Comparison of Mating Systems in Populations of *Gleditsia japonica* var. *koraiensis*

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ABSTRACT: The mating systems of two groups of natural populations of *Gleditsia japonica* var. *koraiensis* in Korea were determined using allozyme analysis. The result suggests that *G. japonica* var. *koraiensis* is predominantly outcrossing. The *t*m values of eight populations in Korea varied from 0.667 (*Mdh-1*) to 0.938 (*Idh-1*), giving an average 0.820. Population and individual outcrossing estimates were associated with flowering tree density or degree of spatial isolation. The reason for relatively low outcrossing rates of some populations could be attributed to reduction of effective population sizes of sib for the medicine, small population size, and isolation of flowering mature trees. The heterozygote excesses were observed in some natural populations, whereas other populations exhibited varying degrees of inbreeding and heterozygotes deficit. Thus, selection against homozygotes operated in the progeny populations throughout the life cycle.

Key words: *Gleditsia japonica* var. *koraiensis,* Mating systems, Allozyme analysis

INTRODUCTION

Allozyme variation within plant populations often shows structured pattern in space, presumably reflecting kinship structure that has arisen through by distance due to restricted dispersals (Heywood 1991, Kang and Lee 2006). Once kinship structure is established, restricted seed and pollen dispersals lead to outcrossing between genetic relative, a phenomenon known as biparental in breeding (Uyenoyama 1986). Thus, even populations of obligate outcrossers may routinely experience some level of inbreeding (Heywood 1993). In addition, selfing rate, inbreeding depression, and relative fecundity have been estimated at different life history stages (Maki 1993).

Brown (1990) further classified plant mating systems into five major models: (1) predominant selfing (with outcrossing rate, $t < 0.1$); (2) predominant outcrossing (self-fertilizationrate, $s < 0.5$); (3) mixed selfing and outcrossing; (4) facultative or obligate apomixis; and (5) intragametophytic or haploid selfing.

The mating system is dynamic and can vary in space and time (Liengsiri et al. 1998). For instance, Murawski et al. (1994) reported that population and individual outcrossing estimates were associated with tree density or degree of spatial isolation. Maki (1993) reported the selfing rate or inbreeding in gynodioecious population of *Chionographis japonica* var. *kurohimensi*s. Murawski and Hamrick (1992) also reported tree-to-tree variation and year-to-year outcrossing rates in *Ceiba pentandra*.

The genus *Gleditsia* includes about 50 species of medium to large sized trees distributed throughout tropical and subtropical Asia, Africa, and America (Woodland 1991). *Gleditsia japonica* var. *koraiensis* is tree (> 7m in height) that is distributed in natural habitats of mountains. The species is associated with nitrogen fixing bacteria in rootlet nodules. *G. japonica* var. *koraiensis* is diploid, monoecious or rarely polygamous, and predominantly insect-pollinated that blooms from summer to early fall. Pods with heavy seeds usually dispersed by gravity.

G. japonica var. *koraiensis* has been used as an oriental medicine and is also an economically important for its stems, which has been used historically in Korea for household goods, because of the figured heartwood.

Until recently, most part of the Korean forest has been disturbed by cutting of trees and shrubs for firewood in rural areas (Kim et al. 2006). Now, majority of Korean forests has been revegetated both naturally and artificially. Moreover, uncontrolled and excessive plantation may lead to genetic isolation of once continuous natural populations, which may lose genetic diversity as a result of inbreeding and genetic drift following isolation.

In this study I investigated the mating system of eight populations samples across natural populations in Korea using isozyme markers.

