankment) are limited. Moreover, two edifices may lead to genetic isolation of once continuous population, which following isolation, may lose genetic diversity a result of genetic drift. The Save River population (the mouth of river) is located in Zimbabwe, revealed a higher proportion of gene diversity (0.30) than the Sanyati River drainage system (0.15) (Mukaratirwa *et al.* 1996). Our result is concordant that the genetic diversity trends to increase from the source of the main river to the mouth in Zimbabwe. In subdivided population, the loss of genetic drift (Lewontin 1974). If we assume an isolated model of population, selection or genetic drift can lead to the fixation of alleles at all loci when there is no gene flow (Slatkin 1985). Allozyme differences in *Semibalanus balanoides* were found among populations separated by tens and hundreds of kilometers (Dufresense *et al.* 2002). Barnacle *Balanus glandula* also showed similar a steep genetic cline in central California region (Sotka *et al.* 2004). Other studies have found few to no genetic differences over large swaths of the geographical ranges of barnacles (Ford and Mitton 1993, Wares *et al.* 2001).

However, we found that geographically restricted Korean populations had a level of genetic diversity similar to that of more widespread other barnacle species. This probably implies that the population structure below the local population level may be critical, along with the biological characteristics of the species itself, to determine the level of genetic clines. These patterns may be accelerated by artificial structures such as dams or reservoirs for irrigation. Thus, the downstream populations of the Nakdong River which showed high genetic diversity would not have expanded along river bottoms because of the Namkang Dam and Nakdong Tide Embankment. At the point of ecology, it is very import to maintain and conserve *C. leana* and not a better future for this species.

#### LITERATURE CITED

- Buzas, M.A. and L.C. Hayek. 1996. Biodiversity: an integrated approach. *Biodivers. Lett.* 3: 40-43.
- Dufresense, F., E. Bourget and L. Bernatchez. 2002. Differential patterns of spatial divergence in microsatellite and allozyme alleles: further evidence for locus-specific selection in the acorn barnacle, *Semibalanus balanoides*? *Mol. Ecol.* 11: 113-123.
- Ford, M.J. and J.B. Mitton. 1993. Populatin structure of the pink barnacle, *Tetraclita squamon rubescens*, along the California coast. *Mol. Mar. Biol. Biotech.* 2: 147-153.
- Hamrick, J.L., M.J.W. Godt and S.L. Sherman-Broyles. 1992. Factors influencing levels of genetic diversity in woody plant species. *New Forest.* 6: 95-124.
- Hartl, D.L. and A.G. Clark. 1989. *Principles of Population Genetics*. 2nd ed. Sinauer Associates. Inc. Mass. pp. 1-682.
- Hengeveld, R. 1996. Measuring ecological biodiversity. *Biodivers. Lett.* 3: 58-65.
- Hoelzel, R. and G.A. Dover. 1991. *Molecular Genetic Ecology*, P.1. Oxford University Press. Oxford.
- Huh, M.K. 1998. Allozyme variation and population structure of *Corbicula papyracea* from Nam and Sumjin Rivers. *Korean J. Genetics* 20: 163-172.
- Lessios, H.A. 1992. Testing electrophoretic data for agreement with Hardy-Weinberg expectations. *Marine Biol.* 112: 517-523.
- Lewontin, R.C. 1974. *The Genetic Basis of Evolutionary Change*. Columbia University. New York.
- Margaref, R. 1957. Information theory in ecology. *Mem. Real Acad. de Cienc. Artes Barcelona* 23: 373-449.
- Mukaratirwa, S., H.R. Siegismund, T.K. Kristensen and K. Chandiwana. 1996. Population genetics and genetic variability of *Bulinus globosus* (Gastropoda: Planorbidae) from the two main river systems in Zimbabwe. *J. Hered.* 87: 288-294.
- Poudevigne, I. and J. Baudry. 2003. The implication of past and present landscape patterns for biodiversity research: introduction and overview. *Landscape Ecol.* 18: 223-225.
- Raymond, M. and F. Rousset. 1995. GENEPOP version 1.2: A population genetics software for exact tests and ecumenicism. *J. Hered.* 86: 248-249.
- Shannon, C.E. 1948. A mathematical theory of communication. *Bell. Syst. Tech. J.* 27: 379-423.
- Slatkin, M. 1985. Rare alleles as indicators of gene flow. *Evolution* 39: 53-65.
- Soltis, D.E., C.H. Haufler, D.C. Darrow and G.J. Gastony. 1983. Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. *Am. Fern J.* 73: 9-27.
- Sotka, E.E. J.P. Wares, J.A. Barth, R.K. Grosberg and S.R. Palumbi. 2004. Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle *Balanus glandula*. *Mol. Ecol.* 13: 2143-2156.
- Stabile, J., J.R. Waldman, F. Parauka and I. Wirgin. 1996. Stock structure and homing fidelity in Gulf of Mexico sturgeon (*Acipenser oxyrinchus desotori*) based on restriction fragment length polymorphism and sequence analyses of mitochondrial

DNA. *Genetics* 144: 767-775.

Wares, J.P., S.D. Gaines and C.W. Cunningham. 2001. A comparative study of asymmetric migration events across a marine biogeographic boundary. *Evolution* 55: 295-306.

Wright, S. 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution* 19:

395-420.

Yeh, F.C., R.C. Yang and T. Boyle. 1999. POPGENE version 1.31. Microsoft Windows-based Freeware for Population Genetic Analysis.

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