

Spatial Autocorrelation within Three Populations of *Sasa borealis* in Korea

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Spatial autocorrelation was applied to microgeographic variations of *Sasa borealis* populations in Korea. Separate counts of each type of join (combination of genotypes at a single locus) for each allele, and for each distance class of separation, were tested for significant deviation from random expectations by calculating the Standard Normal Deviation. Moran's *I* was significantly different from the expected value in 25 of 150 cases (16.7%). Seven of these values (4.7%) were negative, indicating genetic dissimilarity among pairs of individuals in the ten distance classes. Populations of *S. borealis* are small in Korea, and are distributed with occasional cutting of seed-bearing stems used for sieves. Thus, artificial disturbance may contribute to the fact that the *S. borealis* population of Jirisan is unusual in lacking spatial genetic structure.

Key words – Spatial autocorrelation, Moran's *I*, *Sasa borealis*

Genetic structure is an integral part of the processes of population genetics[10]. Population structure interacts with a number of factors: microenvironmental heterozygosity[2], mortality due to stochastic events[23], and mating systems that feature limited dispersal of seed or pollen[10]. The most important factors are gene flow and natural selection, which influence spatial patterns of the genetic population structure [3,8,17]. In theory, genetic differentiation over short distances may occur either as a result of spatially variable selection or localized genetic drift, and provided that gene flow is sufficiently restricted[6]. Many early, direct studies suggested that actual gene-dispersal distances are greater than the observed pollen- and propagule-dispersal distances[16]. The potential for genetic differentiation via genetic drift within populations of outcrossing plant species may be substantially less than previous thought[6].

Indirect evidence for genetic correlations between neighboring plants has been obtained from data on mating systems [10]. Localized seed and pollen dispersal produced family clusters within these populations[9]. Several studies revealed decreased seed set and seed survivorship from matings between genetically similar, near-neighbors, which has been interpreted as inbreeding depression[13,15,21].

Sasa borealis Makino (Bambusaceae) is mainly distributed in East Asia such as Japan and South Korea. Especially the species is only found in cold regions of the Korean Peninsula. The species does not have been an abundant plant over its

range in Korea and reduction of populations is serious. Many manufactures substituted bamboo for plastic or iron goods. Populations that are reproductively isolated may gradually lose the effective population sizes. The rapid loss of new plants results in the permanent loss of gene pools with potential for species conservation.

S. borealis is a small clumping bamboo and possess pachymorph or determines rhizomes with moderate short necks. Rhizomes have generally long and prostrate stems rooting at the nodes.

In this study, spatial autocorrelation (SA) was analyzed in Korean populations of *S. borealis* to investigate how different levels of gene flow among individuals can be distinguished based on the pattern of population differentiation in an isolation-by-distance model. The spatial distribution was described for alleles at polymorphic enzyme loci in a natural population.

Materials and Methods

Sampling procedure and enzyme electrophoresis

S. borealis was collected from three natural populations which consisted of above 2,000 individuals with diploid genotypes located on a 100×50 square lattice (Table 1). We selected The distance among selected individuals was about 5.0 m, to avoid including those with common lineage. One leaf per plant was sampled. Leaves gathered from natural populations were labeled and then refrigerated in plastic bags for 1 to 2 days, until electrophoresis was carried out.

Electrophoresis was performed using 11.0% starch gel.

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Table 1. Collection localities for populations of *S. borealis* as source for isozyme analysis

| Code | Localities | Latitude |
|------|---|----------|
| SOB | Mt. Sobaek, Youngpung-gun, Gyeongsangbuk-do | 820 m |
| JIR | Mt. Jiri, Sancheong-gun, Gyeongsangnam-do | 1200 m |
| YEH | Mt. Yehang, Haman-gun, Gyeongsangnam-do | 680 m |

Buffer systems and enzyme staining procedures from Soltis *et al.*[20] were used to assay five enzyme systems; fluorescent esterase (FE), malate dehydrogenase (MDH), peroxidase (PER), 6-phosphoglucuronate dehydrogenase (6PGD), and phosphoglucumutase (PGM). The procedures for starch gel electrophoresis were as reported by Soltis *et al.*[20]. For enzymes which resolved more than one zone of activity, the most anodal isozyme is arbitrarily designated 1, with the others sequentially assigned higher numbers. Likewise, alleles were designated sequentially with the most anodally migrating allozyme designated 'a' and progressively slower forms 'b', 'c', and so on. The *S. borealis* isozymes expressed phenotypes that were consistent in subunits structure and genetic interpretation with most isozyme studies in plants, as documented by Weeden and Wendel[22].