MATERIALS AND METHODS

Sampling Procedure and Enzyme Electrophoresis

One leaf and ten pods per each individual of *G. japonica* var. *ko-*

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raiensis were collected from eight natural populations in Korea (Table 1). Enzymes were stabilized and extracted by the addition of a potassium phosphate buffer. In addition, enzymes from radicles of germinating seeds were collected and homogenized with phosphate buffer. Enzyme electrophoresis was performed using 12.0% starch gels. Buffer systems and enzyme staining procedures of Soltis et al. (1983) were used to assay eight enzyme systems; alcohol dehydrogenase (ADH), fluorescent esterase (FE), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), peroxidase (PER), 6-phosphogluconate dehydrogenase (PGD), and phosphoglucomutase (PGM). For enzymes resolved in more than one zone of activity, the most anodal isozyme was arbitrarily designated '1' and subsequent isozymes sequentially assigned higher numbers. Likewise, alleles were designated sequentially with the most anodally migrating allo-

zyme designated 'a' and progressively slower forms 'b', 'c', and so on.

Data Analysis

A locus was considered as polymorphic if two or more alleles were detected, regardless of their frequencies. Multilocus mixed mating program (MLT) was used to estimate single locus (ts) and multilocus outcrossing rates (tm) based on the mixed mating model of Ritland (1990). The model assumptions are: (1) each mating event is a random outcross event (with probability t) or self-fertilization (with probability $s = 1-t$), (2) the probability of an outcross is independent of maternal genotype, (3) outcross pollen allele frequencies are homogeneous among maternal genotypes, (4) selection does not occur between fertilization and the assay of progeny genotypes, and (5) alleles at different loci segregate independently (for multilocus estimates) (Liengsiri et al. 1998).

The observed inbreeding coefficient (F) of Wright (1965) was calculated for both progeny and parental populations as $F = 1 - (Ho/He)$, where Ho is the observed heterozygosity, $He = 1 - pi^2$ is the expected heterozygosity under random mating, and pi is the frequency of the ith allele (Liengsiri et al. 1998). The expected inbreeding coefficient at equilibrium (Fe) was calculated from multilocus outcrossing rate (tm) by the equation of Fyfe and Bailey (1951) as $Fe = (1 - tm)/(1 + tm)$.

RESULTS

For eight natural populations, single-locus estimates of outcrossing (ts) was 0.837 across 14 polymorphic loci, with individual population values ranging from 0.649 to 1.000 (Table 2). The ts

Table 1. Numeric code, population location, and sample size of *Gledsia japonica* var. *koraiensis*

Code	Location	Sample size (n)
N1	Mt. Jiri, Sancheong Pref.	45
N2	Mt. Odae, Myeongju Pref.	38
N3	Mt. Worak, Mungyeong Pref.	45
N4	Mt. Seorak, Yangyang Pref.	42
N5	Mt. Sobaek, Yeongpoong Pref.	47
N6	Mt. Deogyu, Muju Pref.	42
N7	Mt. Baegam, Baegam Pref.	55
N8	Mt. Baegun, Hamyang Pref.	36

Table 2. Single locus (ts) and multilocus (tm) outcrssing rate and inbreeding coefficient (F) for eight populations of *G. japonica* var. *koraiensis*

Pop.	*Adh*	*Pgd-1*	*Pgd-2*	*Me-1*	*Pgm-1*	*Pgm-2*	*Idh-1*	*Idh-2*	*Fe-2*	*Fe-3*	*Per-1*	*Per-3*	*Mdh-1*	Total
N1	0.804	0.883	0.955	0.778	1.000	0.935	1.000	0.830	0.918	0.767	0.816	0.895	0.843	0.879
N2	0.922	0.859	0.897	0.888	0.870	0.984	0.908	0.778	0.878	0.777	0.943	0.806	0.956	0.882
N3	0.878	0.786	0.863	0.705	0.655	0.829	0.943	0.649	0.838	0.710	0.932	0.714	0.935	0.803
N4	0.909	0.760	0.879	0.691	0.745	0.926	0.801	0.750	0.834	0.773	0.862	0.726	0.955	0.816
N5	0.874	0.812	0.900	0.710	0.910	0.996	0.932	0.845	0.700	0.803	0.820	0.892	0.875	0.851
N6	0.839	0.795	0.904	0.684	0.755	0.907	0.903	0.739	0.857	0.755	0.778	0.861	0.975	0.827
N7	0.963	0.820	0.879	0.702	0.676	0.918	0.915	0.902	0.768	0.684	0.800	0.875	0.925	0.833
N8	0.881	0.731	0.899	0.667	0.855	0.900	0.806	0.676	0.802	0.660	0.761	0.890	0.917	0.803
Mean	0.884	0.806	0.897	0.728	0.808	0.924	0.901	0.771	0.824	0.741	0.839	0.832	0.923	0.837
tm	0.907	0.825	0.914	0.784	0.667	0.900	0.938	0.712	0.846	0.770	0.889	0.837	0.667	0.820
F	0.049	0.096	0.045	0.121	0.200	0.053	0.032	0.168	0.083	0.130	0.059	0.089	0.200	0.099

