

Genetic Diversity and Population Structure of Two Korean Pond Frog Species, *Rana nigromaculata* and *R. plancyi* (Anura, Ranidae), with a Survey of Temporal Genetic Variation in *R. nigromaculata*

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Korean *R. plancyi* occupies a restricted area in western South Korea and shows a relatively low level of genic variability (%P=15.2, Ho=0.052, He=0.048). In contrast, *R. nigromaculata* is broadly distributed in South Korea. The observed low level of variability of *R. nigromaculata* (%P=14.3, Ho=0.042, He=0.043) is probably due to its recent colonization. Populations of *R. nigromaculata* exhibited considerable genetic differentiation ($F_{ST}=0.149$) and low level of gene flow ($Nm=1.427$) among populations, compared to those of *R. plancyi* ($F_{ST}=0.096$, $Nm=2.354$), which occupies a restricted area. The observed levels of gene flow among populations of *R. nigromaculata* ($Nm=1.427$) over a broad geographic range is relatively higher than other amphibian species. The high level of gene flow is probably the result of the high dispersal abilities of *R. nigromaculata*. A survey of temporal genetic variation of *R. nigromaculata* showed that there was no significant change on the overall average genetic diversity from 1978 (average He=0.044) to 1997 (average He=0.040). Wright's F-statistics also indicated no significant genetic differentiation from 1978 ($F_{ST}=0.118$) to 1997 ($F_{ST}=0.108$). This suggests that the environmental change appears to have had little influence on the genetic composition of *R. nigromaculata* in the study areas during the past 20 years. The low level of temporal variation might be due to the result of high dispersal abilities and wide migration range of this species.

Rana nigromaculata Hallowell (1861) is widely distributed in Korea, China, Russia and Japan, and *R. plancyi* Lataste (1880) is distributed in Korea, eastern China and Taiwan (Zhao and Adler, 1993). In South Korea, *R. nigromaculata* is broadly distributed over all area, whereas *R. plancyi* is restricted to the western region (Yang and Yu, 1978). Since these two species share similar morphologies and habitat, they were considered as subspecies for some time (Okada, 1931). Thereafter, Shannon (1956) treated them as distinct species based on differences of dorsal bulge-lines. Recently, we reported that they are discrete species which are reproductively isolated by differences in their mating call and breeding season at sympatry (Yang et al., 1988).

Since any genetic variation is informative as a reflection of population structure and history, genetic variability for natural populations were detected on many

amphibians (Selander, 1976; Nevo and Yang, 1979; Yang, 1983; Kim, 1988; Yang et al., 1997a, b, c). However, the detailed studies of genetic population structure for amphibia are rare (Larson et al., 1984; Szymura and Barton, 1991; Highton and Hedges, 1995; Driscoll, 1998).

In this study, we performed the genetic analyses using starch gel electrophoresis to assess the genetic diversity and population structure of *R. nigromaculata* and *R. plancyi* in Korea and Japan. In addition, temporal genetic variation in some populations of Korean *R. nigromaculata* was analyzed.

Materials and Methods

Sample collection and protein electrophoresis

A total of 677 specimens of *Rana nigromaculata* and *R. plancyi* were collected from 28 localities in Korea and Japan (Table 1, Fig. 1). Each specimen was identified according to external morphological characters described by Shannon (1956) and Yang et al. (1988). Live specimens were transported to the laboratory and

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Table 1. Collection localities, collection dates, and sample sizes for the analyses of *Rana nigromaculata* and *R. plancyi* from Korea and Japan

