Genetic Relationships among Korean Brown Frog Species (Anura, Ranidae), with Special Reference to Evolutionary Divergences between Two Allied Species Rana dybowskii and R. huanrenensis

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Genetic Relationships among Korean Brown Frog Species (Anura, Ranidae), with Special Reference to Evolutionary Divergences between Two Allied Species *Rana dybowskii* and *R. huanrenensis*

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ABSTRACT—Allozyme analysis for 41 populations of brown frog species, *Rana dybowskii*, *R. huanrenensis*, and *R. amurensis* from Korea and three reference species (Chinese *R. chensinensis* and Japanese *R. dybowskii* and *R. tsushimensis*), were performed to clarify taxonomic status of Korean brown frogs. The level of average genetic differentiation (Nei's D) among local populations of each species in Korea was very low (D<0.012) and Korean and Japanese *R. dybowskii* also showed conspecific level of differentiation (D=0.070). Whereas, much larger, discrete genetic differences were detected in the interspecific comparisons (D>0.370). In the genetic relationships among five species examined, the 24 chromosome brown frogs (*R. dybowskii*, *R. huanrenensis*, and *R. chensinensis*) did not form a monophyletic group. *Rana dybowskii* with the chromosome number of 2n=24 was grouped together with *R. amurensis* with the chromosome number of 2n=26. The hypothesis of reversal change from 24 to 26 in Korean *R. amurensis* seems to better explain the phylogenetic relationships of east Asian brown frogs than the assumption of parallel reduction in chromosome number from 2n=26 to 24 in *R. dybowskii* and in the common ancestor of *R. huanrenensis* and *R. chensinensis*. The genetic, morphological, and reproductive divergences between Korean *R. dybowskii* and *R. huanrenensis* were compared.

Key words: allozyme, brown frog, chromosome number, genetic differentiation, phylogenetic relationship

INTRODUCTION

The Eurasian brown frogs are a morphologically conservative assemblage consisting of the Eurasian *Rana temporaria* and a large number of similar species considered to be related (Frost, 1985; Borkin and Kuzmin, 1988; Green and Borkin, 1993; Nishioka *et al*., 1992; Maeda and Matsui, 1999). The chromosome number of great majority of *Rana* species is 26 and most of brown frog species have the same number. Some of brown frogs, however, are unique in having diploid chromosomes of 2n=24 (Matsui, 1991; Green and Borkin, 1993; Xie *et al*., 1995). These 24 chromosome brown frogs include the European *R. arvalis* and several east Asian species allied to *R. chensinensis*, such as *R. dybowskii*, *R. ornativentris*, *R. pirica*, and *R. huanrenensis* (Kobayashi, 1962; Seto, 1965; Wu, 1982; Green, 1983; Luo and Li, 1985; Lee and Park, 1986; Ma, 1987; Wei *et al*, 1990; Liu *et al*., 1993; Green and Borkin, 1993; Xie *et al*., 1995; Lee and Lee, 1998). These east Asian brown frogs are quite similar in morphology, and are very difficult to identify (Nakamura and Ueno, 1963; Matsui *et al*., 1993, 1998; Xie *et al*., 1995; Yang *et al*., 2000). Indeed, most of them were originally described on the basis of slight morphological differences. Recently, taxonomic status of each species was made clearer by lines of additional information, such as considerable genetic divergences among them (Matsui, 1991; Green and Borkin, 1993; Tanaka-Ueno *et al*., 1998; Matsui *et al*., 1998; Kim *et al*., 1999; Yang *et al*., 2000). Although extensively studied in the laboratory (Kawamura *et al*., 1981), the direct evidence of reproductive isolation in the field among these allied species have never been reported

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because of their geographic isolation due to allopatric distribution.

Until recently, it has been reported that *R. dybowskii* (a 24 chromosome member) and *R. amurensis coreana* (a 26 chromosome member) are distributed in South Korea (Yang and Yu, 1978; Sengoku, 1979; Green and Borkin, 1993; Matsui *et al*, 1998). Most recently, we (Yang *et al*., 2000) reported a new Korean brown frog member (*R. huanrenensis* Fei, Ye and Huang, 1990) which was morphologically and karyologically (2n=24) very similar to *R. dybowskii*.

In this study, we investigate the degree of inter- and intraspecific genetic variation and to clarify the genetic relationships among three species of Korean brown frogs. For comparisons, Japanese *R. dybowskii* and *R. tsushimensis* and Chinese *R. chensinensis* are also incorporated to the analysis. In addition, we surveyed the levels of morphological, genetic, and reproductive divergence between sympatricsamples of *R. dybowskii* and *R. huanrenensis* from South Korea.

MATERIALS AND METHODS

Collection and field notes

Five brown frogs were collected from 41 localities in Korea, Japan, and China (Table 1). During most collecting trips in Korea and Japan, the notes and photographs on color pattern of each specimen and records of each breeding site were taken.

Protein Electrophoresis

For an electrophoretic examination, a total of 849 specimens belonging to 41 populations of five species were employed. These include 10 populations of *Rana huanrenensis* from South Korea, 17 populations of *R. dybowskii* from South Korea and Japan, 2 populations of *R. chensinensis* from China, 11 populations of *R. amurensis* from South Korea, and 1 population of *R. tsushimensis* from Japan (Table 1).

Live samples were transported to the laboratory and were stored at –70°C until use. In the laboratory, the tissues of liver, heart and skeletal muscle were removed from each specimens and homogenized by glass homogenizer in an equal volume of distilled water and were centrifugated at 18,000 rpm for 30 min at 4°C to obtain the supernatant for electrophoresis. Voucher specimens were fixed in 10% formalin, preserved in 70% ethanol, and deposited in Yang's collection at Inha University. The supernatant was subjected to horizontal starch-gel (12%) electrophoresis and histochemical staining procedures (Yang *et al*., 1997: Appendix I). Multiple loci were numbered sequentially, and alleles were designated alphabetically with "a" being the fastest migrant.

Individual genotypes were used to calculate allele frequencies for each population, these in turn were used to calculate matrices of genetic similarity (Rogers, 1972) and genetic distance (Nei, 1978). Three different methods were employed to infer relationships among populations. First, Nei's (1978) distance was clustered according to the UPGMA algorithm (Sneath and Sokal, 1973). Then, modified Rogers' distance (Wright, 1978) was analyzed by the Neighbor-joining (NJ) method (Saitou and Nei, 1987), and finally, we employed Felsenstein's (1993) DNAML procedure with allele frequencies for the maximum-likelihood (ML) analysis. These analyses were performed by use of BIOSYS-1 (Swofford and Selander, 1981) and PHYLIP vers. 3.5 C computer packages (Felsenstein, 1993).

Morphology

In order to detect morphological differences between Korean *Rana dybowskii* and *R. huanrenensis*, conditions of vocal sacs and the pattern of coloration on the body were examined for these two species.

RESULTS

Genetic variation and relationships among brown frogs

Genetic variation — By-products of 18 loci were scored from 13 enzymes and general proteins. Observed allelic frequencies are given in Appendix II.

Based on allelic frequencies listed in Appendix II, the degree of genetic variation of each population was estimated (Table 2). The genetic variability of *R. dybowskii* was P=26.0% (22.2–33.3%), Ho=0.118 (0.070–0.183), and He=0.122 (0.078–0.153). The genetic variabilities of *R. huanrenensis* and *R. amurensis* were P=22.2% (16.7–27.8%), Ho=0.063 (0.046–0.073), He=0.067 (0.058–0.081) and P=22.2% (5.6–33.3%), Ho=0.080 (0.029–0.120), He=0.086 (0.035–0.124), respectively. In Korean brown frogs, Kanseong population of *R. dybowskii* had the highest genetic variability (P=36.4%, Ho=0.165, He=0.165) while Koseong population of *R. amurensis* showed the lowest variability (P=9.1%, Ho=0.048, He=0.042). On the other hand, Chinese *R. chensinensis*, a reference species, showed P=22.3%, Ho=0.078, He=0.075. Another reference species Japanese *R. tsushimensis*, was more variable, with P=27.8%, Ho=0.094, He=0.097, than in Korean brown frog species.

