A Cline of Genetic Diversity of *Corbiculidae* according to Artifical Ediface (Namkang Dam)

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1. Introduction

Corbiculidae, the one of edible fresh water mussels are egg-laying freshwater bivalvias native to East Asia. Their small size, ease of breeding and shortness of generation time make them useful experimental animals for genetic research. As with other freshwater bivalvia, Corbiculidae are thought that a swift stream and inclination of river are barriers to the migration of these species, and that local populations of these species are thus unfined to their own watershed and isolated from one another. Yet no information is available regarding the genetic structure and possible genetic variation of population of this Corbiculidae.

The main forces responsible the spatial arrangement of polymorphism are random event, historical occurrences, and selection pressures. The random process is represented by the isolated-by distance model, which results in a simple spatial arrangement. It predicts that neighboring populations are more similar than distant ones. Historical explanations, like periods of isolation, local extinction, and recolonation, depend on the spatial structure whereas selection is often habitats dependent. This habitats dependence is not necessarily a spatial relationship, because the geographic arrangement of habitats can be pathy in different ways.

To distinguish between the processes responsible for genotypic and phenotypic spatial variation, spatial patterns of *Corbicula leana* Prime and *C. papyracea* Heude, we have compiled and quantatively analysed what known of the genetic structure of populations to examine whether any trends occur both within and among populations. In several main rivers of Korea, *C. leana* and *C. papyracea* were always the dominant species in several years

ago. As the moment exotic species such as *C. fluminea* Muller, *C. leana* Prime, *C. papyracea* are found. The lower part of stream is now being destroyed at an alarming rate by means of environmental pollution. Therefore, the results derived from this study could provide information for conservation strategy.

The purpose of this study was: 1) to estimate how much total genetic diversity is maintained in the species; 2) to assess genetic structure of *Corbiculidae*; and 3) to provide differentiational information on the genetic variation in these species populations from the two main rivers in Korea.

2. Materials and Methods

The materials were collected either by handpicking or using a scoop. Electrophoresis was performed using 10% starch gel. Gel and electrode buffers systems and enzyme staining procedures from Soltis et al. (1983) were used to analysis for the following enzyme systems; fluorescent esterase (FE), acid phosphatases (ACP), malate dehydrogenase (MDH), alcohol dehydrogenase (ADH), leucine aminopeptidase (LAP), glutamate oxaloacetate transaminase (GOT), 6-phosphoglugonate dehydrogenase (PGDH), phosphoglucomutase (PGM), isocitrate dehydrogenase (IDH)lactate dehydrogenase (LDH), malic enzyme (ME), shikimate dehydrogenase (SKDH), and glucosphophate isomerase (GPI).

Four standard genetic parameters were estimated using a computer program developed by Loveless and Schnabel; percent polymorphic loci (P), mean number of alleles per locus (A), effective number of alleles per locus (Ae), and gene diversity (He) (Hamrick et al. 1992). Subscripts refer to species (s) or population (p) level parameters. Observed heterozygotes (Ho) were compared to Hardy-Weinberg expected value using Wright's fixation index (F) or inbreeding coefficients. Nei's gene diversity formulae (H_T, H_S, D_{ST}, and G_{ST}) were used to evaluate the distribution of genetic diversity within and among populations. Nei's genetic identity (I) was calculated for each pairwise combination of populations. We used the PC-SAS program to conduct a cluster analysis on genetic distances via the unweighted pairwise groups method arithmetic average (UPGMA). Correction between geographical and genetic distances was tested using Mantel's test as advocated by Smouse et al. (1986). This method compares matrices pairwise; one matrix contains genetic distances while the other contains geographical distances. The genetic structure within and among populations was also evaluated using Wright's F-statistics. The $F_{\rm IT}$ and $F_{\rm IS}$ coefficients measure excesses of homozygotes or heterozygotes relative to the panmictic expectations within the entire population samples and with populations, respectively. The $F_{\rm ST}$ coefficient estimates relative population differentiation. Two indirect estimates of gene flow were calculated. One estimate of Nm (the number of migrants per generation) was based on $F_{\rm ST}$ (Wright 1951) and the other estimate was based on the average frequency of "rare" alleles found in only one population (Barton and Slatkin 1986; Slatkin 1985).

