# Spatial Autocorrelation within a Korean Population of *Alnus hirsuta*

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The present study was investigated microgeographic variations to spatial autocorrelation in the Korean alder, *Alnus hirsuta*. 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 (SND). Moran's *I* was significantly different from the expected value in 24 of 120 cases (20.0%). 17 of these values (14.2%) were significantly negative, indicating genetic dissimilarity among pairs of individuals in the ten distance classes. Many Korean populations of alder are small and are distributed by men for firewood. This occasional cutting of seed-bearing stems may bring a high level of gene flow. In addition, stump sprouting ability also may contribute to the fact that the Chengkwang population at Gijang is unusual in lacking spatial genetic structure.

**Key words** – *Alnus hirsuta, genetic structure, spatial autocorrelation, standard normal deviation* (SND)

Genetic structure in populations is an integral part of population genetics[11]. Population structure interacts with a number of factors: microenvironmental heterozygosity[4], mortality due to stochastic events[27], and mating systems that feature limited dispersal of seed or pollen[11]. Especially, the most important factors are gene flow and natural selection, which influence spatial patterns of the genetic population structure[4,9,22]. In theory, genetic differentiation over short distances may occur either as a result of spatially variable selection or localized genetic drift. It occurs that gene flow is sufficiently restricted[8]. Many early, direct studies suggested that actual gene-dispersal distances are greater than the observed pollen- and propagule-dispersal distances[20]. The potential for genetic differentiation via genetic drift within populations of outcrossing plant species may be substantially less than that previously thought theroetical results[8].

Indirect evidence for genetic correlations between neighboring plants has been obtained from data on mating systems[11], suggesting that localized seed and pollen dispersal have 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 caused by matings among genetically similar neighbors[16,19,26].

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Alder is an early-successional monoecious species which fixes nitrogen symbiotically with the actinomycete *Frankia* [17]. Bousquet *et al.*[2] suggested that Asia and Indies are probably the origin of alder. Actually, Asian regions such as China, Korea, and Japan are well known for various alder species. The genus *Alnus* in Korea is comprised of 15 species. Little is known about the levels of genetic variation and the population structure of these species, despite its economical importance such as furniture, forestation, firewood, and windbreak forest and its transcontinental distribution.

The present study was carried out to examine spatial autocorrelation (SA) in Korean populations of *Alnus hirsuta* Turcz. (alder, Betulaceae). The spatial distribution was described for alleles at polymorphic enzyme loci in a natural population.

## Materials and Methods

### Sampling Procedure and Electrophoresis

Sampling was conducted from May 2000 to August 2001, at Chengkwang-ri, Gijang-up, Busan province. One leaf per plant was sampled. The distance between selected individuals was about 5.0 m, in order to avoid including individuals those with common lineage. Leaves gathered from natural populations were labeled and stored in plastic bags then refrigerated for 1 to 2 days in a refrigerator until electrophoresis was carried out.

Homogenization, starch gel electrophoresis, and enzyme

assay procedures were followed according to the methods of Soltis *et al.*[25]. Leaves were homogenized to release enzymes from the cell and organellar membranes with a Tris-HCl grinding buffer-PVP solution. Electrophoresis was performed using 10% starch gels, and a total of seven enzyme systems were assayed: alcohol dehydrogenase (ADH), malate dehydrogenase (MDH), glutamate oxaloacetate transaminase (GOT), peroxidase (PER), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucomutase (PGM), and isocitrate dehydrogenase (IDH).

For enzymes resolving in more than one zone of activity, the most anodal isozyme was arbitrarily designated as '1' and subsequent isozymes were sequentially assigned higher numbers. Likewise, alleles were designated sequentially, with the most anodally migrating allozyme designated as 'a' and progressively slower forms 'b', 'c', and so on.

