

Genetic Diversity and Speciation of *Rana rugosa* (Amphibia; Ranidae)

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Horizontal starch gel electrophoresis for 29 populations (n=543) of the wrinkled frog, *Rana rugosa*, from Korea and Japan was performed to assess the degree of genic variation and genetic diversity, and to understand the biogeographic pattern of distribution and speciation. A sum of 22 presumptive loci was screened from 17 enzymes and general proteins. Four loci, *Aco*, *Est-3*, *Me-2*, and *Pgm*, demonstrated high levels of polymorphism. The degree of average genetic variation of *R. rugosa* was $P=22.7\%$ (9.1-40.9%), $H_o=0.086$ (0.048-0.165) and $H_e=0.090$ (0.042-0.168). In the south-eastern region of the Korean peninsula (Chongsong, Yongchon, Ulsan, Kyongju, Pohang, Yongdok and Ulchin), a few unique alleles in the *Mpi* locus were detected and their biogeographic implications were considered. The degree of genetic differentiation among the Korean populations was moderate ($S=0.900$), whereas the degree of genetic diversity between Korean and Japanese populations was notably high ($S=0.687$, $D=0.293$). This result corresponds with the data obtained by the mitochondrial cytochrome *b* gene sequence (Lee et al., 1999) suggesting that the Korean and Japanese *R. rugosa* might have evolved a specific level of genetic differentiation since their geographic isolation.

The first reports on species-level isozyme variation in *Drosophila* by Lewontin and Hubby (1966) and the human population by Harris (1996), heralded an explosion of studies measuring the extent of such variation in a wide range of organisms. Almost thirty years of allozyme studies have demonstrated enormous variability among higher organisms in their levels of genetic polymorphism and heterozygosity (Selander et al., 1971; Selander, 1976; Yang and Patton, 1981; Yang, 1983; Park, 1988; Yang and Min, 1989; Min, 1991; Kim, 1992; Shim, 1994; Sumida and Nishioka, 1994; Yang et al., 1997a, b, c). It is possible to estimate the similarity or difference among populations by using various measures of genetic distance, or to detect the genetic structure of populations by calculating how their levels of heterozygosity depart from those predicted by the preconceived model of random mating. An appreciation of the general principles of taxonomy and an understanding of the taxonomy of the group being studied is fundamental for the formulation of effective biodiversity conservation programmes. Furthermore, as the importance of species diversity and genetic diversity has been recently emphasized, the research on the characteristics of genetic variation and genetic composition of the natural population has been available for establishing a strategy for species

conservation, with a new academic field of "Conservation Genetics" being formed (Hedrick, 1976, 1986; Frankel and Soule, 1981; O'Brien et al., 1983; Schonewald-Cox et al., 1983; Vrijenhoek et al., 1985; Wildt et al., 1987; Loeschcke et al., 1994; Gray, 1996; Maxted, 1996).

The wrinkled frog, *Rana rugosa*, is a small dark-brownish frog which usually inhabits plains and mountains near bodies of water. It is widely distributed in Korea, Japan, northeastern China, and southernmost Primorskii, USSR (Maeda and Matsui, 1990).

In this study, horizontal starch gel electrophoresis for 29 populations of the wrinkled frog, *Rana rugosa*, from Korea and Japan was performed to assess the degree of genic variation and genetic diversity, and to understand the biogeographic pattern of distribution and speciation.

Materials and Methods

Collection

A sum of 543 adults of *R. rugosa* were collected from 29 localities in Korea and Japan for genetic analysis (Table 1, Fig. 1).

Protein gel electrophoresis

Live frog samples were transported to the laboratory and then frozen and stored at -70°C . The tissues of liver, heart, kidney and skeletal muscle from each specimen

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Table 1. Collection localities, collection dates and sample sizes for electrophoresis of 29 populations of *Rana rugosa* from Korea and Japan