Statistical Measures of Genetic Structure

The spatial structuring of allozyme variation was quantified by Moran's *I*, a coefficient of SA[18,19]. As applied in this study, Moran's *I* quantifies the genetic similarity of pairs of spatially adjacent individuals related to the population sample as a whole. The value of *I* ranges between +1 (complete positive autocorrelation, i.e., paired individuals have identical values) and -1 (complete negative autocorrelation). Each plant was assigned a value depending on the presence or absence of a specific allele. If the *i*th plant was a homozygote for the allele of interest, the assigned p_i value was 1. If the individual was a heterozygote, the value 0.5 was assigned, and if the allele was absent, the value 0 was assigned.

Pairs of sampled individuals were classified according to the Euclidian distance d_{ij} , so that the class *k* included d_{ij} satisfying $k-1 < d_{ij} < k+1$, where *k* takes 1 to 10. The interval for each distance class was 5.0 m. Moran's *I* statistic for the class *k* was calculated as follows: $I(k) = n \sum_i \sum_{j(i \neq j)} W_{ij} Z_i Z_j / S \sum Z_i^2$, where Z_i is $p_i - p$ (p is the average of p_i), W_{ij} is 1 if the distance between the *i*th and *j*th plants is classified into class *k*; otherwise, W_{ij} is 0, *n* is the number of all samples, and *S* is the sum of W_{ij} ($\sum_i \sum_{j(i \neq j)} W_{ij}$) in class *k*. Under the randomization hypothesis,

$I(k)$ has the expected value $u_1 = -1/(n-1)$ for all *k*. Its variance, u_2 , has been given, for example, in Sokal and Oden[18]. Thus, if an allele is distributed randomly for class *k*, the normalized $I(k)$ for the standard normal deviation (SND) for plant genotype, $g(k) = \{I(k) - u_1\} / u_2^{1/2}$, asymptotically has the standard normal distribution[4]. Hence, SND $g(k)$ exceeding 1.96, 2.58, and 3.27 are significant at probability levels 0.05, 0.01, and 0.001, respectively.

For diallelic loci, only those with allele frequencies < 0.95 and > 0.05 were employed, and then only one allele was considered because the second allele would contribute identical information. For multiallelic loci, all alleles at that locus, regardless of their frequencies, were used for the spatial analysis.

Results

From the individuals sampled, twelve loci were found for *S. borealis*. Three alleles were also found for *Pgm-2* and *6Pgd* (Table 2). *Per-2* and *Mdh-3* loci also expressed two alleles. The remaining seven loci (*Fe-2*, *Per-1*, *Per-3*, *Per-4*, *Mdh-1*, *Mdh-2*, and *Pgm-1*) were monomorphic in all populations. The SA coefficient, Moran's *I*, for a polymorphic locus is presented in Figs. 1-3. Fig. 1 for population SOB shows the distribution of SA for *S. borealis* across the distance classes.

Moran's *I* was significantly different from the expected value in only 25 of 150 cases (16.7%). Only seven of these values (4.7%) were negative, indicating genetic dissimilarity among pairs of individuals in the eight distance classes. Seventeen of the significant values (68.0%) were positive, indicating a partially genetic similarity among individuals in the distance class VII, i.e., pairs of individuals separated by more than 35.0 m. Overall, the Korean *S. borealis* populations have significant genetic structure in most spatial classes.