Genetic relationships — Based on allelic frequencies listed in Appendix II, average genetic similarities (Rogers' S) and distances (Nei's D) among populations of five brown frog species were calculated (Appendix III). In the Korean brown frogs, *R. huanrenensis*, *R. dybowskii* and *R. amurensis*, the degree of genetic differentiation within a species was small (D=0.034: Appendix III), but differentiations among these Korean brown frogs were very distinct (D=0.584 between *R. huanrenensis* and *R. dybowskii*, D=0.788 between *R. huanrenensis* and *R. amurensis*, and D=0.500 between *R. dybowskii* and *R. amurensis*) due mainly to *Gp-4*, *aGpd*, *Mdh*, and *Ldh-1* loci that were ascertained as diagnostic among these Korean species.

When populations of 24 chromosome species from outside of Korea were included, genetic dissimilarities between *R. huanrenensis* (populations 1–10; Appendix III) and *R. dybowskii* (pops. 11–30) included fixed allelic differences at *Gp-4*, *Mdh*, and *Iddh* loci and diagnostic differences at the 95% confidence level (Ayala and Powell, 1972) at *Ldh-1* locus. *Rana huanrenensis* and *R. chensinensis* (pops. 28 and 29) included fixed allelic difference at *Ldh-1* locus and diagnostic differences at *Iddh*, *Aat-1*, and *Acoh*. Fixed allelic differences at *Gp-4*, *Ldh-1*, *Mdh*, and *Iddh* and diagnostic differences at *Aat-1* were found between *R. dybowskii* and *R. chensinensis* (Appendix II). Among populations of three brown frog species with 24 chromosomes (Appendix III),

Table 1. Collection localities, collection dates, and sample sizes (N) for electrophoretic and morphological analyses of *Rana huanrenensis*, *R. dybowskii*, *R. chensinensis*, *R. amurensis*, and *R. tsushimensis* from Korea, Japan, and China.

	Collection localities	Date	N
	Rana huanrenensis (2n=24)		
1.	Jangseong: Bukha-myon, Jangseong-gun, Chollanam-do	Mar. 29, 1995	27
2.	Pohang: Bokyung-sa, Pohang-shi, Kyongsangbuk-do	Apr. 25, 1997	28
3.	Cheongsong: Daejeon-sa, Cheongsong-gun, Kyongsangbuk-do	Mar. 28, 1997	30
4.	Yeongdeog: Namjung-myon, Yeongdeog-gun, Kyongsangbuk-do	Apr. 25, 1997	26
5.	Pyeongchang: Chinbu-myon, Pyeongchang-gun, Kangwon-do	May 19, 1994	8
6.	Hanso-ri: Hanso-ri, Bekjeon-myon, Jeongseon-gun, Kangwon-do	Apr. 27, 1995	29
7.	Oban-ri: Oban-ri, Dong-myon, Jeongseon-gun, Kangwon-do	Mar. 27, 1997	29
8.	Inje: Baekdam-sa, Buk-myon, Inje-gun, Kangwon-do	Apr. 14, 1995	7
9.	Kapyeong: Hwaak-ri, Buk-myon, Kapyeong-gun, Kyonggi-do	Apr. 4, 1998	16
10.	Donghae: Bicheon-dong, Donghae-shi, Kangwon-do	Feb. 26, 1998	30
	R. dybowskii (2n=24)		
11.	Yangpyeong: Yongmun-myon, Yangpyeong-gun, Kangwon-do	Apr. 3, 1997	16
12.	Cheongyang: Taechi-myon, Cheongyang-gun, Chungchongnam-do	Mar. 30, 1997	21
13.	Muju: Ansung-myon, Muju-gun, Chollabuk-do	Mar. 12, 1997	21
14.	Jangseong: Bukha-myon, Jangseong-gun, Chollanam-do	Mar. 29, 1995	33
15.	Kurye: Hwaom-sa, Kurye-gun, Chollanam-do	Mar. 6, 1994	14
16.	Haenam: Masan-myon, Haenam-gun, chollanam-do	Mar. 16, 1997	14
17.	Jeju: Sogwipo-shi, Jeju-do	Mar. 23, 1996	30
18.	Keoje: Shinhyun-up, Keoje-shi, Kyongsangnam-do	Mar. 15, 1997	27
19.	Hadong: Ssangkye-sa, Hadong-gun, Kyongsangnam-do	Mar. 15, 1997	25
20.	Yangsan: Naewon-sa, Yangsan-gun, Kyongsangnam-do	Mar. 10, 1995	29
21.	Donghae: Bicheon-dong, Donghae-shi, Kangwon-do	Mar. 21, 1997	29
22.	Inje: Baekdam-sa, Buk-myon, Inje-gun, Kangwon-do	Apr. 14, 1995	14
23.	Kanseong: Kanseong-up, Koseong-gun, Kangwon-do	Apr. 15, 1995	7
24.	Keojin: Keojin-up, Koseong-gun, Kangwon-do	Mar. 2, 1997	28
25.	Kapyeong: Hwaak-ri, Buk-myon, Kapyeong-gun, Kyonggi-do	Apr. 4, 1998	13
26.	Wonju: Chiak-Mt., Wonju-shi, Kangwon-do	May 23, 1997	10
27.	Tsushima Isl.: Tokoya, Tsushima-Isl., Nagasaki-pref., Japan	Mar. 9, 1998	1
	R. chensinensis (2n=24)		
28*.	Ningxia Hui: Yinnan-pref., Ningxia Hui-prov., China	—	5
29*.	Qinghai: Haidong-pref., Qinghai-prov., China	—	5
	R. amurensis (2n=26)		
30.	Haenam : Masan-myon, Haenam-gun, Chollanam-do	Mar. 16, 1997	26
31.	Kangwha : Naega-myon, Kangwha-gun, Incheon	Apr. 4, 1997	30
32.	Sorae : Sorae, Shihung-shi, Kyonggi-do	May 22, 1995	5
33.	Yangpyeong : Yongmoon-myon, Yangpyeong-gun, Kyonggi-do	Sep. 26, 1997	20
34.	Cheongju : Sangdang-dong, Cheongju-shi, Chungchongbuk-do	Mar. 12, 1997	30
35.	Yeongdong : Chupungryong-myon, Yeongdong-gun, Chungchongbuk-do	Sep. 28, 1997	30
36.	Cheongyang : Chongsan-myon, Cheongyang-gun, Chungchongnam-do	Mar. 29, 1997	30
37.	Yangsan : Changan-up, Yangsan-gun, Kyongsangnam-do	Mar. 10, 1995	6
38.	Kyeongju : Kangdong-myon, Kyeongju-shi, Kyongsangbuk-do	Jun. 20, 1997	35
39.	Kangnung : Yuchon-dong, kangnung-shi, Kangwon-do	Sep. 27, 1997	30
40.	Koseong : Keojin-up, Koseong-gun, Kangwon-do	Mar. 21, 1997	25
	R. tsushimensis (2n=26)		
41.	Tsushima Isl.: Tokoya, Tsushima-Isl., Nagasaki-pref., Japan	Mar. 9, 1998	10

* Part of frozen tissues (RM 5176, 5178, 5180, 5308, 5309, 5431, 5432, and 5435, and TP 19669 and 19670) deposited in the MVZ (Museum of Vertebrate Zoology, University of California, Berkely).

average genetic distances among local populations of a single species were low (D=0.008 in *R. huanrenensis*, D=0.005 in Korean *R. dybowskii*, D=0.070 in Korean and Japanese *R. dybowskii*, D=0.053 in *R. chensinensis*), whereas the average genetic distances among three species were distinctly high (D=0.584 between *R. huanrenensis* and *R.*

Table 2. Genetic variation of 41 populations in *Rana huanrenensis*, *R. dybowskii*, *R. chensinensis*, *R. amurensis* and *R. tsushimensis* from Korea, Japan, and China.

