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Genetic Variation and Conservation of the Endangered Species *Cotoneaster wilsonii* (Rosaceae) from Ulleung Island

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ABSTRACT: The genetic diversity plays a significant role in determining a species' survival and perseverance. Endangered species often lack genetic variation, which makes them vulnerable to numerous dangers of extinction including selection, genetic drifts and human interference. Knowing an endangered species' genetic background greatly enhances conservation efforts since it reveals why, what and how to conserve that species. *Cotoneaster wilsonii* is an endangered plant species endemic to Ulleung island, but not enough genetic research has been done on this taxon for its effective conservation plans. In this study, three populations of *C. wilsonii* in Ulleung island underwent allozyme analysis through starch gel electrophoresis. 10 loci were analyzed and F-statistics was calculated. Overall data indicated that *C. wilsonii* possessed low genetic diversity with intense inbreeding, heterozygote deficiency and low differentiation among populations. These results implied that *C. wilsonii* was recently introduced to the Ulleung island from ancestor species, and did not have much time to differentiate. Current status of *C. wilsonii* habitats is very fragile and vulnerable, with increasing tourism constantly threatening the species' survival. It is very likely that *C. wilsonii* will become extinct in near future unless organized conservation protects its populations and genetic diversity.

Keywords: *Cotoneaster wilsonii*, genetic variation, conservation.

Introduction

The knowledge of genetic variation is the key to the establishment of an effective conservation plan (Hamrick et al., 1991). Setting up a plan that includes all individuals in every population of a species is both impossible and wasteful. Population that is most genetically diverse has the highest probability of surviving since genetic diversity is directly connected to adaptive prospect of that population. In addition, it will have the largest portion of the genetic diversity existing within the species. Therefore, elucidating levels of genetic variation among populations is a good way to start for an effective and efficient conservation plan (Barret and Kohn, 1991). After enough genetic data have been gathered, the most genetically diverse population can be targeted for conservation, which will contain most of the genetic diversity and have the highest probability of survival and persistence.

Ulleung island is a pentagonal volcanic island located in the East Sea approximately 150 km from Korean peninsula. Created in Cenozoic period and about 1.8 million years old, it is a tholoide which mainly consists of trachyte, andesite, and basalt. It is 73 km^2 large, and the highest peak is Sunginbong (984 m) located in the center. Due to its volcanic characteristic, it has relatively little flat grounds, and its coasts are mostly high cliffs (Sun and Stuessy, 1998).

Along with its unique and limited environment, Ulleung island holds many rare and endangered species. More than 700 vascular plant species inhabit in Ulleung island, and 37 angiosperms among them are endemic species (Lee and Yang, 1981). Unfortunately, not enough research, monitoring and conservation plans have been done to effectively protect those species. Worsening the problem, increasing number of tourists and following harmful developments such as road and fence construction are constantly threatening those endangered species. In order to prevent further loss of species and environment, a research-based conservation plan is urgently needed.

Cotoneaster wilsonii is a member of genus *Cotoneaster* which is known to inhabit in some part of China and Russia. It was traditionally reported as one of the endemic species of Ulleung island, but recent studies suggested that *C. wilsonii* is indeed the same species as *C. multiflorus*, which is a widespread species in central and northern China (Kim et al., 2002). Whether *C. wilsonii* is endemic to Ulleung island or

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not is still a controversial issue.

Cotoneaster wilsonii is a deciduous species that grows up to 1.5m with oval, alternate leaves that have slender edges and some fur on the back side. It also has linear, needle-like stipules that range from 1 to 4 mm. It has white flowers whose petals are of circular shape and 3 mm long on average. There are two furless styles with shorter stamens around them. The flowers bloom between May and June, and the fruition occurs during September and October. Its fruit is red and round-shaped, approximately 6 mm in diameter. The main method of reproduction in *Cotoneaster* is apomixis though self and cross pollination through insect pollinators are possible (Bartish et al., 2001).

Currently, *C. wilsonii* is considered a first-class endangered species endemic to Ulleung island by Korean government. Kim et al. (2002) reported two populations found in Namyang and Tonggumy of *C. wilsonii* inhabiting in Ulleung island whose size and genetic diversity were very low. Indeed, the existing *C. wilsonii* populations were very small, and their habitats were open to destruction. No protection other than wooden panels indicating habitat of *C. wilsonii* was present, and it was very likely that current habitats would perish under increasing tourism. For example, one habitat near Songgotbong consisted of 20 or more individual *C. wilsonii*. But, the Songgotbong, which was a famous rock climbing site, was being visited by a large number of tourists whose careless activities might harm or pollute nearby *C. wilsonii* habitat. More natural causes also put *C. wilsonii* at stake. A habitat located in Namyang was a steep cliff, almost impossible for tourists to inflict any kinds of harm. Instead, a strong and violent sea wind could destroy remaining populations for the ground on which they inhabit was made out of dry and shallow soil. Likewise, various dangers of population loss and ultimate extinction were observed. However, virtually no conservation attempts have been conducted that could safely secure current populations due to lack of research that provided fundamental information about *C. wilsonii*.