Collection locality	Date	Sample size
Korea		
1. Kapyong: Kapyong-up, Kapyong-gun, Kyonggi-do	Sep. 28, 1997	20
2. Chonan.: Songak-myon, Chonan-gun, Chungchongnam-do	June 17, 1994	5
3. Nonsan: Yangchon-myon, Nonsan-gun, Chungchongnam-do	Apr. 27, 1997	21
4. Muju: Ansong-myon, Muju-gun, Chollabuk-do	Mar. 12, 1997	30
5. Chongup: Chongup-shi, Chollabuk-do	June 21, 1997	30
6. Changhung: Changhung-gun, Chollanam-do	May 5, 1993	17
7. Changsong: Bukha-myon, Changsong-gun, Chollanam-do	May 18, 1995	26
8. Kwangju: Kwangju-shi, Chollanam-do	June 18, 1998	8
9. Haenam: Samsan-myon, Haenam-gun, Chollanam-do	June 7, 1997	20
10. Sangju: Sangju-gun, Kyongsangbuk-do	Sep. 28, 1997	20
11. Yangyang: Hyunbuk-myon, Yangyang-gun, Kangwon-do	Sep. 27, 1997	30
12. Chongson: Bukpyong-myon, Chongson-gun, Kangwon-do	May 28, 1995	5
13. Kosong: Chukwang-myon, Kosong-gun, Kangwon-do	Apr. 18, 1998	17
14. Wonju: Wonju-shi, Wonju-gun, Kangwon-do	Sep. 26, 1997	31
15. Chindo: Kogun-myon, Chindo-gun, Chollanam-do	July 24, 1994	13
16. Namhae: Samdong-myon, Namhae-gun, Kyongsangnam-do	Oct. 4, 1997	23
17. Ulsan: Pumsu-myon, Uiju-gun, Kyongsangnam-do	Sep. 4, 1998	11
18. Pohang: Shinkwang-myon, Pohang-shi, Kyongsangbuk-do	Sep. 5, 1998	17
19. Yongchon: Chongdong-myon, Yongchon-shi, Kyongsangbuk-do	Sep. 4, 1998	9
20. Kyongju: Yangbuk-myon, Kyongju-shi, Kyongsangbuk-do	Sep. 5, 1998	26
21. Ulchin: Onchong-myon, Ulchin-gun, Kyongsangbuk-do	Sep. 6, 1998	10
22. Yongdok: Chuksan-myon, Yongdok-gun, Kyongsangbuk-do	Apr. 25, 1997	37
23. Hwasu-ri: Yongduk-up, Yongduk-gun, Kyongsangbuk-do	Mar. 28, 1994	35
24. Chongsong: Chongsong-gun, Kyongsangbuk-do	Sep. 5, 1998	30
25. Tonghae: Samhwa-dong, Tonghae-shi, Kangwon-do	Sep. 5, 1998	6
26. Okkye: Okkye-myon, Tonghae-myon, Kangwon-do	Sep. 6, 1998	16
27. Samchok: Kundok-myon, Samchok-gun, Kangwon-do	Sep. 4, 1998	15
28. Kangnung: Wangsan-myon, Kangnung-shi, Kangwon-do	Sep. 6, 1998	5
Japan		
29. Kyoto: Sizuichi-Sizuhara, Sakyō, Kyoto, Japan	Aug. 3, 1997	10

were removed and homogenized by a glass homogenizer in a volume of distilled water and were centrifuged 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 (Selander et al., 1971; Yang, 1983) (Table 2). Genetic data were

analyzed using BIOSYS-1 (Swofford and Selander, 1981). Loci were numbered sequentially with integers beginning with one for the most anodal form, and alleles were designated alphabetically. Individual genotypes were used to calculate allele frequencies for each population, these in turn were used to estimate the degree of genetic variability and to calculate matrices of genetic similarity (Rogers, 1972) and genetic distance (Nei, 1972). Rogers' (1972) similarity coefficients were then clustered by the unweighted pair group method using arithmetic average linkages (UPGMA: Sneath and Sokal, 1973) to provide a general estimate of the overall genetic relationships among populations.

Results

A sum of 22 presumptive loci were inferred from 17 enzymes and general proteins. Observed allelic frequencies for the variable loci are given in Appendix I. Five loci of the 22 genetic loci scored, *Ak*, *Lap*, *Ipo*, *Pept*, *Me-1*, were monomorphic across all populations. The remaining 17 loci exhibited varying degrees of electrophoretic variation. Four of them, *Pgm*, *Aco*, *Est-3*, *Me-2*, were highly polymorphic in most populations. Two alleles occurred at the *Ck* locus. Allele *Ck^a* was fixed in all Korean populations, but the Japanese population had *Ck^a* and *Ck^b* alleles at frequencies of 0.40 and 0.60, respectively. The *Gdh* locus was variable only in the Ulsan population. Seven alleles were found at the *Mdh* locus and they are completely different between Korean (*Mdh-a, b, c, e, g* alleles) and Japanese (*Mdh-d, f* alleles) populations. Diagnostic

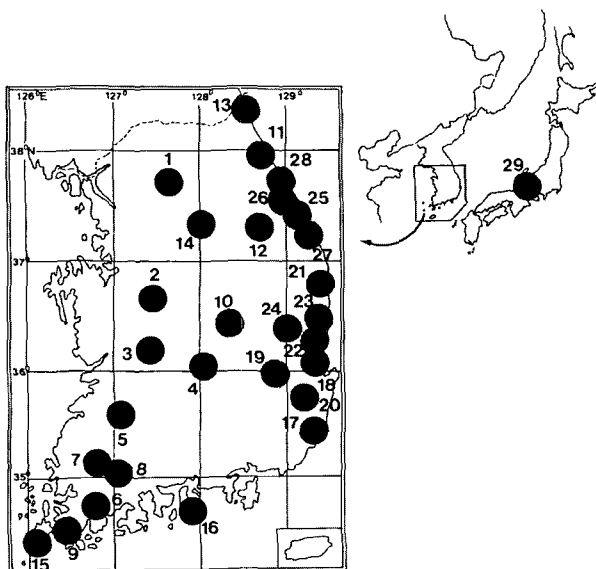


Fig. 1. Distribution range of *Rana rugosa* and collection sites in Korea and Japan. Numbers of collection locality are specified in Table 1.