Population	N	Mean N of Alleles (N)	% Polymorphism (P)	Mean Heterozygosity	
				Observed (Ho)	Expected (He)
Rana huanrenensis					
1. Jangseong	27	1.3	27.8	0.064	0.080
2. Pohang	28	1.6	22.2	0.046	0.060
3. Cheongsong	30	1.5	22.2	0.065	0.065
4. Yeongdeog	26	1.4	22.2	0.073	0.060
5. Pyeongchang	8	1.4	27.8	0.069	0.072
6. Hanso-ri	29	1.6	16.7	0.050	0.058
7. Oban-ri	29	1.6	16.7	0.073	0.066
8. Inje	7	1.3	22.2	0.063	0.081
9. Kapyeong	16	1.4	27.8	0.066	0.066
10. Donghae	30	1.6	16.7	0.063	0.058
R. dybowskii					
11. Yangpyeong	16	1.7	22.2	0.108	0.113
12. Cheongyang	21	1.9	22.2	0.138	0.120
13. Muju	21	1.8	22.2	0.116	0.131
14. Jangseong	33	2.0	22.2	0.121	0.131
15. Kurye	14	1.6	27.8	0.095	0.117
16. Haenam	14	1.4	27.8	0.087	0.118
17. Jeju	30	1.4	22.2	0.070	0.078
18. Keoje	27	1.6	27.8	0.130	0.122
19. Hadong	25	1.7	27.8	0.109	0.124
20. Yangsan	29	1.8	27.8	0.111	0.125
21. Donghae	29	1.9	22.2	0.113	0.109
22. Inje	14	1.6	22.2	0.119	0.120
23. Kanseong	7	1.5	27.8	0.183	0.153
24. Keojin	28	1.9	33.3	0.129	0.135
25. Kapyeong	13	1.7	27.8	0.120	0.123
26. Wonju	10	1.6	33.3	0.133	0.126
27. Tsushima Isl.	1	–	–	–	–
R. chensinensis					
28. Ningxia	5	1.2	16.7	0.078	0.067
29. Quing	5	1.3	27.8	0.078	0.083
R. amurensis					
30. Haenam	26	1.4	16.7	0.068	0.070
31. Kangwha	30	2.1	33.3	0.107	0.115
32. Sorae	5	1.2	16.7	0.067	0.057
33. Yangpyeong	20	1.6	33.3	0.094	0.100
34. Cheongju	30	1.6	27.8	0.100	0.103
35. Yeongdong	30	1.6	22.2	0.083	0.082
36. Cheongyang	30	1.7	27.8	0.089	0.108
37. Yangsan	6	1.5	33.3	0.120	0.124
38. Kyeongju	35	1.7	22.2	0.076	0.084
39. Kangnung	30	1.3	16.7	0.044	0.058
40. Koseong	25	1.2	5.6	0.029	0.035
R. tsushimensis					
41. Tsushima Isl.	10	1.3	27.8	0.094	0.097

dybowskii, D=0.386 between *R. huanrenensis* and *R. chensinensis*, and D=0.485 between *R. dybowskii* and *R. chensinensis*).

Between Korean *R. amurensis* (pops. 30–40) and Japanese *R. tsushimensis* (pop. 41), both with 26 chromosomes, genetic dissimilarities included fixed allelic differ-

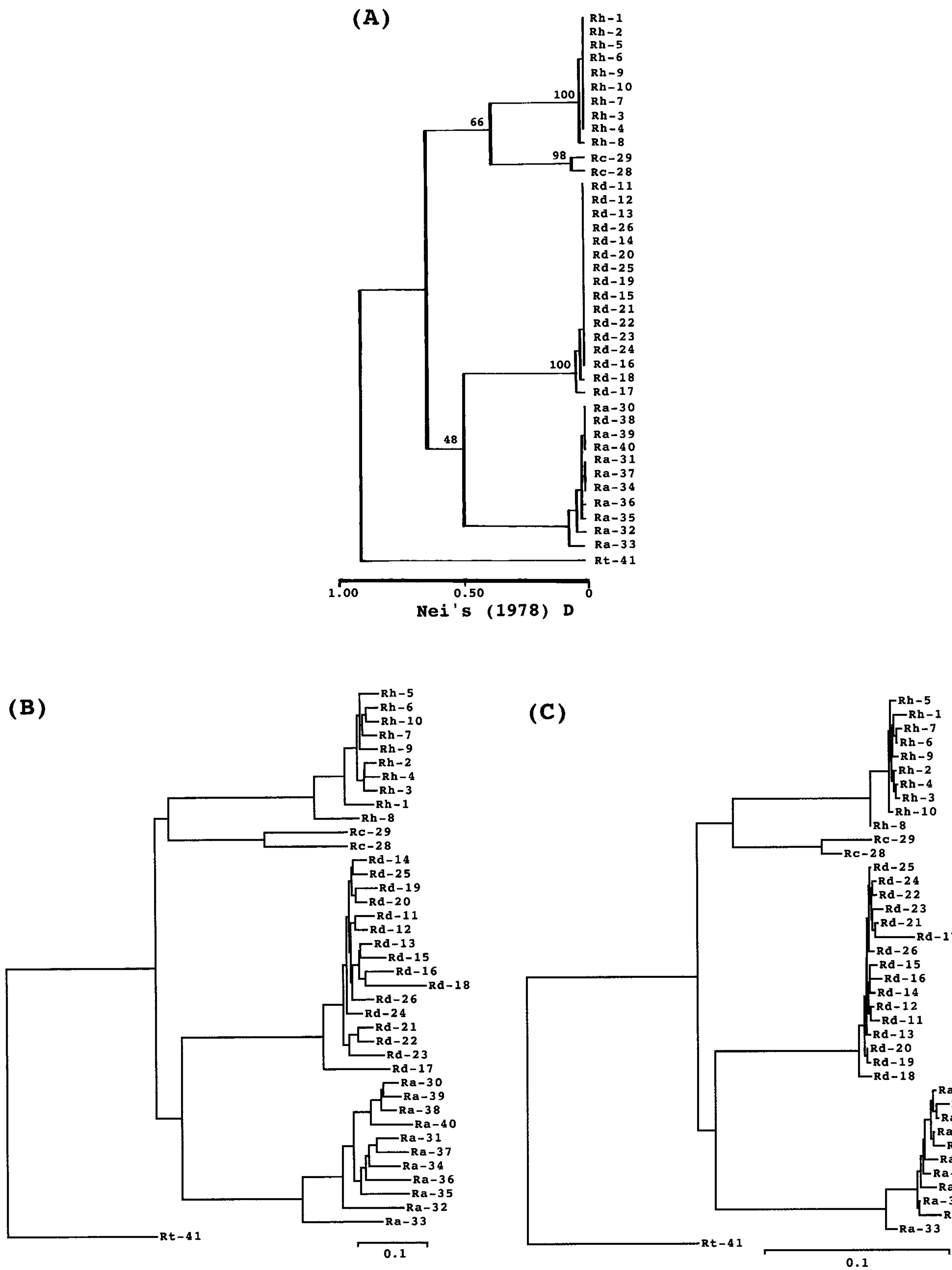

Fig. 1. A UPGMA tree (A), a neighbor-joining tree (B), and a maximum-likelihood tree (C) among a total of 41 populations of *Rana huanrenensis* (Rh), *R. dybowskii* (Rd), *R. chensinensis* (Rc), *R. amurensis* (Ra), and *R. tsushimensis* (Rt). For population number, refer to Table 1. Nodal values on the UPGMA tree (A) indicate percent support for branches in 100 bootstrap replicates.

ences at *Got-1*, *Gp-4*, *Idh*, *Sod*, *Ldh-1*, and *Ldh-2* loci and diagnostic differences (at the 95% confidence level) at *Mdh*, *Pgm-1*, and *Pgm-2* loci (Appendix II). The average genetic differentiation among these two 26 chromosome species were distinctly high (mean D=0.935).

Fig. 1A shows the UPGMA tree based on Nei's unbiased genetic distance. Although the bootstrap support for most of the nodes, except for monophyly of each species (not shown in the figure), was weak, *Rana tsushimensis* exhibited the earliest divergence among all populations examined. The remaining populations were divided into two distinct groups; One group included *R. huanrenensis* and *R. chensinensis*, and the other included *R. dybowskii* and *R. amurensis*. Topologies of NJ (Fig. 1B) and ML (Fig. 1C) trees based on modified Rogers' distance and allele frequenicies, respectively, were similar to that of UPGMA tree in that *R. tsushimensis* first diverged and *R. amurensis* and *R. dybowskii*, and *R. chensinensis* and *R. huanrenensis*, respectively, formed a separate subcluster.