### Statistical Measures of Genetic Structure

The spatial structuring of allozyme variation was quantified by Moran's *I*, a coefficient of spatial autocorrelation[23,24]. Moran's *I* quantifies the genetic similarity of pairs of spatially adjacent individuals relative 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 has 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 individuals sampled (total number of pairs) 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: $I(k) = n\sum i\sum j(i\neq j)WijZiZj/S\sum Zi^2$, where *Zi* 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* $\{\sum i\ \sum j(i \neq j)Wij\}$ in class k. Under the randomization hypothesis, *I*(k) has the expected value $u1 = -1/(n-1)$ for all k. Its variance, $u2$, has been given in Sokal and Oden[23]. Thus, if an allele is distributed randomly for class k, the normalized *I* (k) in the standard normal deviation (SND) for plant genotype, SND $g(k)=\{I(k)-u1\}/u2^{1/2}$, asymptotically has the standard normal distribution[5]. Hence, SND g (k) exceeding 1.96, 2.58, and 3.27 are significant in probability levels of 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 to 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, three alleles were found for *Idh-2*, with frequencies of 0.735 (allele *a*), 0.204 (allele b), and 0.061 (allele *c*). Three alleles were also found for *Pgm-2*, with frequencies of 0.914 (allele *a*), 0.029 (allele *b*), and 0.057 (allele *c*). *Pgd-1* and *Pgm-1* loci expressed two alleles. ADH, GOT, and PER were monomorphic at all sites. The spatial autocorrelation SA coefficient, Moran's *I*, for a polymorphic locus is presented in Table 1. Moran's *I* was significantly different from the expected value in only 24 of 120 cases (20.0%). 17 of these cases (14.2%) were significantly negative, indicating genetic dissimilarity among pairs of individuals in the ten distance classes. Only seven of the significant values (5.8%) were positive, indicating a partially genetic similarity among individuals in the distance class 6, i.e., pairs of individuals separated by more than 30 m. Overall, the Korean alder population lacked 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. Figs. 1 and 2 show the distribution of spatial autocorrelation for alder across the distance class 10. For all distance classes, only four SND statistics were significant. Two alleles, *Idh-2c* and *Pgm-2b*, showed significantly positive SND values for distance class 2. *Mdh-1a* and *Mdh-1b* had also significantly positive SND values for distance classes 5 and 6, respectively (Fig. 1). The aggregation of an identical allele, called a "patch", in those loci. The nine significantly negative SND values, indicate an excess of different alleles pairs at the nine loci (*Idh-2b*, *Idh-2c*, *Mdh-1a*, *Pgd-1a*, *Pgm-1a*, *Pgm-1b*, *Pgm-2a*, *Pgm-2b*, and *Pgm-2c*) for nine classes except class 10. This suggests that neighbor patches with different alleles appear at predominant 45- to

Table 1. Spatial autocorrelation coefficients (Moran's *I*) of 12 alleles among populations of *Alnus hirsuta* for ten distance classes

| | Class | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Alleles | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| *Idh-2a* | 0.003 | 0.027 | 0.023 | -0.010 | 0.004 | -0.021 | -0.022 | 0.034 | -0.009 | 0.015 |
| *Idh-2b* | 0.000 | 0.005 | -0.036 | 0.007 | -0.017 | -0.008 | -0.046 | -0.050* | -0.030 | -0.041 |
| *Idh-2c* | 0.101*** | 0.076** | 0.041 | -0.078** | 0.011 | -0.009 | 0.029 | 0.003 | -0.048 | 0.025 |
| *Mdh-1a* | 0.008 | 0.034 | 0.040 | 0.030 | 0.055* | 0.097** | 0.032 | -0.051* | -0.036 | -0.042 |
| *Mdh-1b* | -0.013 | -0.009 | -0.026 | -0.037 | 0.085** | -0.003 | 0.014 | -0.022 | 0.015 | 0.006 |
| *Pgd-1a* | 0.064* | -0.007 | 0.041 | -0.008 | -0.054* | -0.021 | -0.056* | -0.023 | -0.110*** | -0.023 |
| *Pgd-1b* | 0.002 | 0.023 | -0.028 | -0.001 | 0.019 | -0.033 | 0.026 | -0.042 | -0.014 | -0.000 |
| *Pgm-1a* | 0.018 | -0.029 | -0.054* | -0.066** | -0.045 | -0.030 | 0.008 | -0.038 | 0.004 | 0.007 |
| *Pgm-1b* | 0.010 | -0.009 | -0.046 | -0.035 | -0.082** | -0.057* | -0.044 | -0.005 | 0.010 | -0.028 |
| *Pgm-2a* | 0.015 | 0.040 | -0.039 | 0.005 | -0.061* | -0.038 | -0.048 | -0.081** | -0.040 | -0.027 |
| *Pgm-2b* | -0.057* | 0.086** | -0.005 | -0.022 | 0.032 | 0.040 | -0.052* | -0.043 | -0.067* | -0.000 |
| *Pgm-2c* | -0.002 | 0.020 | -0.007 | -0.018 | -0.084** | -0.067* | -0.037 | -0.005 | 0.014 | 0.012 |

*p<0.05, **p<0.01, ***p<0.001.

The distance classes are 0-5.0 m (class 1), 5.0-10.0 m (class 2), 10.0-15.0 m (class 3), 15.0-20.0 m (class 4), 20.0-25.0 m (class 5), 25.0-30.0 m (class 6), 30.0-35.0 m (class 7), 35.0-40.0 m (class 8), 40.0-45.0 m (class 9), and 45.0-50.0 m (class 10).

50 m apart on average.

