

## GENETIC DIVERSITY OF RESTORED ENDANGERED SPECIES, *PENTHORUM CHINENSE* IN THE RIVERBED

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**ABSTRACT:** The Ministry of Land, Infrastructure and Transport has done the business of digging on the river road to increase the flowing quantity of Ibi River every year since 2000. It is reported to the dug region the *Penthorum chinense* populations which is the threatened species the restored in 2001. Digging is done from 2000 to 2010 every year. Therefore, we investigated *P. chinense* population of which extent to which region grew. As a result, it was shown some the growth of the plant of 200 individuals in the 2002 digging region, 4000 individuals in the 2001 digging region and 1000 individuals in the 2000 digging region, and so on. Though it is thought that the population formed in 2000 is seed bank origin, whether the populations formed in 2001 and later the burial seed origin or formed with the seed of an existing population, it is uncertain. Then we sampled 8 populations of the species from these areas, and studied them for allelic variation at 16 enzyme loci. There was no significant correlation between the actual population size and genetic diversity parameters, suggesting that the effective population size was very small even for the large populations. However, population that restored at the riverbed and population that approved on the embankment were able to distinguish obviously in the populations that had been approved in 2002. As for the population approved to the embankment, it was shown that genetic variation was very high in the population approved to the riverbed thought it was hardly admitted.

**Keywords:** Population size, Burial seeds, Restoration, Endangered species, River management

### 1. INTRODUCTION

The Ministry of Land, Infrastructure and Transport has dug up the river in Ibi River (Center of Japan) for the flood prevention every year since 2000 to 2010. This area has been disturbed by flood damage, so the digging can make the flow rate of the river increased and stop a flood, and provide the low marsh area in the river. In the new marsh area by the 2000 digging it was observed that a population of *Penthorum chinense* recovered in 2001. From 2000 to 2010 the digging was done by The Ministry of Land, Infrastructure and Transport every year, and new populations of *P. chinense* were appeared in new marsh area. And sometime new populations were appeared on the bared embankment. *P. chinense* has been described to the red list as vulnerable level (VU). A few studies reported that the populations of *P. chinense* have restoration by buried seeds [1]–[3]. In general, the populations restored it from the buried seeds are thought that genetic diversity is high. However, the character of the genetic diversities was hardly reported.

On the other hand, the natural population of *P. chinense* was observed in the river. The seeds of the species are so small that dispersed very long distance easily. It was suggested that the new populations were constructed by the seeds from natural population. Then, it paid attention to

genetic diversities of the *P. chinense* populations approved by digging in the river in this research. Then we investigated the genetic diversity of the species with three questions, 1) Is there a relationship between the location and genetic distance? 2) Are the new populations appeared from burial seeds? 3) Is there a relationship between population size and genetic diversity? It was paid attention to these three points and analyzed.

### 2. MATERIALS AND METHODS

#### 2.1 The Study Site and field census

The study was carried out on the Ibi River at Gifu Pref. (from 35° 20' 25''N, 136° 38' 92''E, altitude 9.3 m to 35° 17' 50''N, 136° 37' 7''E, altitude 0.2 m). The Ibi River is very steep and wide range river system, water system of the extension 121km and 1840 km<sup>2</sup> in the valley area. Because this river region had frequently received the flood damage, bank repair and a river dredge are continuing. Our study was done the area 36.2km to 32.3km from the mouth of the river (Table 1).

We counted the number of *P. chinense* on 20<sup>th</sup>, Oct. in 2004, when the leaves of the species turned red and yellow, so we can find the species easily to

find out.

## 2.2 The Study Plant

*Penthorum chinense* Pursh (Fig. 1) is a polycarpic herb plant distributed in eastern Asia [4], but its population has been decreasing in Japan and Red Data Book of Japanese vascular plants listed the species a 'vulnerable' level species [5][6]. This species is often distributed in marsh river flood plain, salt marsh area, wetland around paddy fields, and abandoned paddy fields in Japan. The species reproduces both sexually by seeds and vegetatively by rhizomes [7].

Table 1 The characters of study site, established year, habitat condition, the distance from the mouth of the river

Study site	Established year	habitat	distance (km)
A	2001	Marsh	32.2-32.6
B	2002	Bank	32.6-33.0
C	2002	Marsh	32.6-33.0
D	2003	Bank	34.6-35.8
E	2003	Marsh	34.6-35.8
F	2002	Marsh	32.8-33.8
G	2002	Marsh	36.2-37.0
H	Natural	marsh	18.8



Fig. 1 The flowers of *P. chinense*.