Table 2. Buffer systems and enzymes for the analysis of horizontal starch gelelectrophoresis of *Rana rugosa*

Buffer system	E. C. No.*	Enzyme	Condition
Continuous tris citrate II (pH 8.0)	1.1.1.42	Isocitrate dehydrogenase (<i>Idh</i>)	100 V / 3 hrs
	2.7.5.1	Phosphoglucumutase (<i>Pgm</i>)	
	1.4.1.2	Glutamate dehydrogenase (<i>Gdh</i>)	
	3.4.11.1	Leucine amino-peptidase (<i>Lap</i>)	
	2.7.4.3	Adenylate kinase (<i>Ak</i>)	
	2.7.3.2	Creatine kinase (<i>Ck</i>)	
LiOH (pH 8.1)	1.1.1.37	Malate dehydrogenase (<i>Mdh</i>)	250 V / 3 hrs
	3.1.1.1	Esterase (<i>Est-1,3</i>)	
	N. S.**	General protein (<i>Gp-3,4</i>)	
	2.6.1.1	Glutamate oxaloacetate isomerase (<i>Got-1,2</i>)	
Discontinuous tris citrate (pH 8.2)	4.2.1.3	Aconitase (<i>Aco</i>)	200 V / 3 hrs
	1.15.1.1	Indophenol oxidase (<i>Ipo</i>)	
	1.1.1.27	Lactate dehydrogenase (<i>Ldh-1,2</i>)	
	5.3.1.8	Mannose-6-phosphate isomerase (<i>Mpi</i>)	
	3.4.11.11	Peptidase (<i>Pept</i>)	
Tris maleic EDTA (pH 7.4)	1.1.1.40	Malic enzyme (<i>Me-1,2</i>)	100 V / 4 hrs
	1.1.99.5	α -Glycerol-3-phosphate (<i>aGpd</i>)	

*Enzyme commission number, **Non specific

differences at *Gp-4* and *Ldh-2* were also found between Korean and Japanese *R. rugosa*. Four alleles

occured at the *Gp-3* locus. The *Gp-3^b* is near fixation in most Korean populations, but *Gp-3^c* and *Gp-3^d* alleles were present in the Japanese population. At the *Mpi* locus, there were 5 alleles. The *Mpi^e* allele was found at high frequencies throughout populations, but in the south-eastern area of the Korean peninsula, (Chong-song, Yongchon, Ulsan, Kyongju, Pohang, Yongdok,

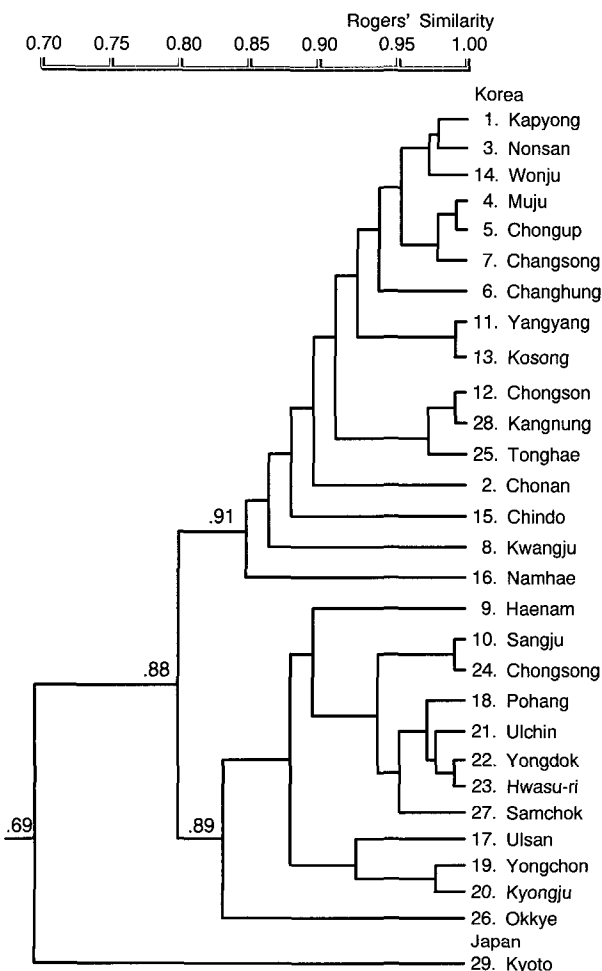


Fig. 2. A phenogram of 29 populations of *Rana rugosa* in Korea and Japan based on Rogers' genetic similarity coefficients (S).