Collection localities	Dates	Sample sizes
<i>Rana nigromaculata</i>		
1. Kanghwa Isl.: Daun-ri, Hajom-myon, Kanghwa-gun, Incheon-shi	Jun. 27, 1996	21
2. Kyodong Isl.: Wolsun-ri, Kyodong-myon, Kanghwa-gun, Incheon-shi	Oct. 8, 1996	20
3. Shido Isl.: Bukdo-myon, Ongjin-gun, Incheon-shi	Aug. 2, 1994	20
4. Poryong: Bongduk-ri, Nampo-myon, Poryong-shi, Chungchongnam-do	Jun. 2, 1996	30
5. Yonki: Seochang 1-ri, Jochiwon-up, Yonki-gun, Chungchongnam-do	Jun. 27, 1996	29
6. Tae'an: Jeongdang-ri, Anmyon-up, Tae'an-gun, Chungchongnam-do	Jun. 19, 1996	30
7. Nonsan: Anshim 6-dong, Yonmu-up, Nonsan-gun, Chungchongnam-do	Jun. 26, 1996	30
8. Changsong: Shinsong-ri, Bukha-myon, Changsong-gun, Jollanam-do	May 18, 1995	14
9. Kwangju: Hwaam-dong, Buk-ku, Kwangju-shi	Jun. 4, 1996	24
10. Chindo Isl.: Chosang-ri, Uishin-myon, Chindo-gun, Chollanam-do	Jun. 6, 1996	30
11. Cheju Isl.: Kosan-ri, Hankyong-myon, Bukcheju-gun, Cheju-do	Jun. 20, 1996	27
12. Hadong: Ssanggye-sa, Hwagae-myon, Hadong-gun, Kyongsangnam-do	Jun. 7, 1996	29
13. Namhae Isl.: Youngji-ri, Samdong-myon, Namhae-gun, Kyongsangnam-do	Jun. 16, 1996	30
14. Keoje Isl.: Yoncho 3-ri, Yoncho-myon, Keoje-gun, Kyongsangnam-do	Jun. 17, 1996	30
15. Pusan: Kilchon-ri, Changan-up, Yangsan-gun, Pusan-shi	Jun. 14, 1996	30
16. Kyongju: Yangdong-ri, Kangdong-myon, Kyongju-shi, Kyongsangbuk-do	Jun. 25, 1996	30
17. Sangju: Yangchon-dong, Sangju-shi, Kyongsangbuk-do	Jun. 13, 1996	12
18. Ulsan: Kusan 1-ri, Keunnam-myon, Ulsan-gun, Kyongsangbuk-do	Jun. 26, 1996	29
19. Wonju: Hakkok-ri, Socho-myon, Wonju-shi, Kangwon-do	May 22, 1996	23
20. Chuncheon: Udu-dong, Chuncheon-shi, Kangwon-do	Jul. 11, 1996	20
21. Yangyang: Asungjeon-ri, Hyunbuk-myon, Yangyang-gun, Kangwon-do	May 12, 1995	17
22. Kosong: Kosong-gun, Kangwon-do	Jun. 18, 1996	30
23. Suncheon: Samsan-dong, Suncheon-shi, Chollanam-do	Jun. 7, 1997	30
24. Japan: Sizuichi-Sizuhara, Sakyo, Kyoto, Japan	Aug. 2, 1997	30
<i>R. plancyi</i>		
25. Kyodong Isl.: Wolsun-ri, Kyodong-myon, Kanghwa-gun, Incheon-shi	Oct. 8, 1996	5
26. Nonsan: Anshim 6-dong, Yonmu-up, Nonsan-gun, Chungchongnam-do	Jun. 26, 1996	30
27. Pyongtaek: Suksong-ri, Osong-myon, Pyongtaek-shi, Kyongki-do	Jun. 26, 1996	20
28. Kanghwa Isl.: Daun-ri, Hajom-myon, Kanghwa-gun, Incheon-shi	Jun. 26, 1996	7
	Total	677

were stored at -70°C until electrophoresis was carried out. In the laboratory, the tissues of liver, heart and skeletal muscle from each specimen 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 elec-