Comparisons between *R. dybowskii* and *R. huanrenensis*

Morphology— Intraspecific morphological variation was much less notable than interspecific one. *Rana huanrenen-*

Fig. 2. Ventral views of *Rana huanrenensis* (A and B) and *R. dybowskii* (C and D) from South Korea in breeding season showing the grayshyellow throat and chest of male *R. huanrenensis* (A) compared to the milky-white throat and chest of male *R. dybowskii* (C) and the greenishyellow throat and chest of female *R. huanrenensis* (B) compared to the reddish-yellow throat and chest of female *R. dybowskii* (D).

Table 3. Morphological and ecological diagnostic characters between *Rana huanrenensis* and *R. dybowskii* in breeding season

Characters	*Rana huanrenensis*	*Rana dybowskii*
Female ventral color	minute black dots densely distributed over throat and yellowish green chest	red color patched over throat and chest
Egg mass nature	relatively small and tightly clustered	relatively large and loose
Egg deposition	egg mass attached on the submerged rock in montane streams	egg mass floating on still water mainly in rice field

sis was morphologically very similar to *R. dybowskii*, but differs from the latter in the ventral color pattern (Fig. 2). In males, *R. dybowskii* had a milky white ground (Fig. 2C), whereas the ground color of male *R. huanrenensis* was yellowish gray with minute black dots densely distributed over the throat and chest (Fig. 2A). In the breeding season, females of *R. huanrenensis* had throat and chest covered with yellowish green (Fig. 2B), whereas in females of *R. dybowskii*, the red color patched over the throat and chest, which color turned to black patches in alcohol (Fig. 2D). In addition to these differences in coloration, male *R. dybowskii* had paired internal vocal sacs, while male *R. huanrenensis* lacked vocal sacs.

Protein electrophoresis — *R. huanrenensis* and *R. dybowskii* showed a discrete genetic difference (Nei's D=0.585: Appendix III) and no evidence of gene flow between these two species was found in the sympatric areas surveyed (Jangseong, Inje, Kapyeong, and Donghae; see Table 1, Appendix II).

Ecological notes — *R. huanrenensis* is sympatric with *R. dybowskii* in some parts of South Korea such as Tonghae, Inje, Jangseong, and Kapyeong (see Table 1), and therefore, ecological comparison of the two species is pertinent. *R. dybowskii* altitudinary ranges very wide, from plains to montane regions, where they breed in still waters in rice fields and small pools in early spring. On the other hand, *R. huanrenensis* occurs only at valley in relatively high montane regions, where the species spawn on the rocks in streams. Eggs of the species laid in relatively small and tightly clustered egg mass, and each egg mass is attached on the submerged rock in small streams in early spring (Table 3).

DISCUSSION

The Eurasian brown frogs are very difficult to classify (Matsui, 1991; Green and Borkin, 1993; Tanaka-Ueno *et al*., 1998). Especially, members with 24 chromosomes are morphologically quite similar to each other and have a complicate taxonomic history, but now, taxonomic status of each member is made more clear than before by the presence of distinct genetic divergences among them (Matsui, 1991; Green and Borkin, 1993; Tanaka-Ueno *et al*., 1998; Matsui *et al*., 1998; Kim *et al*., 1999). It has long been known that the frogs with 24 chromosomes include several east Asian species allied to *R. chensinensis*, such as *R. ornativentris*, *R. dybowskii*, *R. pirica*. However, it has been known recently that *R. huanrenensis*, originally described from China (Fei *et al*., 1990), is also a member of this group (Xie *et al*., 1995) and co-occurs with *R. dybowskii* in South Korea (Yang *et al*, 2000). Before this finding, *R. huanrenensis* has been known only from the type locality, Huanren County, Liaoning Province, China for nearly 10 years. The significant range extention to Korea was recorded from localities that were well-known for the presence of *R. dybowskii* (Yang *et al*., 2000).

In South Korea, *R. huanrenensis* has been misidentified as *R. dybowskii* because of difficulties in identification. However, as shown in the present study, *R. huanrenensis* is actually well differentiated morphologically from *R. dybowskii* chiefly by the ventral color pattern. Moreover, males of these two species clearly different in the presence or absence of vocal sacs.

Since the separation of gene pools is the essence of species formation, a study of speciation must involve the examination of the level of reproductive isolation between the taxa compared. Allozymic analysis has been used extensively for such an examination at the zones of sympatry, and the contact zones of amphibian species that are problematic in taxonomic status (Wake *et al*, 1980; Yang and Park, 1988; Yang *et al*, 1988, 1997; Good, 1989). In our result, genetic divergence between *R. huanrenensis* and *R. dybowskii* included fixed allelic differences at *Gp-4*, *Mdh*, and *Iddh* loci, and these three loci are diagnostic genetic markers to identify them. No evidence of gene flow between these two species was found at the zone of sympatry. *R. huanrenensis* and *R. dybowskii* are completely isolated reproductively by their microhabitats, especially of the spawning site, and breeding habits. Particularly, the different condition of vocal sacs in males of the two species means the presence of clear differences of mating signals between them.

The east Asian brown frogs include two chromosomal groups (Kuramoto, 1979; Nishioka *et al*., 1986; Matsui, 1991; Green and Borkin, 1993). *R. dybowskii*, *R. huanrenensis*, and *R. chensinensis* have 2n=24 chromosomes, while *R. amurensis* and *R. tsushimensis* have 2n=26 (Lee and Park, 1986; Nishioka *et al*., 1986; Xie *et al*., 1995; Yang *et al*., 2000). It is generally believed that the fundamental chromosome number in *Rana* is 2n=26 (Morescalchi, 1973;

Wilson *et al.*, 1974; Kuramoto, 1979, 1989; Schmid, 1980; Green, 1983; Park, 1990). From the study of *R. dybowskii*, Green (1983) proposed that the karyotypes with 24-chromosomes could have arisen in east Asia, based on the location of secondary constrictions and chromosome bands. Meanwhile, from the banding patterns of Eurasian and North American brown frogs, Nishioka *et al.* (1986, 1987) similarly suggested the chromosome number reduction from 2n=26 to 2n=24. Chromosome evolution through reduction in number resulted from inversion/fusion has also been reported in other anuran species (King, 1990; Bogart and Tandy, 1981; Blommers-Schlosser, 1978). Considering this pattern of chromosome evolution as a single event, it could be presumed that the species with putative derived chromosome number (2n=24) form a monophyletic group. However, our results indicate that *R. dybowskii* with 2n=24 is genetically closer to *R. amurensis* with 2n=26 (D=0.500) than to *R. huanrenensis* (D=0.584) or to *R. chensinensis* (D=0.584) both with 24 chromosomes. Reflecting this situation, *R. amurensis* did not form a cluster, but was included in a cluster containing other brown frogs with 24 chromosomes in all the three trees we obtained.

These results imply that the interspecies relationships incidental to the chromosomal evolution are not in accordance with relationship inferred from genetic analyses. In view of our results, two assumptions of chromosomal evolution in brown frogs around Korea would emerge. One possibility is that the chromosome number reduction has evolved independently at least two times (parallel reduction in chromosome number from 2n=26 to 24). Namely, *R. tsushimensis* first differentiated from the common stock of brown frogs around Korea with 2n=26 chromosomes. Subsequently, through a reduction of primary chromosome number, divergence of an ancestor of the *R. huanrenensis* and *R. chensinensis* lineage (2n=24) occured from an ancestral species (2n=26) common to the Korean *R. amurensis* and *R. dybowdkii* lineage. Finally, speciation of *R. dybowskii* (2n=24) and Korean *R. amurensis* (2n=26) occurred while also accompanying a secondary chromosome number reduction in the *R. dybowskii* lineage.

Another possibility is that the common ancestor of all these four species, after diverged from *R. tsushimensis*, reduced the chromosome number from 26 to 24 before the separation of the *R. huanrenensis* and *R. chensinensis* lineage and the Korean *R. amurensis* and *R. dybowskii* lineage. Subsequent speciation of the latter lineage would have included the reversal change in chromosome number from 24 to 26 in Korean *R. amurensis*.