Therefore, this study focuses on genetic analysis of three *C. wilsonii* populations found in Songgotbong, Namyang, and Dodong, in order to provide basic information about genetic variation of existing *C. wilsonii* populations, and rank the conservational significance among them. Using these data, this research aims for some future conservation plans to save this endangered species.

Materials and Methods

C. wilsonii sample groups of 20 individuals on average were obtained from three different sites within Ulleung island (Table 1). They were living mainly in cliffs, mountain sides, and other sloped areas. Population samples were mainly of young leaves and were stored inside an icebox until the experiment.

For running the starch gel electrophoresis, samples were pulverized and ground in porcelain dish using extracting buffer containing 0.1 M tris-HCl, pH 7.5, 1 mM EDTA (tetrasodium salt), 10 mM MgCl, 10 mM KCl, 14 mM 2-mercaptoethanol, and 5-10 mg/ml solid polyvinylpyrrolidone (Gottlieb, 1981). Four chromatography paper wicks approximately 15 mm long and 3-4 mm wide were dipped into each sample solution, and were stored under –60°C refrigerator before the actual electrophoresis.

Each wick was then processed through electrophoresis with 13% starch gel using two buffer systems. An electrode buffer

Table 1. Collection sites for three populations of *Cotoneaster wilsonii* for starch gel electrophoresis.

Number	Location	Number of individuals sampled
1(Songgotbong)	Mt. Songgotbong, Ulleung-do, Korea	16
2(Namyang)	Namyang, Ulleung-do, Korea	26
3(Dodong)	Dodong, Ulleung-do, Korea	21

Table 2. Summary allele-frequency data for ten polymorphic loci among three populations of *Cotoneaster wilsonii* in Ulleung island, Korea.

Locus	Songgotbong	Namyang	Dodong
MDH-1			
a	0.107	0.000	0.000
b	0.893	1.000	1.000
MDH-2			
a	0.909	0.773	0.917
b	0.091	0.227	0.083
GA3PD			
a	1.000	1.000	1.000
ME-1			
a	0.000	0.000	0.071
b	1.000	1.000	0.929
ADH-1			
a	1.000	1.000	1.000
6PGD2			
a	0.000	0.100	0.222
b	1.000	0.900	0.778
PGI-1			
a	1.000	1.000	1.000
ALD-1			
a	1.000	1.000	1.000
MNR-1			
a	0.133	0.500	0.667
b	0.867	0.500	0.333
PGM-1			
a	1.000	1.000	1.000

Table 3. Mean sample size per locus (N), mean number of alleles per locus (A), percentage of polymorphic loci (P), mean observed heterozygosity (H_O), and mean expected heterozygosity (H_E) in three populations of *Cotoneaster wilsonii* in Ulleung island, Korea.

Population	N	A	P	H_O	H_E
1. Songgotbong	10.1	1.3	30.0	0.040	0.061
2. Namyang	10.7	1.3	30.0	0.045	0.108
3. Dodong	10.4	1.4	40.0	0.017	0.113

of 0.065 M L-histidine and 0.007 M citric acid, adjusted to pH 6.5 with NaOH was diluted by 1 : 3 ratio for System I. Another electrode buffer of 0.18 tris, 0.1 M Boric acid, and 0.004 M EDTA, pH 8.6, also diluted according to 1 : 3 ratio was used for System II. The whole process of electrophoresis was carried out inside a refrigerator under –4°C. Gel systems were run at 40 mA for about 5 hours. Enzyme-activity staining and agarose overlays generally followed the protocols of Soltis et al. (1983). Loci and alleles were numbered sequentially and lettered alphabetically beginning with the most anodal form. Total of nine enzymes were assayed by using System I and II; glyceraldehydes-3-phosphate dehydrogenase (GA3PD), 6-phosphogluconate dehydrogenase (6PGD), malate dehydrogenase (MDH), aldolase (ALD) were assayed with System I, while menadione reductase (MNR), malic enzyme (ME), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM) and alcohol dehydrogenase (ADH) were resolved with System II.

The mean number of alleles per locus (A), percentage of polymorphic loci (P), average observed heterozygosity (H_O), and mean expected heterozygosity (H_E) of the 20 samples from each group were studied by using BIOSYS-1 program (Swofford and Selander, 1981). Also, to study the population differentiation, Wright's F-statistics were calculated. It includes F_{IS}, an index of inbreeding, F_{IT}, the overall inbreeding coefficient, and F_{ST}, a measure of the genetic differentiation among subpopulations (Wright, 1965). UPGMA tree was produced by input of Nei's genetic identity values into the BIOSYS-1 program.