Table 3. Genetic variation of 29 populations in *Rana rugosa* in Korea and Japan

Population	N	Mean No. of Alleles	Polymorphism (%)	Mean Heterozygosity	
				Ho (S.E.)	He (S.E.)
Korea					
1. Kapyong	20	1.3	18.2	0.059 (.030)	0.070 (.031)
2. Chonan	5	1.4	31.8	0.100 (.047)	0.129 (.046)
3. Nonsan	21	1.5	22.7	0.065 (.028)	0.097 (.039)
4. Muju	30	1.3	18.2	0.065 (.032)	0.083 (.040)
5. Chongup	30	1.4	18.2	0.080 (.036)	0.091 (.040)
6. Changhung	17	1.5	27.3	0.096 (.036)	0.121 (.043)
7. Changsong	26	1.3	18.2	0.072 (.036)	0.073 (.036)
8. Kwangju	8	1.4	36.4	0.108 (.041)	0.115 (.037)
9. Haenam	20	1.4	22.7	0.086 (.038)	0.083 (.035)
10. Sangju	20	1.4	27.3	0.075 (.035)	0.084 (.036)
11. Yangyang	30	1.2	18.2	0.055 (.026)	0.054 (.026)
12. Chongson	5	1.1	13.6	0.073 (.045)	0.076 (.043)
13. Kosong	17	1.3	18.2	0.059 (.031)	0.058 (.031)
14. Wonju	31	1.5	18.2	0.060 (.030)	0.066 (.029)
15. Chindo	13	1.2	22.7	0.094 (.046)	0.095 (.039)
16. Namhae	23	1.5	27.3	0.085 (.033)	0.092 (.035)
17. Ulsan	11	1.5	36.4	0.165 (.055)	0.165 (.047)
18. Pohang	17	1.2	18.2	0.075 (.037)	0.071 (.033)
19. Yongchon	9	1.5	31.8	0.162 (.059)	0.138 (.047)
20. Kyongju	26	1.5	31.8	0.115 (.044)	0.126 (.043)
21. Ulsan	10	1.2	22.7	0.068 (.031)	0.087 (.039)
22. Yongdok	37	1.3	22.7	0.097 (.045)	0.085 (.038)
23. Hwasu-ri	35	1.3	18.2	0.058 (.029)	0.060 (.030)
24. Chongsong	30	1.4	22.7	0.088 (.037)	0.093 (.038)
25. Tonghae	6	1.2	18.2	0.106 (.052)	0.079 (.037)
26. Okkye	16	1.2	9.1	0.048 (.030)	0.042 (.025)
27. Samchok	15	1.1	13.6	0.064 (.036)	0.059 (.032)
28. Kangnung	5	1.1	13.6	0.055 (.033)	0.055 (.032)
Japan					
29. Kyoto	10	1.5	40.9	0.164 (.052)	0.168 (.051)
Average	543	1.3	22.7	0.086 (.038)	0.090 (.037)

and Uljin), a few unique alleles of Mp^f , Mp^d , and Mp^e were detected.

The degree of genetic variabilities of each population was estimated (Table 3) based on allelic frequencies listed in Appendix I. The average degree of genetic variation of Korean *R. rugosa* was $P=22.7\%$ (9.1-36.4%), $H_o=0.086$ (0.048-0.165), and $H_e=0.090$ (0.042-0.165). The highest and lowest values of genetic diversity were found in Ulsan ($P=36.4\%$, $H_o=0.165$, $H_e=0.165$) and Okkye ($P=9.1\%$, $H_o=0.048$, $H_e=0.042$) populations of all the Korean populations, respectively. The Japanese population showed a higher level of genetic variation ($P=40.9\%$, $H_o=0.164$, $H_e=0.168$) than those of Korean populations.

The average genetic similarities (Rogers' S) and distances (Nei's D) among populations of *R. rugosa* were estimated (Appendix II). The degree of genetic differentiation among the Korean populations was moderate ($S=0.900$), whereas the genetic divergence between Korean and Japanese populations was notably high ($D=0.293$, $S=0.687$). The UPGMA clustering based on Rogers' genetic similarities is shown in Fig. 2. In this phenogram, populations of *R. rugosa* were divided into three groups: the south-eastern part of the Korean peninsula, the northeastern part of Korea, and the Kyoto population of Japan.

Discussion

The genetic variabilities in allopatric populations have been investigated from various amphibian species of the genus *Rana* (Nevo et al., 1984; Nevo and Beiles, 1991; Nishioka et al., 1992; Sumida and Nishioka, 1994). The present study showed that the genetic variabilities in 29 populations of *R. rugosa* were slightly lower than those of *Rana dybowskii* (Yang et al., 1998), *Hyla japonica* (Yang et al., 1997a), and *Bombina orientalis* (Yang et al., 1997b), but higher than those of *Rana nigromaculata*, *Rana plancyi* (Yang et al., 1999), and *Hyla suweonensis* (Yang et al., 1997a) in Korea.