3. Results

3.1 Genetic diversity

Twenty-two putative loci encoding 13 enzyme systems in C. leana were screened. Seventeen of the 22 loci studied (77.3%) showed detectable polymorphism in at least two populations. 59.1% of the loci were polymorphic within populations. A considerable waterway differentiation was observed in the distribution of individual enzyme variants. At least the five loci (Est-3, Mdh-1, Lap-1, Idh-2 and Pgd-2), there is a conspicuous differences between the allele frequencies found at the Sumjin River populations and those found in the populations along the Nam River. The average number of alleles per locus (A) was 1.82 across populations. The mean genetic diversity within populations was 0.216. Genetic diversity levels at the species and at the population were high (Hes = 0.270; Hep = 0.216, respectively). Striking observation is the geographic variation in the genetic diversity. The genetic diversity trends to increase from the source of the main river to the mouth. There is a positive correlation between genetic diversity and geographic distance (r = 0.28). The eight populations of the Sumjin River revealed a higher proportion of genetic diversity (mean = 0.262) than the nine populations of the Nam River (mean = 0.175). With an r value 0.28 only 8% of the variation in genetic distance values is explained by geographic distance. But, genetic distance was correlated with geographic distance subpopulations (r = 0.69 for the Sumjin River populations; r = 0.73for populations $1 \sim 4$, the upper populations of the Namkang River; r = 0.77for populations $5 \sim 9$, the lower populations of the Namkang River).

Twenty-two putative loci encoding 13 enzyme systems in *C. papyracea* were screened. Seventeen of the 22 loci studied (77.3%) showed detectable

polymorphism. 65.8% of the loci were polymorphic within populations. A considerable waterway differentiation was observed in the distribution of individual enzyme variants. At least the four loci (Est-1, Est-2, Mdh-1, and Lap-1) there is a conspicuous differences between the allele frequencies found at the Sumiin River populations and those found in the populations along the Nam River. A was 1.86 across populations. The mean genetic diversity within populations was 0.294. The Population SA had the highest expected diversity (0.380), while population NA had the lowest (0.191). Genetic diversity at the species level was high, whereas the value at the population level was low (Hes = 0.315; Hep = 0.294). Populations of the Sum River showed relatively high value of genetic diversity (Hep = 0.328). whereas the value in the populations of the Nam River were distinctly lower (Hep = 0.275). Striking observation is the geographic variation in the genetic diversity. The genetic diversity trends to increase from the source of the main river to the mouth (r = -0.84). There is a positive correlation between genetic diversity and geographic distance. There is a positive correlation between genetic distance and geographic distance (r = 0.30). With an rvalue 0.30 only 9% of the variation in genetic distance values is explained by geographic distance. But, genetic distance was correlated with geographic distance subpopulations (r = 0.64 for the Sumjin River populations; r = 0.70for the Namkang River populations).

3.2 Genetic structure

Analysis of fixation indices, calculated for all polymorphic loci in each population, showed a substantial deficiency of heterozygosites relative to Hardy-Weinberg expectations. For example, 91.5% of fixation indices were positive (162/177), and 114 of those departed significantly from zero (p < 0.05). In contrast, of 15 negative fixation indices, no one was significantly different from zero (p < 0.05). As expected from the chi-square tests, F_{IS} , a measure of the deviation from random mating within 17 populations, was 0.415, range from 0.237 for Fe-1 to 0.691 for Acp-1. The observed moderate, and positive F_{IS} value indicates that heterozygosity were significantly in deficit at all loci. At the level of the sample as a whole, however, Wright's F; coefficients showed that significant deficiencies of heterozygosites exist for seven loci of the seventeen polymorphic loci. On a per locus basis, G_{ST} ranged from 0.056 for Est-2 to 0.516 for Mdh-1 with a mean of 0.178, indicating that about 18% of the total allozyme variation was among

populations. Thus, the majority of genetic variance (82%) resided within populations. Genetic identity values among pairs populations range from 0.836 to 0.998. The similarity among populations can be seen in the UPGMA dendrogram, where total populations cluster at a below genetic distance 1.402. Although the populations in two island groups (populations 1 and others) were as expected, other groups showed little relationship to the UPGMA dendrogram. The estimated of gene flow based on G_{ST} was moderate among Korean populations of C. leana (Nm = 1.15).

Genetic variability in gastropodas varies with the kinds of environmental in which species live, increasing from the terrestial environments. Of course *C. leana* and *C. leana* are not gastropod but pelecypoda. But only both gastropod and pelecypoda were evolved from marine to freshwater successfully. Within each environment, the variability is generally higher in outcrossers than in selfers.

Most populations in our study experience annual, severe demographic bottlenecks due to floods. This has resulted in substructuring of *C. leana* and *C. papyracea* populations into multiple genetically differentiated populations within as well as among populations of the two river courses and a limited differentiation between Namkang Dam. The Nam River and its major tributaries must be considered as potential avenues of intermittent migration (during floods and passive migration) and gene flow of a magnitude sufficient to prevent genetic differentiation of populations, and also sufficient to keep genetic diversity high in the face of genetic drift. Genetic diversity is lower in the Nam River because of an artificial edifice (Namkang Dam).

It is necessary that the degree of genetic differentiation among *C. leana* populations of primary importance for the conservation of genetic diversity and the evolutionary potential of the species under conservation. Based on a the data available such as a considerable high G_{ST} value compared with a mean value of *C. leana* species, it is recommended that several populations of the species in Korea should be preserved, giving priority to populations primary with high variation such as populations mostly in the Sumjin River. These populations could be used as source of genetic diversity for the restoration of genetically depauperate populations.