The population with the highest values of genetic diversity would be the first target for future conservation. Also, the population with unique alleles or the one that is genetically farthest from the other populations would be considered genetically significant, and thus be targeted for the conservation, too.

Results

Out of 10 loci resolved, only locus MDH could be consistently resolved and scored for 2 interpretable loci (MDH-1 and MDH-2). Five loci (ME, MDH-1, MDH-2, PGD-2, MNR) out of ten were polymorphic, making 50% of the entire loci studied polymorphic. Total of 15 alleles were found at all loci combined, with the average of 1.5 alleles per locus and the average of 2 alleles per polymorphic locus. The allele a of locus MDH-1 was unique to population 1 (Songgotbong) while the allele a of locus ME was unique to population 3 (Dodong). Population 2 (Namyang) had no unique allele.

The mean number of allele per locus within each population ranged from 1.3 to 1.4, with the highest being the population 3 (Dodong) and the lowest being population 1 (Songgotbong) and 2 (Namyang). The Population 3 (Dodong) was most polymorphic with 40% of its loci examined having 2 alleles each. The rest had only 30% of their 10 loci evaluated polymorphic. The mean heterozygosity expected by Hardy-Weinberg equilibrium ranged from 0.061 to 0.113 and averaged 0.094. The mean heterozygosity observed ranged from 0.017 to 0.045 and averaged 0.034. All population showed lower observed heterozygosity than expected by Hardy-Weinberg equilibrium. This clearly indicated severe heterozygote deficit.

Observed genotype proportions were compared to those expected by the Hardy-Weinberg equilibrium by computing the fixation index F for each polymorphic locus in each population. Statistical difference of the fixation index from 0 was calculated by the chi-square test (Table 4). With three examined populations, ten tests could be validly conducted. Of the valid tests, four showed accordance to Hardy-Weinberg proportions while remaining six were significantly different from Hardy-Weinberg equilibrium. In every statistically significant cases, the value of F exceeded 0, indicating heterozygote deficiency.

To evaluate the magnitude of genetic difference among three populations of *C. wilsonii*, the method of F-statistics was used (Nei, 1977; Wright, 1978). F_{IS} value indicated the degree of divergence from Hardy-Weinberg expectations within individual populations. F_{IT} value indicated the degree of divergence from Hardy-Weinberg expectation over all population. F_{ST} indicated allele frequency difference among populations (Allendorf and Luikart, 2007). Although few negative values for F_{IS} and F_{IT} were found in some loci, total F_{IS} and F_{IT} averaged 0.622 and

Table 4. Values of the fixation index F for polymorphic loci in populations of *Cotoneaster wilsonii*. Consistency with Hardy-Weinberg equilibrium, i.e., statistical difference of fixation indices from 0, was evaluated using chi-square analysis and is indicated via asterisks.

Locus	Songgotbong	Namyang	Dodong
MDH-1	-0.120	------	------
MDH-2	-0.100	-0.294	-0.191
MNR	1.000*	1.000*	1.000
6PGD2	------	1.000*	1.000
ME	------	------	1.000*

*$P < 0.001$

Table 5. Summary of F-statistics at 9 loci from three population of *Cotoneaster wilsonii*. F_{IS}, an index of inbreeding, F_{IT}, the overall inbreeding coefficient, and F_{ST}, a measure of the genetic differentiation among populations.

Locus	F_{IS}	F_{IT}	F_{ST}
MNR-1	1.000	1.000	0.202
MDH-1	-0.120	-0.037	0.074
MDH-2	-0.200	-0.155	0.038
ME-1	1.000	1.000	0.049
6PGD2	1.000	1.000	0.086
Mean	0.622	0.671	0.128

Table 6. Mean values for Nei's (1978) unbiased genetic identity coefficient for pair-wise comparisons of three populations of *Cotoneaster wilsonii* in Ulleung island Korea.

Population	Songgotbong	Namyang	Dodong
1 Songgotbong	------		
2 Namyang	0.985	------	
3 Dodong	0.966	0.998	------

0.672, respectively (Table 5). This again signifies an ongoing heterozygote deficiency. In addition, a low mean F_{ST} value (0.128) indicates that there is much less differentiation among populations than within each population (Nickrent and Wiens, 1989).

Nei's unbiased genetic identity coefficients calculated in pairwise manner showed that the three populations of *C. wilsonii* were very similar to each other. The closest populations were Namyang and Dodong, whose Nei's unbiased genetic identity coefficient was 0.998. The farthest ones were Songgotbong and Dodong, whose coefficient was measured to be 0.966. A UPGMA phenogram based on genetic identity values were drawn to visualize genetic relationships among three populations of *C. wilsonii* (Fig. 1).