## 2.3 Electrophoresis

Fresh leaves were collected from 30 individuals per population at August in 2003. Leaves were kept on ice during 4 hours transportation to the laboratory, after which they were refrigerated 2 days until electrophoresis. The following enzyme systems were examined: malate dehydrogenase (MDH), phospho-glucose isomerase (PGI), phospho-glucumutase (PGM), aldolase (ALD), triose-phosphate isomerase (TPI), alcohol

dehydrogenase (ADH), acid phosphate (ACP), isocitrate dehydrogenase (IDH) and malic enzyme (ME). Leaves were used to resolve the following 16 putative loci: mdh-1, mdh-2, mdh-3, pgi-1, pgi-2, pgm, ald, tpi-1, tpi-2, adh, acp-1, acp-2, acp-3, idh, me and mr. Samples were ground in a cold extraction buffer described by Odrzykoski and Gottlieb [8]. The enzymes were resolved on 10.8% starch gel. System 5 of Soltis et al. [9] were used. Staining procedures followed previous works [9]-[11].

## 2.4 The Statistical analysis

For each populations we calculated the number of alleles per locus ( $A$ ), percentage of polymorphic loci ( $P$ ), and gene diversity ( $h$ ). We used all loci data in the calculation of  $A$ , and regarded a locus as polymorphic if the frequency of its most frequent alleles is under 0.95. In addition, total gene diversity [12] was calculated for species level. The population genetic structure was analyzed by initially calculating Nei's  $G_{ST}$  value [12]. Values for genetic identities ( $I$ ) and standard genetic distance ( $D$ ) were computed for each pairwise comparison of all populations. The neighbor joining method [13] based on  $D$  was used for constructing a phenogram for *P. chinense*.

## 3. RESULTS

### 3.1 Field Census

The number of individuals was shown in Table 2. The number of individuals ranged from 85 to 11000.

Table 1 This is the example for table formatting

Population	A	B	C	D	E	F	G	H
No. of individuals	412	45	950	120	1780	850	312	52

### 3.2 Genetic Diversity

Sixteen loci were scored: mdh-1, mdh-2, mdh-3, pgi-1, pgi-2, pgm, ald, tpi-1, tpi-2, adh, acp-1, acp-2, acp-3, idh, me and mr. In all populations, mdh-1, pgi-1, pgi-2, adh, acp-1 and acp-2, six loci were polymorphic. In all population mdh-3, tpi-2 and acp-3 were monomorphic. Allele frequencies at the polymorphic loci are listed in Appendix 1.

Table 3 summarizes the resultant values of  $A$ ,  $P$  and  $h$  for each population. And total gene diversity ( $H_T$ ) of *P. chinense* was 0.487. The levels of genetic diversity in *P. chinense* was higher than that of other endangered species, for example, *Aster kantoensis* growing in the river bed were

1.53 (*A*), 0.36(*P*) and 0.142 (*h*) [14]. And other endangered species showed *A* (1.44 to 2.01), *P* (0.199 to 0.65) and *h* (0.037 to 0.43) [15]-[18].

Table 3 Mean number of polymorphic loci (*A*), proportion of polymorphic loci (*P*), and gene diversity within a population (*h*) at 16 loci for examined populations of *P. chinense*.

Population	<i>P</i>	<i>A</i>	<i>h</i>
A	0.538	2.385	0.210
B	0.769	2.385	0.286
C	0.923	2.615	0.286
D	0.769	2.846	0.343
E	0.692	2.538	0.334
F	0.692	2.154	0.383
G	0.846	2.385	0.413
H	0.769	2.077	0.208

### 3.3 Population Structure

The value of *I* and *D* was shown in Table 4. There is no relationship between real distance and genetic distance. It is suggested that new constructed populations result from burial seeds previous population. Fig. 3 showed the genetic distance among established populations and natural population. The genetic distance between the nearest populations were small, but the longest distant population H was not out of the phenogram.

Table 4 Estimated values of genetic identity (*I*) (upper) and standard genetic distance (*D*) (under triangle).