The dendrogram of *R. rugosa* drawn on the basis of the genetic similarities among the 29 populations by the UPGMA method showed that Korean populations could be divided into the north-western group and south-eastern group (Fig. 2). The north-western group consisted of 16 populations (1, 2, 3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16, 25, and 28). The genetic similarities (S) among these 16 populations ranged from 0.879 between Kwangju and Namhae populations to 0.986 between Yangyang and Kosong populations with a mean of 0.936. The south-eastern group consisted of 12 remaining populations (9, 10, 17, 18, 19, 20, 21, 22, 23, 24, 26, 27). The genetic similarities among them ranged from 0.852 between Ulchin and Okkye populations to 0.975 between Yongdok and Hwasu-ri populations with a mean of 0.922. The average genetic similarity between north-western and south-eastern populations was 0.883.

The Mp^f allele was found throughout most of south Korea and the Mp^e allele was restricted to the south-eastern area of the Korean peninsula (Chongsong, Yongchon, Ulsan, Kyongju, Pohang, Yongdok and Ulchin). The results of mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) analysis (Lee et al., 1992) and the partial sequence of the cytochrome *b* gene of mtDNA (Lee et al., 1999) of *Rana rugosa* also showed the same pattern of geographical distribution. Similar geographical distribution was found in other taxa such as *Zacco platypus* (Min and Yang, 1991a), *Pungitius kaibarae* (Yang and Min, 1990), *Rhinogobius brunneus* (Kim and Yang, 1996) and *Hynobius leechii* (Yang et al., 1997c).

This may indicate that the populations in the south-eastern area are isolated from neighboring populations and probably associated with the geographical history of the Korean peninsula (Kim, 1988). Particularly, the southern region of the Kyongsang province and the northern Kyongsang province located in the south-eastern area of the Korean peninsula, including Yongchon, Kyongju, Yongdok, Pohang, etc., which belongs to the Youngnam region and Kyongsang accumulation layer group, is thought to be different from other regions in formation (Yang and Chang, 1988).

The degree of genetic differentiation between Korean and Japanese *R. rugosa* was found to be much greater ($D=0.293$, $S=0.687$) than the conspecific level. This genetic differentiation between the Korean and the Japanese populations was found not only in *R. rugosa* but also in *Hyla japonica* (Yang et al., 1997a), and even in some fish taxa (Yang and Min, 1990; Min and Yang, 1991b, 1993; Kim and Yang, 1996). The geographic isolation is one of the important mechanisms of speciation, which brings about speciation by accelerating genetic differentiation over a long period of time. Nishioka et al. (1993) reported that as a result of analyzing 40 populations of Japanese *R. rugosa* genetically by isozyme electrophoresis, it was divided into 2 groups, the eastern group and western group including 3 subgroups. Furthermore, as a result of the chromosome analysis by the banding method, 4 kinds of sex chromosomes were identified (Nishioka et al., 1994), and the Japanese *R. rugosa* populations were divided into 4 subgroups, confirming the previous results of isozyme analysis (Nishioka et al., 1993). The Japanese *R. rugosa* population used in this study belonged to the northern subgroup of the eastern group. Further intensive and detailed study on the Korean *R. rugosa*, including chromosome analysis, should be carried out for a better understanding of genetic and evolutionary factors of the speciation status of this organism.

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Genetic Diversity and Speciation of *Rana rugosa*

Appendix I. Allele frequencies of 17 polymorphic loci on 29 populations of *Rana rugosa* from Korea and Japan