Table 3. Wright's fixation indices for eight populations of *G. japonica* var. *koraiensis*

Pop.	Adh	Pgd-1	Pgd-2	Me-1	Pgm-1	Pgm-2	Idh-1	Idh-2	Fe-2	Fe-3	Per-1	Per-3	Mdh-1
N1	0.238	0.208	−0.064	0.213	−0.162	0.228	−0.267	0.163	0.324	0.224	0.116	0.000	0.325
N2	0.277	0.244	0.230	0.069	0.188	0.405*	0.100	0.332	0.277	0.386*	0.454*	0.215	0.300
N3	0.362*	0.188	0.039	0.433*	0.312	0.257	0.189	0.306	0.027	0.503*	0.244	0.181	0.584*
N4	0.085	0.432*	0.225	0.522*	0.246	0.296	0.093	0.244	0.335	0.205	0.504*	0.348	0.114
N5	0.241	0.278	0.243	0.218	0.238	-0.017	0.176	0.068	0.423*	0.411*	0.675*	0.455*	0.221
N6	0.165	0.211	0.267	0.170	0.122	0.137	0.232	0.540*	0.336	0.287	0.508*	0.319	0.120
N7	0.012	0.435*	0.043	0.319	0.343	0.175	0.281	0.557*	0.189	0.559*	0.374*	0.250	0.298
N8	0.151	0.256	0.129	0.254	0.269	0.116	0.269	0.431*	0.183	0.641*	0.369*	0.388*	0.339

*$p<0.05$.

were significantly heterogeneous in three (*Me-1, Pgm-1*, and *Idh-2*) of 14 loci (Table 2). Population N2 had the highest expected diversity (0.882), while population N3 had the lowest (0.803). Multilocus outcrossing rates (*t*m) within loci was 0.820. The expected inbreeding coefficient at equilibrium (*F*e) was 0.099.

Tests for deviations from mating system equilibrium revealed somewhat different results in all natural populations (Table 3). In some cases (primarily at *Fe-3* and *Per-1*), significant heterozygote deficiencies still occurred, suggesting that the presence of other forms of inbreeding in addition to consanguineous mating. In addition, there were heterozygosity observed in two populations (Mt. Jiri and Mt. Sobaek) as indicated by the negative inbreeding coefficient that ranged −0.017 (*Pgm-2*) to −0.267 (*Idh-1*) and it is not significant (Table 3). In contrast, most populations except Mt. Jirisan exhibited varying degrees of inbreeding and heterozygote deficit as indicated the positive inbreeding coefficients. *F* values were positive in most cases for eight natural populations (100/104), and 23.0% of those (23/100) departed significant from zero. Only four of indices were negative, indicating an excess of heterozygosity at *Pgd-2*, *Pgm-2*, and *Idh-1* for populations N1 and *Pgm-2* for populations N5, no one was departed significant from zero ($p<0.05$).