Separate counts of each type of join (combination of genotypes at a single locus) for each allele, and for each distance class of separation, were tested for significant deviation from random expectations by calculating the SND (Table 3). For all distance classes, only 25 SND statistics

Table 2. Gene frequencies for *S. borealis* on five polymorphic loci

| Population | Allele | Locus | | | | |
|------------|--------|-------------|--------------|--------------|--------------|-------------|
| | | <i>Fe-1</i> | <i>Per-2</i> | <i>Mdh-3</i> | <i>Pgm-2</i> | <i>6Pgd</i> |
| SOB | a | 0.2586 | 0.3030 | 0.2000 | 0.1563 | 0.1500 |
| | b | 0.5172 | 0.6970 | 0.8000 | 0.6875 | 0.6100 |
| | c | 0.2241 | 0.0000 | 0.0000 | 0.1563 | 0.2400 |
| JIR | a | 0.2069 | 0.3125 | 0.2500 | 0.1250 | 0.1667 |
| | b | 0.5690 | 0.6875 | 0.7500 | 0.6429 | 0.5185 |
| | c | 0.2241 | 0.0000 | 0.0000 | 0.2321 | 0.3148 |
| YEH | a | 0.0556 | 0.4545 | 0.1833 | 0.1429 | 0.1000 |
| | b | 0.8333 | 0.5455 | 0.8167 | 0.7143 | 0.6750 |
| | c | 0.1111 | 0.0000 | 0.0000 | 0.1429 | 0.2250 |

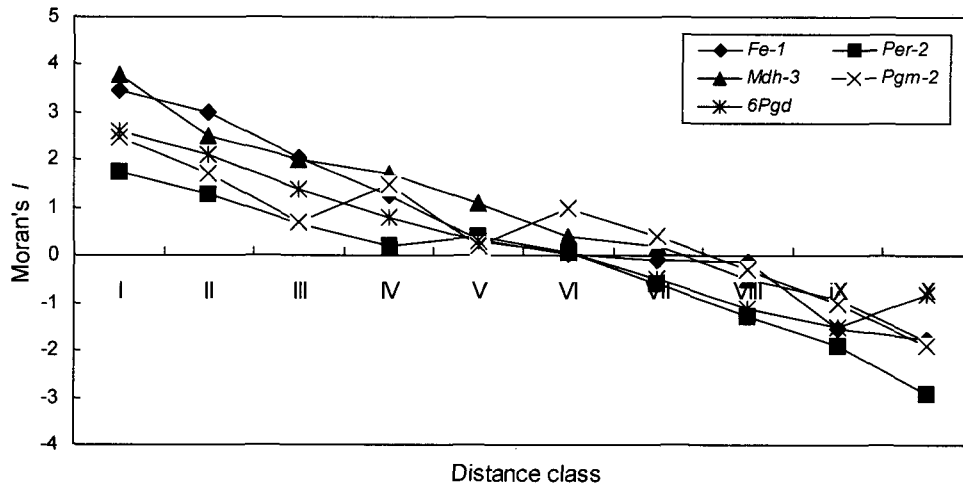


Fig. 1. Correlograms of autocorrelation statistics for population SOB as a function of distance. The distance classes are 0-5.0 m (class I), 5.0-10.0 m (class II), 10.0-15.0 m (class III), 15.0-20.0 m (class IV), 20.0-25.0 m (class V), 25.0-30.0 m (class VI), 30.0-35.0 m (class VII), 35.0-40.0 m (class VIII), 40.0-45.0 m (class IX), and 45.0-50.0 m (class X).