Locus	Korea															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
<i>Idh</i>	b	b(0.80) c(0.20)	b	b	b	b	a(0.04) b(0.96)	b	b	b	b	b	b(0.97) d(0.03)	b	b	
<i>Pgm</i>	b(0.15) c(0.85)	b(0.20) c(0.80)	b(0.17) c(0.83)	b(0.13) c(0.87)	b(0.03) c(0.97)	c	c(0.98) d(0.02)	c	c	b(0.30) c(0.70)	b(0.15) c(0.85)	b(0.30) c(0.70)	b(0.09) c(0.91)	b(0.03) c(0.97)	c	
<i>Ck</i>	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	
<i>Gdh</i>	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	
<i>Mdh</i>	c	c	a(0.05) c(0.90) e(0.05)	c	c	c(0.97) g(0.03)	c	c(0.94) e(0.06)	c	c	c	c	c	c	a(0.42) c(0.58)	
<i>Gp-3</i>	b	a(0.10) b(0.90)	b	a(0.02) b(0.98)	b	b	b	b	b	b	b	b	b	b	b	
<i>Gp-4</i>	a(0.02) c(0.98)	c	a(0.02) c(0.98)	c	c	a(0.21) c(0.79)	c	a(0.12) c(0.88)	a(0.07) c(0.93)	c	c	c	c	a(0.02) c(0.98)	c	
<i>Got-1</i>	d(0.98) e(0.02)	d	d	d	b(0.02) d(0.78) e(0.20)	a(0.03) b(0.15) d(0.82)	d(0.89) e(0.11)	d(0.88) f(0.12)	b(0.15) d(0.78) e(0.07)	d	d	d	d	d	d	
<i>Got-2</i>	b	b	b(0.95) c(0.05)	b	b	b	b	a(0.06) b(0.81) c(0.13)	b	b	b	b	b	b	b(0.98) c(0.02)	b
<i>Est-1</i>	a	a(0.90) b(0.10)	a(0.98) b(0.02)	a	a	a(0.79) b(0.21)	a	a(0.69) b(0.31)	a(0.02) b(0.98)	a(0.05) b(0.95)	a	a	a	a(0.86) b(0.14)	a(0.85) b(0.15)	
<i>Est-3</i>	a(0.27) c(0.73)	a(0.30) c(0.60) d(0.10)	a(0.38) c(0.62)	a(0.43) c(0.57)	a(0.43) c(0.57)	a(0.26) c(0.68) d(0.06)	a(0.13) c(0.87)	a(0.12) c(0.88)	a(0.47) c(0.53)	a(0.35) c(0.65)	a(0.80) c(0.20)	a(0.50) c(0.50)	a(0.77) c(0.23)	a(0.18) c(0.82)	a(0.77) c(0.23)	
<i>Aco</i>	a(0.07) b(0.20) c(0.73)	a(0.60) b(0.10) c(0.30)	a(0.26) b(0.38) c(0.36)	a(0.52) b(0.30) c(0.18)	a(0.42) b(0.32) c(0.26)	a(0.30) b(0.35) c(0.35)	a(0.39) b(0.36) c(0.25)	a(0.75) b(0.25)	a(0.05) b(0.10) c(0.85)	a(0.07) b(0.35) c(0.58)	b(0.27) c(0.73)	c	a(0.12) b(0.29) c(0.59)	a(0.13) b(0.27) c(0.60)	b(0.62) c(0.38)	
<i>Mpi</i>	c	c	c	c	b(0.02) c(0.98)	c	c	c	c	b(0.05) c(0.95)	b(0.02) c(0.98)	c	c	b(0.03) c(0.97)	c	
<i>Ldh-1</i>	b	b	b	b	a(0.03) b(0.97)	b	b	b	b	b	b	b	b	b	b	
<i>Ldh-2</i>	a	a	a	a	a	a	a	a	a	a(0.98) b(0.02)	a	a	a	a	a	
<i>αGpd</i>	a	a	a	a	a	a(0.97) b(0.03)	a	a	a	a	a	a	a	a	a(0.98) b(0.02)	a
<i>Me-2</i>	a(0.80) b(0.20)	a(0.60) b(0.40)	a(0.81) b(0.19)	a(0.68) b(0.32)	a(0.80) b(0.20)	a(0.74) b(0.26)	a(0.75) b(0.25)	a(0.63) b(0.37)	a(0.65) b(0.35)	a(0.93) b(0.07)	a(0.90) b(0.10)	a(0.70) b(0.30)	a(0.94) b(0.06)	a(0.94) b(0.06)	a(0.69) b(0.31)	

Appendix I. Continued

Locus	Korea													Japan
	16	17	18	19	20	21	22	23	24	25	26	27	28	29
<i>Idh</i>	b	b	b	b	b	a(0.35) b(0.65)	b	b	b	b	b	b	b	b
<i>Pgm</i>	b(0.13) c(0.87)	b(0.45) c(0.55)	b(0.15) c(0.85)	b(0.50) c(0.50)	b(0.54) c(0.46)	b(0.35) c(0.65)	b(0.34) c(0.66)	b(0.27) c(0.73)	a(0.02) b(0.33) c(0.65)	b(0.17) c(0.83)	b(0.28) c(0.72)	b(0.27) c(0.73)	b(0.10) c(0.90)	b(0.05) c(0.95)
<i>Ck</i>	a	a	a	a	a	a	a	a	a	a	a	a	a	a(0.40) b(0.60)
<i>Gdh</i>	a	a(0.91) b(0.09)	a	a	a	a	a	a	a	a	a	a	a	a
<i>Mdh</i>	b(0.02) c(0.98)	c	c	c	c	c	b(0.07) c(0.93)	c	c	c	c	c	c	d(0.60) f(0.40)