DISCUSSION

The results say that *G. japonica* var. *koraiensis* has intermediate outcrossing rate. Several authors suggested that intermediate outcrossing rates are evolutionarily unstable and they should evolve either to selfing or to outcrossing (Clegg 1980, Charlesworth and Charlesworth 1987, Holsinger 1991). According to these ideas, mating system of *G. japonica* var. *koraiensis* is expected to evolve into selfing or outcrossing.

Presence or absence of self-incompatibility mechanisms, availability of pollinators and their foraging behavior, and flower density and phonological variation are among the factors that affect the mating system (Brown and Allard 1970, Clegg 1980, Charlesworth and Charlesworth 1987, Escalante and Hamrick 1994). The mean value of *t*m of eight populations in Korea was 0.820. Other North American species of *Gleditsia triacanthos* also showed highly outcrossed mating system (Schnabel and Hamrick 1990). For example, one population of this species was equal to 1.0 (Schnabel 1988). Some Fabaceae species have a similar or lower *t*m than that observed in Korean *G. japonica* var. *koraiensis* populations, e.g. *Phaseolus coccineus* (*t*m = 0.592~0.698, Escalante et al. 1994), *Phaseolus vulgaris* (*t*m = 0.8~1.0, Escalante et al. 1994), *Hedysarum coronarium* (*t*m = 0.830, Yagoubi and Chriki 2000), *Senna multijuga* (*t*m = 0.540~0.838, Riberiro and Lovato 2004).

In this study, *t*m and *t*s varied among populations and revealed a habitat differences, with eight natural populations exhibiting high outcrossing rates. The low outcrossing rates of some populations could be attributed to destruction of habitat, low density, and isolation of reproductively mature trees. Especially, overexploitation of this species can be a major factor that affects the mating system in Korean populations. The life history characteristics of *G. japonica* var. *koraiensis* would lead one to predict the change of mating system. It should be noted that the number of age classes in two groups of populations examined is quite different. Natural populations on a plain are consisted of 10~15 different age classes, while natural populations at high mountain and secluded valleys are consisted of 30~45 years old plants and a few juveniles.

The observed high, significant, and positive *F* value indicates that homozygotes were significantly in excess. If significant deficiencies of heterozygosity for each polymorphic locus are present, this indirectly indicates the existence of inbreeding. Generally, seedling stages are expected to have higher levels of inbreeding than

found in adults (Holsinger 1991, Cruzan et al. 1994, Hall et al. 1994). This level of inbreeding can result from a variety of causes because *G. japonica* var. *koraiensis* is bisexual and mixed mating species: positive assortative mating (i.e., preferential mating among similar genotypes); selection for homozygotes; family structure within a restricted neighborhood, and causing mating among relatives. The significant deficiency of heterozygotes found in many populations may partly be due to the fact that there has been selection favoring homozygotes among populations. This may suggest that selection against homozygotes operated in the progeny populations throughout the life cycle. This allowed few inbred progenies to survive to the adult stage, resulting in more outcrossed adult trees. Selection in favor of heterozygotes typically occurs in more extreme environments. The reproductive strategy of *G. japonica* var. *koraiensis* could explain the observed inbreeding level. Because *G. japonica* var. *koraiensis* is polygamous species, it is expected that all of the inbreeding detected is due to consanguineous and self-mating. Nei et al. (1975) have shown that the reduction in average heterozygosity per locus depends not only on the size of the population bottleneck, but also on the subsequent rate of population growth. If population growth is reduced, reduction in average heterozygosity is large, even given a small number of founder.

LITERATURE CITED

Brown AHD. 1990. Genetic characterization of plant mating systems. In: Plant population genetics, breeding, and genetic resources (Brown AHD, Clegg MT, Kaher AL, Weir BS, eds), Sinaur, Sunderland, Massachusetts. pp 145-162.

Brown AHD, Allard RW. 1970. Estimation of the mating system in open-pollinated maize populations using isozyme polymorphisms. Genetics 66: 133-145.

Charlesworth D, Charlesworth B. 1987. Inbreeding depression and its evolutionary consequences. Ann Rev Eco and Syst 18: 237-268.