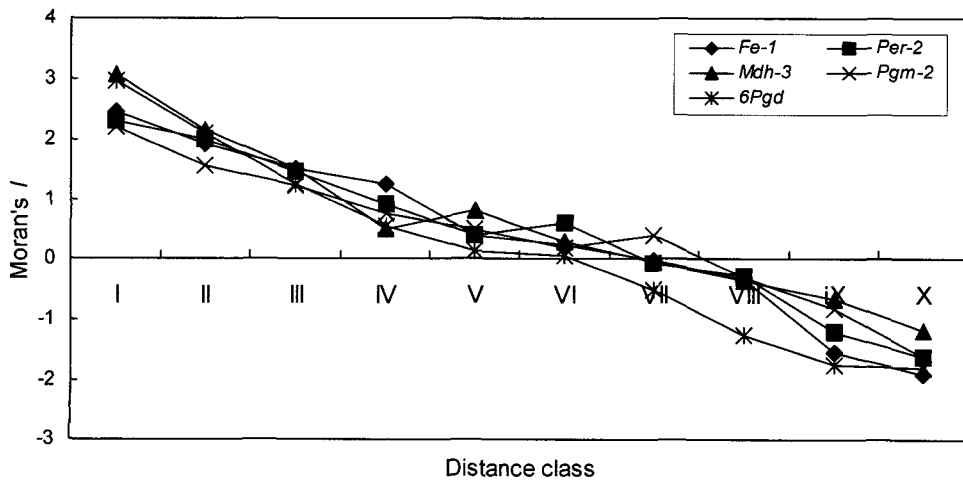


Fig. 2. Correlograms of autocorrelation statistics for population JIR as a function of distance. Distance classes are the same as Fig. 1.

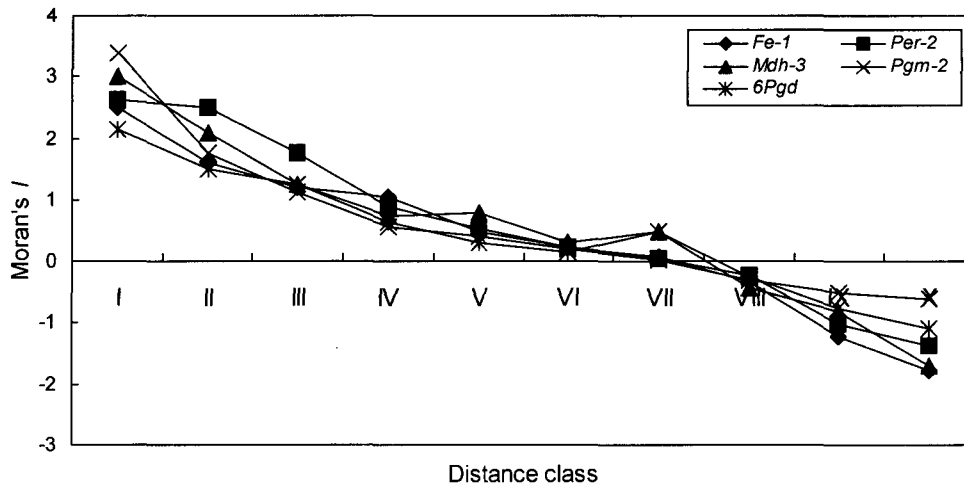


Fig. 3. Correlograms of autocorrelation statistics for population YEH as a function of distance. Distance classes are the same as Fig. 1.

Table 3. The standard normal deviation (SND) for plant genotype at ten distance classes

| Popu- lation | Locus | Distance class | | | | | | | | | |
|-----------------|--------------|------------------|-----------------|-----|----|---|----|-----|------|----------------|-----------------|
| | | I | II | III | IV | V | VI | VII | VIII | IX | X |
| SOB | <i>Fe-1</i> | + ^{**} | + [*] | + | + | + | + | + | - | - | - |
| | <i>Per-2</i> | + [*] | + | + | + | + | + | + | - | - [*] | - ^{**} |
| | <i>Mdh-3</i> | + ^{***} | + [*] | + | + | + | + | + | - | - | - |
| | <i>Pgm-2</i> | + [*] | + | + | + | + | + | + | - | - | - |
| | <i>6Pgd</i> | + [*] | + | + | + | + | + | + | - | - | - |
| JIR | <i>Fe-1</i> | + [*] | + | + | + | + | + | + | - | - | - [*] |
| | <i>Per-2</i> | + [*] | + | + | + | + | + | + | - | - | - |
| | <i>Mdh-3</i> | + ^{***} | + | + | + | + | + | + | - | - | - |
| | <i>Pgm-2</i> | + [*] | + | + | + | + | + | + | - | - | - [*] |
| | <i>6Pgd</i> | + ^{***} | + | + | + | + | + | + | - | - [*] | - [*] |
| YEH | <i>Fe-1</i> | + [*] | + | + | + | + | + | + | - | - | - [*] |
| | <i>Per-2</i> | + | + | + | + | + | + | + | - | - | - |
| | <i>Mdh-3</i> | + ^{**} | + ^{**} | + | + | + | + | + | - | - | - [*] |
| | <i>Pgm-2</i> | + ^{***} | + | + | + | + | + | + | - | - | - |
| | <i>6Pgd</i> | + [*] | + | + | + | + | + | + | - | - | - |