It is yet to be surveyed which of these two assumptions is more probable, but the first assumption parallels with the idea proposed by Green and Borkin (1993) or Nishioka *et al.* (1992) that *R. arvalis* with 2n=24 chromosomes is paraphyletic with east Asian brown frogs having the same 2n=24 chromosomes. However, there are strong disagreements between Green and Borkin (1993) and Nishioka *et al.* (1992). Green and Borkin (1993) suggested parallel reduction to 2n=24 in European *R. arvalis* and all east Asian species including *R. dybowskii*, but according to Nishioka *et al.* (1992), all east Asian brown frogs with 26 chromosomes, excepting *R. tsushimensis* but including *R. amurensis* and even European *R. temporaria*, have that number as a result of reversal change in chromosome number from 24 to 26.

The second assumption more conforms to Green and Borkin (1993) or Tanaka-Ueno *et al.* (1998). These authors considered Japanese *R. ornativentris*, with 24 chromosomes, represents the sister group of other east Asian species having 24 chromosomes. Including *R. ornativentris*, "the parallel chromosome number reduction" hypothesis needs three steps (reductions in *R. ornativentris*, *R. dybowskii*, and the *R. huanrenensis* and *R. chensinensis* lineage), but "reversal change in Korean *R. amurensis*" requires only two steps (one reduction in the common ancestor of all species with 24 chromosomes and one reversal in Korean *R. amurensis*).

Moreover, later divergence of *R. amurensis* among east Asian brown frogs, suggested by our result and Nishioka *et al.* (1992), strongly contradicts to the idea proposed by Green and Borkin (1993) from allozyme analyses and by Tanaka-Ueno *et al.* (1998) from the analyses of mitochondrial DNA. Both of these reports suggested the earliest divergence of Russian *R. amurensis* among east Asian brown frogs. Interestingly, Korean and Russian *R. amurensis* exhibit different degree of genetic differentiation between *R. dybowskii*; The genetic differentiation between Korean *R. dybowskii* and *R. amurensis* we obtained in the present study (D=0.500) was intermediate between those reported between Korean *R. dybowskii* and Russian *R. amurensis* (D=0.874) by Green and Borkin (1993) and between *R. dybowskii* from Tsushima and *R. amurensis* from Mongolia, China, and Russia (D=0.304-0.311) reported by Nishioka *et al.* (1992).

These genetic inconsistencies of Korean and Russian *R. amurensis* suggest a distinct taxonomic status of each population. In order to clarify the problem of chromosome number change, as well as the relationships of local populations of *R. amurensis*, more extensive studies including many more taxa from regions surrounding Korea are strongly required.

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Appendix I. Buffer systems and enzymes for the analysis of horizontal starch gel electrophoresis

Buffer System	E. C. No.*	Enzyme	Condition
Continuous tris citrate II (pH 8.0)	2.7.3.2	Creatine kinase (*Ck-1,2*)	100V/3 hrs
	1.1.1.42	Isocitrate dehydrogenase (*Idh*)	
	5.4.2.2	Phosphoglucomutase (*Pgm-1,2*)	
	1.1.1.14	Iditol dehydrogenase (*Iddh*)	
	3.4.11.1	Leucine amino-peptidase (*Lap*)	
LiOH (pH 8.1)	N. S.**	General protein (*Gp-3,4*)	250V/3 hrs
	1.1.1.37	Malate dehydrogenase (*Mdh*)	
Discontinuous tris citrate (pH 8.2)	2.6.1.1	Aspartate aminotransferase (*Aat-1*)	200V/3 hrs
	4.2.1.3	Superoxide dismutase (*Sod*)	
	1.15.1.1	Aconitate hydratase (*Acoh*)	
	1.1.1.27	Lactate dehydrogenase (*Ldh-1,2*)	
	5.3.1.8	Mannose-6-phosphate isomerase (*Mpi*)	
Tris maleic EDTA (pH 7.4)	1.1.1.8	Glycerol-3-phosphate dehydrogenase (*G3pdh*)	100V/4 hrs
	2.6.1.1	Aspartate aminotransferase (*Aat-2*)	

* E. C. No. : Enzyme commisson number

** N. S.: Non specific

Appendix II. Allele frequencies of 41 populations in *Rana huanrenensis*, *R. dybowskii*, *R. chensinensis*, *R. amurensis* and *R. tsushimensis* from Korea, Japan, and China.

Loci	*Rana huanrenensis*										*Rana dybowskii*		
	1	2	3	4	5	6	7	8	9	10	11	12	13
Acoh	b	a(0.02) b(0.98)	b	a(0.02) b(0.98)	b	b	b(0.96) c(0.02) d(0.02)	b	b	b	a	a	a
Ck-1	a	a	a	a	a	a	a	a	a	a	a	a	a
Ck-2	a(0.93) c(0.07)	a	a	a	a	a	a	a	a	a	a	a	a
Aat-1	h(0.91) j(0.09)	g(0.04) h(0.90) j(0.02) K(0.04)	h	h	h(0.81) j(0.06) K(0.13)	h	c(0.03) h(0.95) K(0.02)	h	h(0.94) j(0.06)	h(0.93) k(0.97)	b(0.03) c(0.03) h(0.59) i(0.03) j(0.06) k(0.26)	b(0.02) c(0.12) g(0.02) h(0.59) j(0.02) k(0.21) l(0.02)	c(0.12) h(0.57) j(0.02) k(0.29)
Aat-2	b	b	b	b	a(0.06) b(0.94)	a(0.03) b(0.97)	b	a(0.07) b(0.93)	b	b	b	b	b
Gp-3	c	c	c	c	c	c	b(0.02) c(0.98)	c	c	c	c(0.97) d(0.03)	c(0.98) d(0.02)	a(0.05) c(0.95)
Gp-4	d(0.07) e(0.93)	d(0.05) e(0.95)	d(0.03) e(0.95) f(0.02)	d(0.04) e(0.96)	e	d(0.02) e(0.96) f(0.02)	e	e	e	e	a	a(0.91) c(0.09)	a(0.95) c(0.05)
G3pdh	b	b	b	b	b	a(0.02) b(0.98)	a(0.03) b(0.97)	b	b	a(0.05) b(0.92) d(0.03)	b(0.47) d(0.53)	b(0.43) d(0.57)	b(0.55) d(0.45)
Idh	b	b	b(0.93) c(0.07)	b	b	b	b	b	b	b	b	b	b
Sod	c	c	c	c	c	c	c	c	c(0.97) d(0.03)	c	c	c(0.95) e(0.05)	c(0.95) e(0.05)
Lap	a	a	a	a	a	a	a	a	a	a	a	a	a
Ldh-1	e	d(0.02) e(0.98)	e	d(0.02) e(0.98)	e	d(0.03) e(0.95) f(0.02)	d(0.02) e(0.98)	e	d(0.06) e(0.94)	d(0.03) e(0.97)	b	b	b(0.98) c(0.02)
Ldh-2	d	d	a(0.03) d(0.97)	a(0.08) d(0.92)	d	d	d	d	a(0.03) d(0.97)	a(0.02) d(0.98)	d	d	d
Mdh	d(0.46) f(0.48) g(0.06)	d(0.32) f(0.59) g(0.09)	d(0.32) f(0.56) g(0.12)	d(0.44) f(0.50) g(0.06)	d(0.19) f(0.62) g(0.19)	d(0.22) f(0.62) g(0.16)	d(0.12) f(0.60) g(0.28)	d(0.36) f(0.50) g(0.14)	d(0.25) f(0.69) g(0.06)	a(0.02) d(0.22) f(0.56) g(0.20)	a(0.06) c(0.94)	c	c
Mpi	d(0.70) e(0.30)	b(0.04) d(0.94) g(0.02)	b(0.02) d(0.98)	b(0.06) d(0.94)	b(0.06) d(0.94)	d(0.97) e(0.03)	d(0.97) e(0.03)	d	b(0.09) d(0.91)	d	b(0.06) d(0.19) e(0.53) f(0.06) g(0.16)	b(0.12) d(0.07) e(0.58) g(0.21) h(0.02)	b(0.05) d(0.09) e(0.50) g(0.31) h(0.05)
Pgm-1	b	b(0.96) c(0.04)	b(0.87) c(0.13)	b(0.94) c(0.06)	a(0.06) b(0.94)	b	b	b(0.93) c(0.07)	b	a(0.02) b(0.98)	c	c	b(0.09) c(0.91)
Pgm-2	b	b	b	b	b	b	b	b(0.43) c(0.57)	b	b(0.98) c(0.02)	c	c	b(0.02) c(0.98)
Iddh	e	e	d(0.02) e(0.98)	e	e	d(0.09) e(0.91)	d(0.15) e(0.85)	e	d(0.09) e(0.91)	e	b	b(0.98) c(0.02)	a(0.02) b(0.98)