Clegg MT. 1980. Measuring plant mating systems. BioScience 30: 814-818.

Cruzan MB, Hamrick JL, Arnold ML, Bennet BD. 1994. Mating system variation in hybridizing irises effects of phenology and floral densities on family rates. Heredity 72: 95-105.

Escalante AM, Coello G, Eguiarte LE. 1994. Genetic structure and mating systems in wild and cultivated populations of *Phaseolus coccineus* and *P. vulgaris* (Fabaceae). Am J Bot 81: 1096-1103.

Fyfe JL, Bailey NTJ. 1951. Plant breeding studies in leguminous forage crops. 1. Natural cross breeding in winter beans. J Agri Sci 41: 371-378.

Hall P, Chase MR, Bawa KS. 1994. Low genetic variation but high population differentiation in a common tropical forest tree species. Conserv Biol 8: 471-482.

Heywood JC. 1991. Spatial analysis of genetic variation in plant populations. Ann Rew Eco Syst 22: 335-355.

Heywood JC. 1993. Biparental inbreeding depression in the self- incompatible annual plant *Gaillardia pulchella* (Asteraceae). Am J Bot 80: 545-550.

Holsinger KE. 1991. Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems. Am Nat 138: 606-622.

Kang HS, Lee DK. 2006. Stand structure and regeneration pattern of *Kalopanax septemlobus* at the national deciduous broad- leaved forest in Mt. Jeombong, Korea. J Ecol Field Biol 29: 17-22.

Kim CG, Choung Y, Joo KY, Lee KS. 2006. Effects of hillslope treats for vegetation development and soil conservation in burned forests. J Ecol Field Biol 29: 295-303.

Liengsiri C, Boyle TJB, Yeh FC. 1998. Mating system in *Pterocarpus macrocarpus* Kurz in Thailand. J Hered 89: 216-221.

Maki M. 1993. Outcrossing and fecundity advantage of females in gynodiecious *Chiongraphis japonica* var. *eurohimensis* (Liliaceae). Am J Bot 80: 629-634.

Murawski DA, Dayanandan B, Bawa KS. 1994. Outcrossing rates of two endemic *Shorea* species from Sri Lankan tropical rain forests. Biotropica 26: 23-29.

Murawski DA, Hamrick JL. 1992. Mating system and phenology of *Ceiba pentandra* (Bombacaceae) in central Panama. J Hered 83: 401-404.

Nei M, Murawama T, Chakraborty R. 1975. The bottleneck effect and genetic variability in populations. Evolution 29: 1-10.

Riberiro RA, Lovato MB. 2004. Mating system in a neotropical tree species, *Senna multijuga* (Fabaceae). Genet Mol Biol 27: 418-424.

Ritland K. 1990. A serious of FORTRAN computer programs for estimating plant mating systems. J Hered 81: 235-237.

Schnabel A. 1988. Genetic structure and gene flow in *Geledtsia tricanthos* L. Ph.D. Dissertation. Univ of Kansas, Lawrence.

Schnabel A, Hamrick JL. 1990. Organization of genetic diversity within and among populations of *Geledtsia tricanthos* (Leguminosae). Am J Bot 77: 1060-1069.

Soltis DE, Haufler H, Darrow DC, Gastony GJ. 1983. Starch gel electrophoresis of ferns: A complication of grinding buffers, gel and electrode buffers, and staining schedules. Am Fern J 73: 9-27.

Uyenoyama MK. 1986. Inbreeding and the cost of meiosis: the evolution of selfing in population practicing biparental inbreeding. Evolution 40: 388-404.

Woodland DW. 1991. Contemporary plant systematics. Prentice-Hall Inc., New Jersey, 582 pp.

Wright S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19: 395-420.

Yagoubi b, Chriki A. 2000. Estimation of mating system parameters in *Hedysarum coronarium* L. (Leguminosae, Fabaceae). Agronmie 20: 933-942.