Population	A	B	C	D	E	F	G	H
A	-	0.976	0.982	0.943	0.959	0.924	0.892	0.930
B	0.024	-	0.990	0.954	0.965	0.942	0.919	0.953
C	0.018	0.010	-	0.962	0.974	0.940	0.918	0.952
D	0.057	0.046	0.038	-	0.980	0.936	0.917	0.936
E	0.041	0.035	0.026	0.020	-	0.956	0.937	0.958
F	0.076	0.058	0.060	0.064	0.044	-	0.964	0.968
G	0.108	0.081	0.082	0.083	0.063	0.036	-	0.965
H	0.070	0.047	0.048	0.064	0.042	0.032	0.035	-

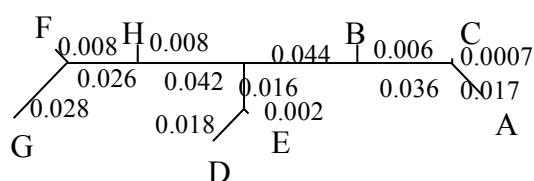


Fig. 2 Phenogram using the neighbor joining method based on Nei's (1987) standard genetic distance.

The result of total population genetic structure  $G_{ST}$  was 0.339. The value of  $G_{ST}$  suggested the high level differentiation was occurred. It is always observed long distance populations. (no differentiation: 0-0.05, low level differentiation: 0.05-0.15, middle level: 0.15-0.25, high level: 0.25-).

### 4. DISCUSSION

In this study, genetic diversity of the populations of *P. chinense* that restored by the excavating work of the river for the flood prevention was clarified. Because the flowing quantity of the river has been made constant by the construction of dams for decades, the disturbance in the river has been suppressed. Therefore, the transition of the vegetation of the river proceeds, and the growth of a peculiar plant in the river that depends on the disturbance has been suppressed. However, if the excavating work of the river was done at moderate frequency, the effect similar to the disturbance was brought, and the restoration of the endangered species can be done. Moreover, it was shown that it was thought that the approval of the populations by digging was a burial seed origin, and genetic diversity was also not low than that of endangered species.

Some highly suggestive research [3][19][20] showed that the soil seed bank could restore the genetic diversity. But the cost of the restoration is high. In our research, the adaptive moderate excavation can be restored the endangered species population with high genetic diversity. The Ministry of Land, Infrastructure and Transport conducted the excavation at winter season when the seedlings were not occurred. And the depth of excavation was 0 to 3m, with loose slop to the low embankment. So, the gradient with water containing was made. The condition could serve the adaptive condition of *P. chinense*. This is suggested that the design of excavation can support the habitat of disturbance dependent species.

In addition, restored populations surprisingly tended to be different according to dug depth and place. It is thought that the populations is adjacent and approved can admit, and each population has been approved from a slightly different seeds at the age by the dug depth region. But sometimes excavation is very dangerous construction because the alien plants can easily invade [21]-[23]. But in

the study site, the accident was not occurred.

Three genetic diversity indexes were very high in all restored populations. Sometimes, endangered species has low genetic diversity and the cause of low genetic diversity lead to extinction vortex. But there is no relationship between population size and genetic diversity, so the condition of the genetic diversity is curious condition. The genetic diversity from the C population that was made at 2002 digging is higher than that of B in this study. The populations of B and C were at the position placed between two spur dikes. Spur dikes were made for the protection of the embankment. It lowers the current velocity of the river. Therefore, the piling up action works, the condition of being easy to retire from a lot of seeds arises. And there is a possibility that the populations with high genetic diversity restored it from the burial seed in population C. Because it is the population B approved to a position that is about 2m higher than C, the piling up of the seed is thought to be only an increase of the flowing quantity of the river though population B is located in similar, too. And the same condition was occurred between population D and E.

The real distance of the population C and F was shorter than that other population. But the genetic distance is very large, 0.058-0.060. So the real distance cannot affect the genetic structure. It suggested that the condition of excavation depth and time of seeds accumulation. And the natural population H is very close to population G, where is the farthest from natural population. Moreover all populations are high differentiation. This sort of differentiation is usually observed among islands [24].

It was shown that the *P. chinense* populations restored it by the river construction as a result of this research. Moreover, it was shown that the genetic diversity of restoration populations was different from that of the natural populations. It will be suggested that attention be necessary for the populations that appears when constructing it like this managing in the future.

## 5. CONCLUSION

We get three main conclusion.

- 1) There is no relationship between the real distance and genetic distance. The populations at the same position with different water level showed small genetic distances.

- 2) The genetic construction of natural population was very different from restored populations. So the restored populations appeared from burial seed banks.

- 3) There is no relationship between population size and genetic diversity.

Based on the conclusion, two proposals about the management method of the excavation.

- 1) The excavation for the flood management should be done with loose slope provide the different water level habitat.
- 2) The excavation should be done in winter when the endangered species is dormant.
- 3) The excavation area should not be large to avoid the uniformity is occurred, that can be provide the genetic diversity.

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