Appendix II. (Continued)

Loci	*Rana dybowskii*													
	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Acoh	a(0.92) b(0.08)	a	a(0.89) b(0.11)	a	a	a(0.94) b(0.06)	a(0.98) b(0.02)	a(0.98) b(0.02)	a	a	a(0.98) b(0.02)	a	a	a(0.50) b(0.50)
Ck-1	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Ck-2	a(0.97) b(0.03)	a	a	a	a	a	a	a	a	a	a	a	a	a
Aat-1	c(0.11) h(0.41) j(0.08) k(0.38) l(0.02)	c(0.14) h(0.61) j(0.07) k(0.18)	h(0.54) j(0.07) k(0.39)	b(0.08) d(0.25) h(0.57) k(0.10)	h(0.46) k(0.46) l(0.08)	c(0.26) h(0.48) k(0.26)	b(0.02) c(0.21) h(0.45) k(0.32)	c(0.02) f(0.02) g(0.02) h(0.31) i(0.02) k(0.60) l(0.01)	c(0.04) f(0.04) h(0.28) j(0.14) k(0.50)	f(0.21) h(0.36) k(0.43)	c(0.09) f(0.05) h(0.48) j(0.07) k(0.27) l(0.04)	c(0.08) f(0.04) h(0.39) j(0.08) k(0.41)	c(0.10) h(0.55) k(0.35)	c(0.50) h(0.50)
Aat-2	b	a(0.04) b(0.96)	b	b	b	b	b	a(0.02) b(0.98)	b	b	b	b	b	b
Gp-3	c	c	c	c(0.98) d(0.02)	c	c	c	c	c	c	c	c	c	c
Gp-4	a(0.97) c(0.03)	a	a	a(0.85) c(0.15)	a	a	a	a(0.88) c(0.12)	a(0.79) c(0.21)	a(0.71) c(0.29)	a(0.84) c(0.14)	a(0.96) c(0.04)	a(0.95) c(0.05)	a
G3pdh	b(0.42) d(0.58)	b(0.64) d(0.36)	b(0.54) d(0.46)	b(0.02) d(0.93) e(0.05)	b(0.83) d(0.17)	b(0.40) d(0.60)	b(0.43) d(0.57)	b(0.41) d(0.55) e(0.04)	b(0.46) d(0.54)	b(0.57) d(0.43)	b(0.39) d(0.59) e(0.02)	b(0.42) d(0.58)	b(0.40) d(0.60)	b(0.50) d(0.50)
Idh	b	a(0.07) b(0.93)	b	b	a(0.02) b(0.98)	b	b	b	b	b	b	b	b	b
Sod	c(0.97) e(0.03)	c	b(0.14) c(0.86)	c	a(0.02) c(0.98)	c(0.98) e(0.02)	a(0.02) c(0.96) e(0.02)	c(0.97) e(0.03)	c	c	c(0.98) e(0.02)	c(0.96) e(0.04)	c(0.95) e(0.05)	c
Lap	a(0.99) b(0.01)	a	a	a	a	a	a	a	a	a	a	a	a	a
Ldh-1	b(0.99) e(0.01)	b	b	b	a(0.02) b(0.98)	b(0.98) e(0.02)	b(0.98) e(0.02)	b	b	b	b	b	b	b
Ldh-2	d	d	d	d	d	d	d	d	d	d	d	d	d	d
Mdh	c	c	c	c	c	c	c	c	c	c	c(0.98) e(0.02)	c	c	c
Mpi	a(0.02) b(0.06) d(0.11) e(0.55) g(0.20) h(0.06)	b(0.14) d(0.07) e(0.50) g(0.25) h(0.04)	b(0.04) d(0.04) e(0.39) g(0.54)	e	d(0.02) e(0.46) g(0.45) h(0.07)	b(0.06) d(0.10) e(0.66) g(0.12) h(0.06)	b(0.03) d(0.07) e(0.54) g(0.29) h(0.07)	b(0.02) d(0.10) e(0.66) g(0.17) h(0.05)	b(0.04) d(0.18) e(0.64) g(0.14)	b(0.07) d(0.14) e(0.51) g(0.21) h(0.07)	b(0.11) d(0.09) e(0.61) g(0.19)	d(0.08) e(0.62) g(0.27) h(0.04)	a(0.05) d(0.10) e(0.45) g(0.35) h(0.05)	d
Pgm-1	c	c	c	c	b(0.39) c(0.61)	b(0.16) c(0.84)	b(0.10) c(0.90)	c	b(0.04) c(0.96)	c	b(0.07) c(0.93)	b(0.08) c(0.92)	c	c
Pgm-2	a(0.03) c(0.97)	c	c	c	c	c	c	c	c	c	c	c	c	c
Iddh	a(0.04) b(0.96)	a(0.07) b(0.93)	b	a(0.25) b(0.75)	b(0.93) c(0.07)	a(0.02) b(0.96) c(0.02)	a(0.03) b(0.94) c(0.03)	b	b	b(0.79) c(0.21)	b(0.93) c(0.07)	b(0.92) c(0.08)	b(0.85) c(0.15)	b

Appendix II. (Continued)

Loci	*R. chensinensis*		*Rana amurensis*										*R. t*	
	28	29	30	31	32	33	34	35	36	37	38	39	40	41
Acoh	a	a	a(0.83) b(0.17)	a(0.63) b(0.37)	a	a(0.40) b(0.60)	a(0.53) b(0.47)	a(0.40) b(0.58) c(0.02)	a(0.17) b(0.45) c(0.38)	a(0.58) b(0.42)	a(0.78) b(0.19) c(0.02) d(0.01)	a(0.93) b(0.07)	a	a(0.65) b(0.35)
Ck-1	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Ck-2	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Aat-1	g	b(0.10) g(0.90)	h(0.08) j(0.92)	g(0.04) h(0.04) j(0.90) l(0.02)	h(0.30) j(0.70)	h(0.15) j(0.85)	g(0.02) h(0.25) j(0.73)	j(0.92) l(0.08)	h(0.10) j(0.88) l(0.02)	g(0.08) j(0.84) l(0.02)	h(0.01) j(0.89) l(0.10)	j	j	a(0.35) e(0.65)
Aat-2	b	b	b	b(0.93) c(0.07)	b	b	a(0.05) b(0.95)	b	b	a(0.25) b(0.75)	b	b	b	a(0.15) b(0.85)
Gp-3	c	c	c	c	c	c	c	c	c	c	c(0.97) d(0.03)	c	c	c
Gp-4	e	e	d(0.98) e(0.02)	d	d	d	d	d	d	d	d	d	d	b
G3pdh	b	a(0.80) b(0.20)	c	c(0.97) d(0.33)	a(0.10) c(0.90)	b(0.05) c(0.88) d(0.07)	a(0.05) c(0.93) d(0.02)	c	a(0.05) c(0.95)	c	c	c	c	c(0.05) d(0.95)
Idh	b	b	b	b	b	b	b	b	b	a(0.08) b(0.92)	b	b	b	c
Sod	c	a(0.10) c(0.90)	d	d(0.96) f(0.02) g(0.02)	d	b(0.05) c(0.88) d(0.07)	d	d	d	d	d	d	d	e
Lap	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Ldh-1	f	f	c	c	c	c	c	c	c	c	c	c	c	e
Ldh-2	a(0.10) d(0.50) e(0.40)	d(0.90) e(0.10)	d	d(0.97) e(0.33)	d	d(0.95) e(0.05)	d(0.97) e(0.03)	b(0.02) d(0.96) e(0.02)	d	d	d	d	d	c
Mdh	f	f	e	b(0.02) e(0.95) g(0.02) h(0.01)	e	e(0.90) h(0.10)	b(0.02) e(0.98)	e	b(0.02) e(0.98)	e(0.92) g(0.08)	b(0.03) e(0.94) g(0.03)	e(0.90) g(0.10)	e	b
Mpi	d(0.80) e(0.20)	b(0.20) d(0.80)	e(0.13) g(0.37) h(0.06) i(0.13) j(0.21) k(0.10)	e(0.18) g(0.07) i(0.30) j(0.42) k(0.03)	i(0.80) j(0.20)	e(0.05) g(0.07) i(0.68) j(0.15) l(0.05)	e(0.13) g(0.07) i(0.55) j(0.25)	e(0.40) g(0.48) i(0.05) j(0.07)	e(0.13) g(0.15) i(0.32) j(0.38) k(0.02)	e(0.08) g(0.08) i(0.25) j(0.59)	d(0.01) e(0.27) g(0.37) i(0.20) j(0.15)	e(0.22) g(0.38) i(0.15) j(0.25)	g(0.20) i(0.06) j(0.56) k(0.18)	a(0.20) c(0.75) g(0.05)
Pgm-1	b(0.90) c(0.10)	b	c	b(0.08) c(0.88) d(0.04)	c	c	c	c	b(0.02) c(0.98)	c	c	c	c	b
Pgm-2	c	c	c	c(0.98) d(0.02)	c	c	c	c(0.95) d(0.05)	c	c	c	c	c	d
Iddh	d	d	d	b(0.02) d(0.98)	d	d	d	d(0.98) e(0.02)	d(0.88) e(0.12)	d	d(0.99) e(0.01)	d	d	d