## Discussion

Although significant aggregation of an identical allele was partially observed at two loci for some classes, no spatial structure of allele frequencies was found for twelve polymorphic loci within the natural populations of alder. The results from this study are not completely consistent with the prediction that plant populations are subdivided into local demes or neighborhoods of related individuals [3,13,15]. Previous reports on the local distribution of genetic variability[10,11,12,21] suggested that microenvironmental selection and limited gene flow are the main

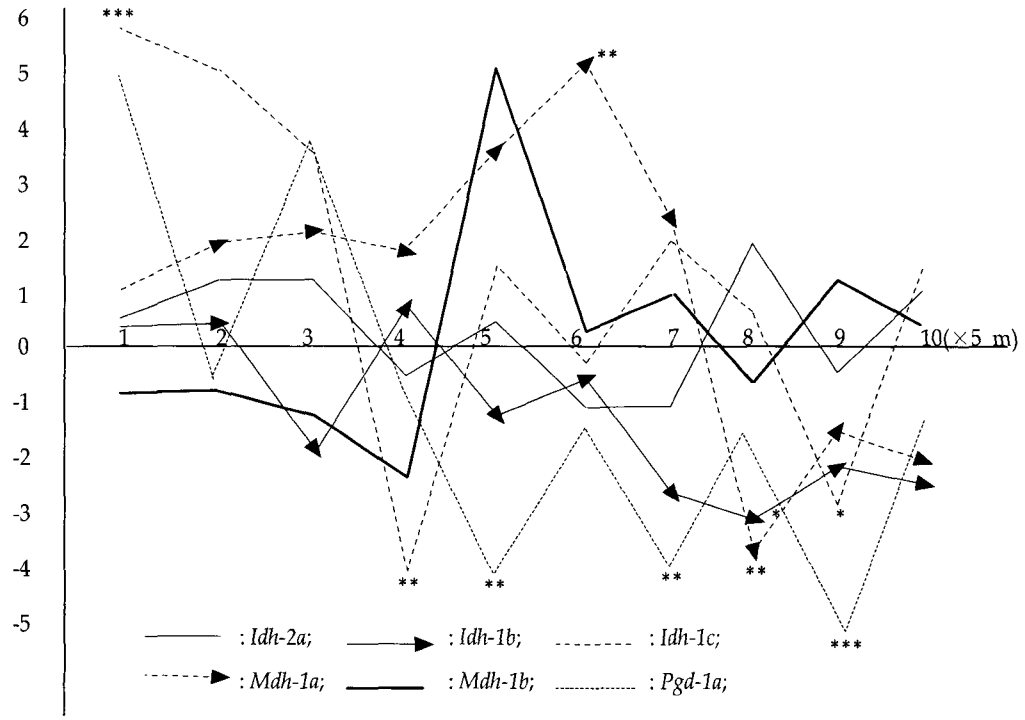


Fig. 1. Correlograms of autorrelation statistics for *A. hirsuta* as a function of distance. * and ** show significance at the 5% and 1% level, respectively.