Appendix I. Continued

Locus	Korea													Japan
	16	17	18	19	20	21	22	23	24	25	26	27	28	29
<i>Gp-3</i>	b	b	b	b	b	b	b	b	b	b	b	b	b	b(0.45) c(0.15) d(0.40)
<i>Gp-4</i>	a(0.02) c(0.98)	c	c	c	c	c	c	c	c	c	c	c	c	a(0.10) b(0.90)
<i>Got-1</i>	b(0.02) c(0.26) d(0.72)	a(0.04) d(0.96)	d	b(0.11) d(0.83) e(0.06)	b(0.09) d(0.83) e(0.08)	d	d	d	d	d	d	d	d	b(0.35) d(0.65)
<i>Got-2</i>	a(0.06) b(0.94)	b	b	b	b	b	b	b	b	b	b	b	b	b(0.75) c(0.25)
<i>Est-1</i>	a(0.83) b(0.17)	a(0.27) b(0.73)	b	a(0.11) b(0.89)	a(0.17) b(0.83)	b	b	b	a(0.02) b(0.98)	a(0.67) b(0.33)	a(0.25) b(0.75)	b	a	a
<i>Est-3</i>	a	a(0.45) c(0.55)	a(0.85) c(0.15)	a(0.22) b(0.06) c(0.72)	a(0.44) c(0.56)	a	a	a	a(0.50) c(0.50)	a(0.25) c(0.75)	a(0.03) c(0.97)	a	a(0.30) c(0.70)	a
<i>Aco</i>	a(0.28) b(0.11) c(0.61)	b(0.18) c(0.82)	b(0.50) c(0.50)	b(0.22) c(0.78)	b(0.27) c(0.73)	b(0.20) c(0.80)	b(0.34) c(0.66)	b(0.13) c(0.87)	b(0.28) c(0.72)	c	c	b(0.33) c(0.67)	c	a(0.85) b(0.15)
<i>Mpi</i>	c	c(0.64) d(0.18) e(0.18)	c(0.65) e(0.35)	a(0.17) c(0.50) e(0.33)	c(0.50) d(0.10) e(0.40)	a(0.50) c(0.50)	a(0.16) c(0.59) e(0.25)	a(0.23) c(0.66) e(0.11)	a(0.03) c(0.79) e(0.18)	c	c	c	c	b(0.30) c(0.55) e(0.15)
<i>Ldh-1</i>	b(0.80) c(0.200)	b	b	b	b(0.98) c(0.02)	b	b	b(0.99) c(0.01)	b	b	b	b(0.70) c(0.30)	b	b
<i>Ldh-2</i>	a	a(0.68) b(0.32)	a	a	a(0.92) b(0.08)	a	a	a	a	a	a	a	a	b
<i>αGpd</i>	a	a(0.96) c(0.04)	a	a	a	a	a	a	a	a	a	a	a	a
<i>Me-2</i>	a	a(0.59) b(0.41)	a(0.97) b(0.03)	a(0.44) b(0.56)	a	a(0.95) b(0.05)	a(0.85) b(0.15)	a(0.91) b(0.09)	a(0.85) b(0.15)	a(0.42) b(0.58)	a(0.03) b(0.97)	a	a(0.60) b(0.40)	a

Appendix II. Rogers' (1972) genetic similarities (above the diagonal) and Nei's (1972) genetic distances (below the diagonal) for 29 populations of *Rana rugosa* in Korea and Japan

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Korea																													
1. Kapyong	-	.941	.971	.961	.957	.946	.959	.917	.917	.928	.965	.968	.959	.968	.920	.916	.894	.890	.895	.892	.861	.874	.888	.919	.952	.900	.886	.974	.697
2. Chonan	.017	-	.946	.962	.939	.931	.940	.928	.891	.900	.920	.938	.923	.930	.906	.891	.877	.863	.872	.858	.840	.855	.857	.887	.930	.882	.857	.943	.698
3. Nonsan	.006	.011	-	.971	.965	.952	.959	.921	.901	.925	.952	.951	.954	.956	.933	.915	.879	.893	.872	.881	.850	.882	.876	.910	.930	.878	.881	.950	.715
4. Muju	.014	.006	.004	-	.973	.946	.965	.928	.906	.911	.947	.954	.951	.943	.931	.908	.883	.884	.871	.872	.841	.869	.869	.901	.930	.878	.871	.955	.720
5. Chongup	.012	.012	.005	.004	-	.952	.973	.921	.911	.907	.942	.938	.949	.948	.923	.915	.870	.879	.865	.880	.836	.864	.864	.896	.912	.861	.867	.941	.726
6. Changhung	.011	.014	.007	.010	.008	-	.957	.942	.921	.908	.917	.924	.925	.951	.925	.904	.871	.874	.878	.872	.830	.860	.857	.890	.924	.873	.861	.933	.710
7. Changsong	.011	.012	.006	.007	.005	.007	-	.939	.900	.901	.929	.935	.938	.957	.919	.895	.861	.867	.870	.862	.826	.851	.850	.883	.921	.880	.853	.945	.706
8. Kwangju	.030	.014	.023	.018	.021	.015	.015	-	.903	.882	.883	.900	.890	.927	.892	.879	.857	.845	.865	.844	.807	.836	.836	.866	.921	.880	.835	.916	.706
9. Haenam	.055	.064	.062	.070	.065	.043	.070	.054	-	.939	.903	.916	.901	.909	.893	.882	.915	.915	.923	.913	.890	.909	.922	.945	.924	.910	.911	.922	.683
10. Sangju	.049	.057	.048	.060	.059	.041	.060	.054	.015	-	.920	.920	.921	.936	.887	.884	.918	.944	.927	.940	.917	.940	.940	.971	.917	.914	.942	.909	.661
11. Yangyang	.014	.032	.015	.023	.020	.027	.034	.058	.061	.058	-	.952	.986	.949	.940	.939	.883	.923	.870	.892	.893	.913	.919	.921	.924	.873	.918	.948	.723
12. Chongson	.006	.026	.018	.027	.027	.027	.030	.046	.057	.057	.015	-	.944	.940	.914	.901	.904	.875	.894	.889	.866	.884	.896	.918	.958	.917	.883	.980	.681