Appendix III. Nei's (1978) genetic distance coefficients (above diagonal) and Rogers' (1972) genetic similarity coefficients (below diagonal) among 41 populations of *Rana huanrenensis*, *R. dybowskii*, *R. chensinensis*, *R. amurensis* and *R. tsushimensis* from Korea, Japan and China

population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
R. huanrenensis																				
1 Jangseong	–	.005	.007	.004	.007	.007	.011	.024	.006	.008	.573	.588	.556	.584	.566	.582	.640	.514	.558	.577
2 Pohang	.965	–	.000	.000	.000	.000	.003	.017	.000	.000	.581	.599	.565	.594	.575	.589	.670	.525	.574	.588
3 Cheongsong	.952	.978	–	.000	.001	.001	.004	.017	.002	.001	.576	.594	.563	.591	.570	.585	.664	.531	.573	.587
4 Yeongdeog	.963	.982	.977	–	.002	.002	.006	.017	.002	.002	.582	.600	.568	.598	.576	.592	.671	.531	.577	.591
5 Pyeongchang	.950	.975	.963	.963	–	.000	.001	.018	.000	.000	.592	.610	.575	.601	.586	.598	.683	.532	.582	.596
6 Hanso-ri	.952	.975	.971	.968	.971	–	.000	.018	.000	.000	.586	.605	.570	.601	.581	.596	.672	.530	.578	.594
7 Oban-ri	.942	.967	.957	.956	.964	.979	–	.021	.002	.001	.583	.601	.567	.597	.578	.594	.669	.527	.573	.589
8 Inje	.925	.946	.948	.947	.943	.943	.930	–	.019	.017	.510	.528	.499	.526	.504	.519	.595	.463	.505	.519
9 Kapyeong	.952	.972	.963	.966	.966	.978	.968	.930	–	.001	.590	.607	.574	.604	.583	.598	.679	.532	.581	.597
10 Donghae	.950	.977	.968	.967	.974	.976	.973	.945	.969	–	.587	.606	.573	.600	.585	.597	.672	.534	.578	.594
R. dybowskii																				
11 Yangpyeong	.547	.541	.538	.538	.539	.535	.541	.563	.538	.541	–	.000	.000	.001	.000	.005	.026	.022	.002	.002
12 Cheongyang	.538	.532	.529	.528	.530	.527	.532	.555	.531	.532	.973	–	.000	.000	.001	.005	.022	.024	.001	.000
13 Muju	.550	.545	.539	.538	.543	.540	.545	.567	.541	.544	.961	.969	–	.001	.000	.002	.033	.011	.002	.000
14 Jangseong	.539	.531	.525	.524	.530	.526	.530	.553	.527	.530	.960	.968	.961	–	.003	.004	.026	.022	.001	.000
15 Kurye	.550	.545	.543	.538	.547	.543	.544	.571	.539	.543	.961	.961	.961	.952	–	.004	.037	.015	.005	.003
16 Haenam	.534	.532	.526	.525	.530	.526	.530	.554	.528	.530	.952	.948	.955	.954	.947	–	.045	.015	.011	.004
17 Jeju	.515	.508	.503	.502	.504	.504	.509	.531	.502	.506	.915	.926	.904	.911	.906	.888	–	.075	.022	.027
18 Keoje	.571	.568	.564	.561	.566	.563	.567	.591	.563	.566	.919	.917	.937	.921	.933	.931	.864	–	.022	.016
19 Hadong	.546	.540	.535	.534	.538	.535	.540	.563	.535	.538	.955	.962	.958	.967	.947	.939	.913	.925	–	.000
20 Yangsan	.540	.535	.530	.529	.533	.530	.535	.558	.531	.534	.960	.968	.972	.973	.957	.951	.910	.939	.978	–
21 Donghae	.525	.519	.513	.512	.519	.515	.518	.543	.514	.518	.955	.966	.950	.965	.940	.944	.918	.917	.954	.958
22 Inje	.538	.531	.525	.524	.529	.525	.529	.553	.525	.529	.958	.959	.949	.958	.938	.938	.917	.916	.950	.954
23 Kanseong	.550	.545	.540	.539	.543	.540	.544	.567	.539	.543	.940	.945	.943	.943	.942	.931	.906	.916	.927	.940
24 Keojin	.546	.540	.534	.533	.538	.534	.538	.562	.535	.538	.959	.974	.960	.964	.949	.939	.927	.922	.966	.970
25 Kapyeong	.534	.528	.522	.521	.527	.523	.527	.551	.524	.527	.959	.969	.967	.974	.949	.949	.916	.938	.966	.979
26 Wonju	.538	.534	.528	.527	.532	.528	.533	.556	.530	.532	.959	.971	.967	.966	.952	.955	.918	.930	.955	.970
27 Tsushima Isl.	–	–	–	–	–	–	–	–	–	–	.903	.895	.891	.898	.895	.897	.845	.852	.899	.896
R. chensinensis																				
28 Ninxia	.653	.664	.659	.660	.665	.666	.671	.687	.674	.660	.599	.592	.599	.586	.599	.578	.566	.631	.596	.597
29 Quing	.611	.629	.616	.624	.631	.634	.641	.647	.648	.634	.589	.588	.589	.581	.580	.572	.572	.600	.590	.592
R. amurensis																				
30 Haenam	.439	.435	.430	.429	.431	.432	.437	.457	.436	.430	.600	.603	.600	.608	.600	.617	.587	.582	.594	.602
31 Kangwha	.452	.448	.444	.443	.447	.446	.449	.473	.450	.444	.580	.581	.584	.586	.578	.586	.569	.563	.584	.586
32 Sorae	.433	.432	.427	.425	.427	.428	.433	.454	.433	.427	.606	.608	.601	.602	.604	.602	.597	.579	.593	.601
33 Yangpyeong	.509	.507	.504	.504	.503	.503	.509	.529	.510	.503	.619	.620	.613	.623	.615	.624	.608	.592	.611	.615
34 Cheongju	.459	.455	.452	.451	.456	.456	.457	.483	.458	.452	.585	.587	.579	.589	.585	.592	.574	.557	.577	.581
35 Yeongdong	.460	.453	.450	.449	.449	.449	.455	.474	.455	.449	.579	.584	.581	.589	.578	.600	.566	.565	.572	.582
36 Cheongyang	.467	.463	.458	.456	.458	.459	.465	.484	.464	.458	.571	.573	.569	.577	.569	.580	.560	.549	.566	.572
37 Yangsan	.431	.429	.428	.422	.432	.429	.431	.458	.429	.424	.561	.564	.557	.568	.571	.571	.552	.539	.556	.561
38 Kyeongju	.441	.436	.431	.429	.432	.432	.439	.458	.437	.431	.605	.608	.604	.610	.602	.618	.591	.584	.596	.604
39 Kangnung	.431	.427	.421	.420	.422	.423	.429	.448	.427	.422	.606	.609	.605	.613	.606	.618	.593	.587	.600	.608
40 Koseong	.419	.417	.412	.411	.413	.414	.419	.439	.418	.412	.595	.597	.592	.594	.594	.596	.584	.573	.583	.593
R. tsushimensis																				
41 Tsushima Isl.	.430	.426	.422	.424	.429	.429	.433	.430	.429	.426	.372	.378	.375	.382	.367	.375	.390	.375	.389	.385