*: p<0.05; **: p<0.01; ***: p<0.001.

were significant. Two alleles, *Fe-1a* and *Mdh-3b*, showed significantly positive SND values for distance class II. The aggregation of n identical alleles, called a "patch", resulted in those loci. The significantly negative SND values indicate an excess of different allele pairs at *Per-2* and *6Pgd* loci for class IX. This suggests neighbor patches in which different alleles are predominant 40.0 to 45.0 m apart, on average.

Discussion

Although significant aggregation of an identical allele was partially observed at some loci for some classes, spatial

structure of allele frequencies was found for either of five polymorphic loci within the natural population of *S. borealis*. The results from this study are partly consistent with the supposition that plant populations are subdivided into local demes, or neighborhoods of related individuals[2,7,12]. Previous reports on the local distribution of genetic variability suggested that microenvironmental selection and gene flow are the main factors causing substructuring of alleles within a population[10,11,16]. Local genetic differentiation at isozyme or other marker loci, caused by microenvironmental heterogeneity, has been observed in a variety of plant species[3]. Those loci showed significant aggregation

of an identical allele, which persisted for generations, as long as the same microenvironmental conditions continued. This persistence was demonstrated, for example, in the *Got-1* locus in lodgepole pine[10] and for the genetic variation in quantitative traits of *Impatiens capensis*[1]. In the present study, the *Mdh-3* locus showed significant aggregation in the two *S. borealis* populations. This aggregation, however, did not persist at all loci, thereby excluding microenvironmental selection as being the main cause for the allele aggregation.

Although aggregation of an identical allele was partially observed at some loci for some classes, the average Moran's *I* values for each distance class in this study indicate that Korean *S. borealis* populations apparently are less structured. Possible contributing factors include differences in density, topography, and human interference. Although *S. borealis* populations were located on a mountain, populations were distributed with tractors used for manufacture. If gene flow is limited at most loci where allele aggregation is observed in adult plant populations, allele aggregation also is expected in a pollen cloud[5]. This occasional cutting of seed-bearing stems may cause high levels of gene flow.

Why is the population of *S. borealis* unusual, in that it lacks significant genetic structure at most spatial classes? A likely explanation is that gene flow has been sufficiently extensive to prevent the random divergence of local gene frequencies. Even a small amount of gene flow is enough to counteract the diversifying effects of genetic drift or weak selection[23]. In simulations, Ohsawa *et al.*[14] and Epperson[9] showed that local genetic differentiation is very sensitive to the degree of actual gene dispersal. The indirect estimate of gene flow, based on the mean *Gst* (the proportion of total genetic diversity partitioned among populations), was high ($Nm=14.06$) in *S. borealis* populations. The levels of gene flow calculated in the present study are of sufficient magnitude to counterbalance genetic drift or weak selection, thus playing a major role in shaping the genetic structure of the *S. borealis* population[23].

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초록 : 한국 조릿대집단의 공간적 상관관계

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조릿대(*Sasa borealis*)집단에서 미세지리적 변이에 대한 공간적 상관관계를 조사하였다. 각 거리등급당 대립유전자좌위에서 연결계수(하나의 대립유전자좌위에서 유전자형의 조합)를 산출하였으며 그 계수가 임의 예상값에 유의성을 가지는지 검증하였다. 150 경우 중 25경우(16.7%)가 예상값과 유의하게 차이를 나타내었다. 이들 값 중에서 8경우(4.7%)는 음의 값으로 거리등급에서 개체쌍이 유전적 비친화성이 있음을 나타낸다. 조릿대는 죽세공에 쓰이므로 인위적 벌채에 의해 한국내 자연집단은 유효 집단이 유지되지 못하는 등 집단 파괴가 이루어지고 있다. 특히 조릿대가 잘 발달되어온 지리산 집단의 경우 공간적 유전 구조가 결여되어 있었다.