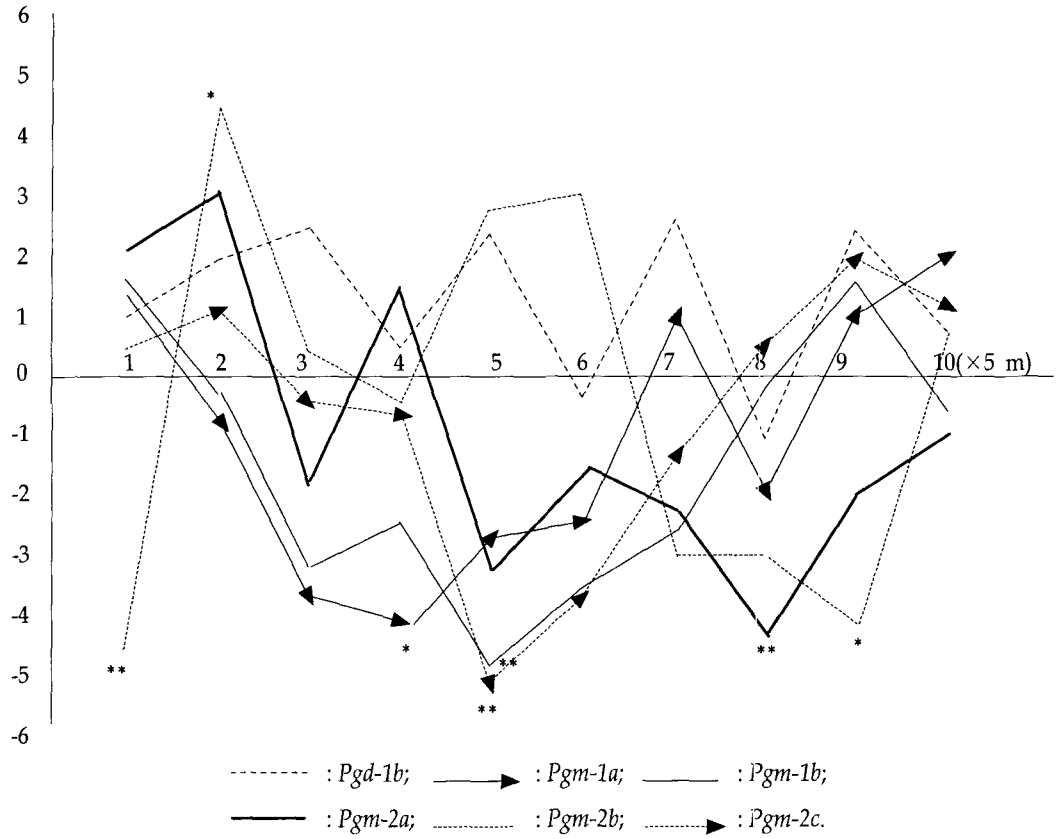


Fig. 2. Correlograms of autorrelation statistics for *A. hirsuta* as a function of distance. * and ** show significance at the 5% and 1% level, respectively.

factors causing substructure of alleles within a population [11,12,21]. Local genetic differentiation at isozyme or other marker loci, caused by microenvironmental heterogeneity, has been observed in a variety of plant species[4]. Those loci showed a significant aggregation of an identical allele, and the aggregation which persisted for generations, as long as the same microenvironmental conditions persisted. This persistence was demonstrated, for example, in the *Got-1* locus in lodgepole pine[11] and for the genetic variation in quantitative traits of *Impatiens capensis*[1]. In the present study, the *Idh-2c* and *Mdh-1a* loci showed significant aggregation in the Korean alder population. These aggregation, however, did not persist at all loci. Thus, it rules out the possibility of excluding microenvironmental selection from as being the main because of allele aggregation.

The average Moran's *I* values for each distance class in this study apparently indicate that the Korean alder population apparently is less structured compared with the Canadian alder[14]. Possible contributing factors between Korean alder and Canadidian alder populations include differences in density, topography, and human interference. These populations were distributed with men used for firewood. In addition, stump sprouting ability also may contribute to the fact that the alder population is unusual in lacking spatial genetic structure. If gene flow is limited, at most loci where allele aggregation is observed in adult plant populations, allele aggregation also is expected also in a pollen cloud[6]. This phenomenon was observed at the Canadian inland sites, and supported the pattern of limited gene flow found in alder patterns[14]. On the other side, the Korean alder populations were located on mountain with slope and on rocky upland slopes. Alder in Korea is used widely as a firewood for farmhouse. This occasional cutting of seed- bearing stems may bring cause high levels of gene flow.

## Conclusion

Why is the Korean population of alder is unusual, in that it lacks significant genetic structure at most spatial classes? A more likely explanation for the lacking spatial

structure is that gene flow has been sufficiently extensive enough to prevent the random divergence of local gene frequencies. Even a small amount of gene flow is sufficient enough to counteract the diversifying effects of genetic drift or weak selection[7,27]. In a recent simulations, Ohsawa *et al.*[18] and Epperson[10]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 ($Nm$ = 4.58) in Korean alder populations[Choi, unpublished]. The levels of gene flow that I have calculated in the present study are of sufficient magnitude to counterbalance genetic drift or weak selection, and thus playing a major role in shaping the genetic structure of the alder population[7,27]. Thus, high levels of gene flow caused by humans as well as insects may contribute to the lack of spatial genetic structure that in the Chengkwang population of Korean alder.

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## 초록 : 한국내 물오리나무(*Alnus hirsuta*) 집단의 공간적 상관관계

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한국내 물오리나무(*Alnus hirsuta*) 집단의 미소지리적 변이를 공간적 상관관계에 적용하였다. 분리된 등급간 대립유전자의 각 형태의 산출을 정규분포 편차 통계에서 임의분포를 하고 있는지를 검정하였다. 모든 경우에서 예상값이 유의성을 가지는지 Moran의 *I*값으로 추정한 결과 120 경우 중 24 경우(20.0%)에서 유의성을 나타내었다. 이들 값 중에서 17 경우는 음의 값을 나타내었는데 이는 한국의 물오리집단이 10등급 간격에서 유사하지 않다는 점을 시사하였다. 그 한 원인으로는 화목(火木)을 위한 인간의 간섭을 추정할 수 있다. 열매를 맺는 가지나 줄기의 간헐적인 벌목에 의해 유전자 유동이 조장되었기 때문일 것이다. 한편, 그루터기나 밑둥으로부터 영양번식을 할 수 있는 능력 또한 유전적 구조를 어지럽히는 한 요인으로 작용한 것으로 사료된다.