Population	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
R. huanrenensis																					
1 Jangseong	.610	.586	.561	.570	.588	.587	–	.385	.445	.813	.769	.827	.638	.751	.756	.738	.810	.809	.841	.872	.834
2 Pohang	.623	.598	.568	.584	.602	.593	–	.364	.417	.824	.784	.830	.648	.762	.779	.752	.819	.825	.855	.878	.841
3 Cheongsong	.621	.597	.565	.581	.602	.589	–	.377	.434	.826	.787	.828	.646	.759	.780	.751	.819	.827	.858	.882	.839
4 Yeongdeok	.628	.604	.572	.586	.607	.596	–	.378	.433	.835	.796	.837	.656	.769	.791	.762	.832	.837	.868	.890	.844
5 Pyeongchang	.627	.602	.574	.593	.609	.603	–	.363	.415	.836	.793	.846	.656	.774	.788	.760	.824	.836	.865	.890	.843
6 Hanso-ri	.631	.606	.576	.589	.609	.599	–	.356	.406	.830	.788	.834	.651	.763	.784	.757	.822	.831	.862	.887	.834
7 Oban-ri	.626	.601	.572	.586	.605	.596	–	.346	.393	.818	.777	.822	.642	.754	.773	.748	.811	.818	.847	.873	.816
8 Inje	.553	.530	.499	.514	.533	.522	–	.317	.369	.752	.714	.756	.582	.688	.712	.680	.740	.753	.783	.806	.840
9 Kapyeong	.634	.608	.578	.592	.612	.603	–	.348	.399	.816	.775	.823	.645	.753	.770	.744	.811	.818	.848	.870	.827
10 Donghae	.628	.604	.575	.590	.608	.599	–	.368	.411	.841	.799	.845	.660	.775	.794	.765	.834	.841	.872	.897	.838
R. dybowskii																					
11 Yangpyeong	.006	.005	.006	.001	.001	.000	.056	.466	.504	.492	.512	.483	.442	.507	.526	.544	.535	.489	.487	.507	.991
12 Cheongyang	.005	.004	.003	.000	.000	.000	.063	.475	.509	.487	.508	.481	.443	.503	.519	.540	.531	.483	.482	.504	.971
13 Muju	.006	.005	.004	.001	.000	.000	.062	.456	.498	.488	.512	.484	.446	.508	.522	.544	.536	.486	.484	.506	.971
14 Jangseong	.002	.002	.004	.001	.000	.000	.058	.480	.514	.486	.504	.486	.435	.502	.511	.532	.525	.481	.481	.503	.971
15 Kurye	.012	.010	.006	.004	.004	.002	.061	.462	.515	.490	.514	.484	.442	.508	.524	.544	.533	.488	.486	.507	1.015
16 Haenam	.010	.011	.008	.008	.003	.001	.068	.491	.533	.478	.505	.486	.440	.499	.501	.527	.524	.475	.475	.500	.996
17 Jeju	.029	.028	.034	.020	.024	.026	.109	.533	.535	.512	.523	.502	.461	.520	.535	.560	.552	.502	.504	.531	.947
18 Keoje	.024	.023	.018	.023	.016	.019	.093	.410	.473	.519	.542	.521	.478	.546	.551	.579	.570	.516	.514	.538	.971
19 Hadong	.007	.006	.010	.001	.000	.003	.059	.462	.491	.505	.518	.500	.452	.517	.531	.552	.545	.500	.501	.524	.940
20 Yangsan	.005	.005	.005	.001	.000	.000	.062	.464	.496	.492	.513	.491	.448	.512	.522	.546	.537	.488	.487	.510	.950
21 Donghae	–	.000	.002	.004	.000	.004	.077	.478	.513	.496	.514	.495	.450	.514	.523	.547	.535	.489	.489	.512	.981
22 Inje	.975	–	.000	.001	.000	.004	.071	.454	.492	.479	.496	.480	.433	.500	.508	.531	.520	.473	.471	.493	.975
23 Kanseong	.950	.959	–	.000	.000	.000	.074	.433	.479	.468	.490	.465	.421	.488	.501	.522	.510	.464	.463	.483	.979
24 Keojin	.965	.967	.952	–	.000	.000	.066	.460	.489	.477	.495	.474	.430	.494	.506	.528	.519	.472	.471	.494	.950
25 Kapyeong	.970	.967	.949	.975	–	.000	.071	.465	.499	.483	.503	.484	.442	.505	.512	.537	.528	.478	.476	.499	.953
26 Wonju	.958	.949	.952	.965	.974	–	.064	.474	.504	.478	.504	.475	.437	.499	.512	.534	.525	.476	.474	.496	.956
27 Tsushima Isl.	.884	.886	.873	.885	.886	.890	–	–	–	–	-	–	–	–	–	–	–	–	–	–	–
R. chensinensis																					
28 Ninxia	.589	.603	.614	.599	.596	.592	–	–	.053	.571	.568	.565	.513	.591	.616	.637	.598	.572	.567	.577	.803
29 Quing	.584	.592	.598	.594	.590	.589	–	.905	–	.524	.522	.509	.477	.541	.574	.582	.548	.527	.521	.527	.780
R. amurensis																					
30 Haenam	.601	.605	.614	.607	.603	.611	–	.548	.574	–	.007	.020	.074	.014	.014	.023	.010	.001	.001	.006	.929
31 Kangwha	.580	.588	.590	.592	.587	.583	–	.542	.567	.951	–	.019	.060	.005	.015	.012	.000	.007	.010	.012	.904
32 Sorae	.601	.608	.615	.610	.605	.611	–	.554	.584	.941	.922	–	.072	.012	.053	.041	.025	.021	.022	.025	.932
33 Yangpyeong	.616	.622	.628	.623	.617	.623	–	.574	.598	.888	.896	.888	–	.053	.073	.065	.066	.072	.082	.092	.935
34 Cheongju	.583	.587	.593	.590	.583	.589	–	.533	.560	.943	.955	.952	.917	–	.019	.012	.006	.013	.021	.028	.927
35 Yeongdong	.580	.584	.591	.586	.584	.591	–	.520	.544	.952	.940	.903	.898	.940	–	.017	.018	.010	.019	.036	.937
36 Cheongyang	.571	.578	.583	.579	.574	.578	–	.513	.540	.940	.946	.916	.893	.947	.939	–	.011	.021	.031	.036	.976
37 Yangsan	.564	.568	.574	.568	.563	.568	–	.518	.544	.936	.952	.908	.882	.943	.928	.930	–	.011	.013	.012	.940
38 Kyeongju	.603	.608	.616	.610	.606	.613	–	.546	.570	.978	.951	.934	.888	.942	.959	.938	.939	–	.001	.012	.928
39 Kangnung	.607	.612	.619	.613	.610	.616	–	.553	.578	.977	.943	.938	.878	.930	.944	.929	.934	.975	–	.006	.930
40 Koseong	.594	.601	.608	.600	.597	.603	–	.551	.577	.968	.941	.944	.870	.926	.929	.928	.934	.955	.972	–	.941
R. tsushimensis																					
41 Tsushima Isl.	.377	.377	.378	.387	.382	.383	–	.439	.451	.404	.423	.391	.402	.414	.399	.384	.414	.405	.396	.387	–