Genetic Diversity and Speciation of Rana rugosa

Appendix II. Continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Korea																													
13. Kosong	.014	.027	.011	.017	.013	.021	.027	.049	.062	.056	.001	.019	-	.958	.944	.941	.873	.921	.859	.885	.885	.903	.907	.911	.916	.864	.913	.945	.730
14. Wonju	.004	.020	.007	.016	.012	.008	.008	.024	.048	.038	.022	.017	.018	-	.924	.919	.880	.897	.883	.892	.860	.880	.885	.913	.939	.894	.888	.950	.700
15. Chindo	.031	.040	.020	.027	.026	.028	.037	.059	.063	.061	.019	.041	.017	.034	-	.905	.858	.898	.845	.849	.847	.882	.873	.884	.901	.849	.879	.916	.716
16. Namhae	.037	.045	.032	.036	.030	.040	.054	.065	.062	.063	.013	.038	.012	.042	.033	-	.845	.896	.836	.868	.874	.891	.900	.877	.888	.837	.922	.899	.734
17. Ulsan	.047	.057	.054	.063	.066	.052	.070	.066	.026	.021	.053	.042	.058	.049	.065	.067	-	.893	.936	.936	.884	.905	.904	.934	.906	.901	.878	.898	.659
18. Pohang	.079	.087	.071	.081	.078	.066	.094	.092	.029	.020	.059	.086	.058	.068	.058	.054	.036	-	.901	.927	.933	.962	.957	.952	.877	.864	.949	.872	.691
19. Yongchon	.065	.071	.073	.082	.085	.064	.082	.071	.029	.026	.084	.061	.089	.064	.089	.100	.015	.046	-	.947	.892	.916	.909	.938	.919	.918	.880	.894	.631
20. Kyongju	.059	.075	.062	.077	.074	.062	.080	.081	.035	.016	.061	.059	.064	.053	.078	.066	.017	.023	.020	-	.905	.925	.921	.951	.881	.878	.908	.878	.668
21. Ulchin	.102	.109	.101	.112	.110	.101	.132	.126	.046	.041	.073	.095	.077	.098	.087	.066	.048	.024	.058	.038	-	.956	.964	.933	.855	.852	.936	.848	.660
22. Yongdok	.088	.094	.082	.092	.091	.082	.113	.107	.033	.028	.061	.084	.064	.084	.065	.054	.032	.006	.042	.024	.013	-	.975	.962	.873	.870	.957	.866	.685
23. Hwasu-ri	.084	.095	.085	.096	.093	.083	.116	.106	.028	.028	.058	.077	.062	.081	.071	.050	.033	.011	.046	.027	.009	.003	-	.955	.888	.884	.960	.880	.683
24. Chongsong	.056	.066	.058	.070	.069	.052	.075	.067	.014	.004	.057	.057	.059	.048	.064	.061	.015	.012	.018	.009	.028	.014	.015	-	.909	.906	.941	.902	.659
25. Tonghae	.016	.030	.030	.037	.038	.027	.035	.036	.030	.041	.035	.012	.039	.023	.049	.060	.028	.076	.035	.055	.089	.075	.069	.042	-	.948	.876	.971	.653
26. Okkye	.064	.068	.080	.085	.093	.067	.080	.063	.038	.054	.100	.057	.106	.069	.101	.128	.037	.102	.027	.076	.116	.098	.095	.053	.018	-	.871	.920	.601
27. Samchok	.085	.094	.080	.091	.087	.079	.110	.105	.032	.026	.057	.084	.059	.079	.065	.043	.045	.014	.064	.037	.024	.012	.011	.020	.079	.108	-	.868	.672
28. Kangnung	.005	.025	.018	.026	.025	.022	.024	.038	.053	.060	.020	.003	.023	.014	.041	.046	.046	.092	.063	.070	.107	.095	.087	.063	.007	.048	.095	-	.682
Japan																													
29. Kyoto	.276	.258	.245	.238	.237	.248	.266	.263	.332	.327	.245	.296	.235	.275	.238	.229	.303	.303	.383	.321	.337	.310	.316	.335	.332	.433	.317	.300	-