Discussion

Overall, *Cotoneaster wilsonii* displayed lower genetic diversity, as it usually is with endangered species. Its low number of alleles per locus (1.5) and number of alleles per polymorphic locus (2.0) indicated that genetic potential itself was quite low.

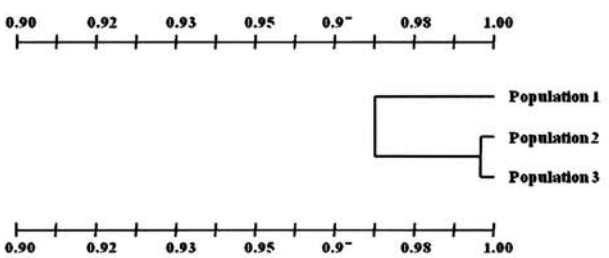

Fig. 1. UPGMA phenogram derived from Nei's genetic identity of three populations of *C. wilsonii*.

Additionally, significant deficit of heterozygosity was observed with all three populations. This also signified an intense inbreeding within each population.

Of the three populations of *C. wilsonii* studied, the population 3 from Dodong showed the highest genetic diversity values; its mean number of alleles per locus (1.4), percentage of polymorphic loci (40%), and heterozygosity expected by Hardy-Weingberg equilibrium (0.113) were higher than those of the rest. The population 3, as well as the population 1, had a unique allele on the locus ME-1. Conserving the genetic diversity of the population 3 would capture 14 alleles (93%) out of 15 documented from *C. wilsonii* in this research.

Although a deficit of heterozygote denoted that populations were inbreeding (Frankel et al., 1995), the F-statistics values for *C. wilsonii* suggested that there was a relatively small genetic difference among three populations, and diverged recently. The reason why *C. wilsonii* shows both the inbreeding pattern and the genetic similarity among populations is not certain, but it is possible that the first generation from the ancestor species arrived at the Ulleung island recently, and there has not been enough time for the founder population to expand and differentiate. It is also possible that the proper pollinators were not present, or the environment such as sea cliff was not optimal for *C. wilsonii* to prosper.

It is true that the population 3 turned out to be the most genetically diverse group, and deserve the priority of conservation as aforementioned, but the overall situation brought new points for conservation plan of *C. wilsonii*. The total population of *C. wilsonii* consisted of three separated ones, each having a little more than 30 individuals. This shows the species' very vulnerable status. Not only a future selection, but also minor damages from tourism could risk the species' survival and ultimately cause its extinction. For example, a new equipment store for rock climbing could be built near Songgotbong, and during the construction, the entire habitat might be destroyed. Similarly, a strong storm might hit Ulleung island as it always does every summer, and the population in Namyang area might perish overnight. Current size and genetic diversity suggest that almost anything could happen that would drive *C. wilsonii* to extinction. Therefore, the most appropriate conservation plan should consider both the protection of the current populations and increment of its size and diversity.

Protection of the current habitat should be the focus of in situ conservation. The habitat of population 1 near Songgotbong, which is a famous rock climbing site, needs not only physical barrier that could stop heedless tourists and climbers from trespassing the habitat, but also a visual sign that clearly inform tourists and local residents of the vulnerability of the habitat

of *C. wilsonii*. The habitat of population 2, located in Namyang region, is situated on the steep cliff, only accessible by climbing up the steep hill. There is a possibility that sudden strong sea wind or violent typhoon wipe the whole population off. A protection net or fence could stop violent wind from harming the species, but the cheaper and the more permanent measure would be introducing population 2 to a new, safer habitat. The population 3 is in relatively safer circumstances, and there is currently a banner notifying *C. wilsonii* habitat in front of it. Nonetheless, some sort of physical boundaries should be constructed in order to ensure preservation of each individual.

It is already known, and was again observed during collection in this research that *C. wilsonii* is capable of fruition although it reproduces through apomixes (Kim et al., 2002). This enables ex situ conservation of genetic diversity through transportation of seeds. Mainly focusing on seeds of population 3, genetic diversity of the total population should be captured by gathering seeds from three populations between September and October and sowing those seeds in ex situ condition. Also, population size increase could be done in ex situ conservation along with increment of diversity. After enough number of individuals and genetic diversity has been achieved, they can be transplanted back to the Ulleung island to make a new population, which would provide easier access to inter-populational cross with shorter distances between them, and increase the total population of the species, making the taxon genetically safer and more stable. Further research about the ecology of *C. wilsonii* is needed to create an effective environment for ex situ conservation and future rehabilitation.

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