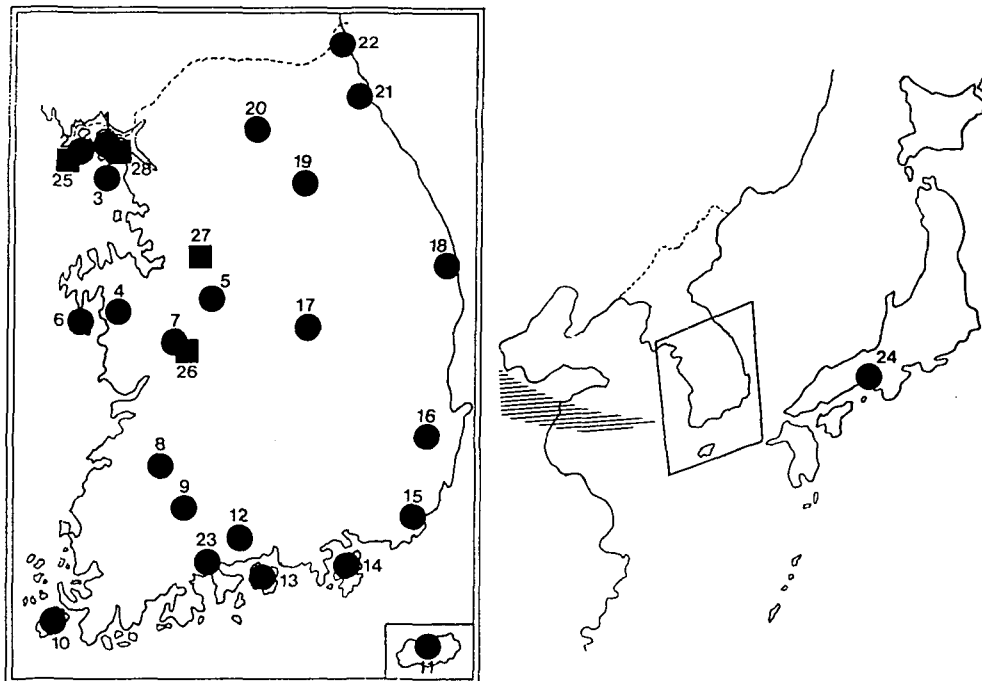


Fig. 1. A map showing the collection localities of 28 populations of *Rana nigromaculata* (●) and *Rana plancyi* (■) in Korea and Japan. Numbers refer to collection localities in Table 1.

Table 2. Number of specimens (N), mean number of alleles per locus (A), percentage of polymorphic loci (%P), observed mean heterozygosity (Ho), Hardy-Weinberg expected mean heterozygosity (=genetic diversity: He), and deviations from expected Hardy-Weinberg proportions (F) for 28 populations of *Rana nigromaculata* and *R. plancyi*

Population	N	A	%P	Ho	He	F
<i>Rana nigromaculata</i>						
1. Kanghwa Isl.	21	1.3	14.3	.034	.039	.129
2. Kyodong Isl.	20	1.3	14.3	.039	.048	.188
3. Shido Isl.	20	1.3	17.9	.027	.035	.229
4. Poryong	30	1.4	7.1	.027	.035	.229
5. Yonki	29	1.3	10.7	.036	.041	.122
6. Taeon	30	1.2	10.7	.042	.045	.067
7. Nonsan	30	1.2	14.3	.035	.037	.054
8. Changsong	14	1.3	10.7	.046	.041	-.122
9. Kwangju	24	1.4	10.7	.048	.052	.077
10. Chindo Isl.	30	1.2	10.7	.031	.027	-.148
11. Cheju Isl.	27	1.1	3.6	.011	.010	-.100
12. Hadong	29	1.3	17.9	.046	.052	.115
13. Namhae Isl.	30	1.2	10.7	.020	.021	.048
14. Keoje Isl.	30	1.4	17.9	.044	.049	.102
15. Pusan	30	1.3	14.3	.049	.047	-.043
16. Kyongju	30	1.5	25.0	.079	.077	-.026
17. Sangju	12	1.3	21.4	.065	.067	.030
18. Ulsan	29	1.4	17.9	.049	.053	.075
19. Wonju	23	1.3	10.7	.037	.033	-.121
20. Chunchon	20	1.3	17.9	.059	.050	-.180
21. Yangyang	17	1.2	14.3	.044	.041	-.073
22. Kosong	30	1.3	14.3	.055	.047	-.170
23. Sunchon	30	1.5	17.9	.049	.047	-.043
24. Japan	30	1.3	17.9	.042	.040	-.050
Average		1.3	14.3	.042	.043	.023
<i>R. plancyi</i>						
25. Kyodong Isl.	5	1.1	10.7	.036	.042	.143
26. Nonsan	30	1.1	14.3	.045	.035	-.285
27. Pyongtaek	20	1.3	21.4	.066	.063	-.048
28. Kanghwa Isl.	7	1.1	14.3	.061	.053	-.151
Average		1.2	15.2	.052	.048	-.083

trophoresis. 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 electrophoresis (12%) and histochemical staining procedures (Selander et al., 1971; Yang et al., 1988: Appendix I).

Data analysis

Multiple loci were numbered sequentially, and alleles were designated alphabetically with "a" being the fastest migrant. Allele frequencies, mean number of alleles per locus (A), percentages of polymorphic loci at 95% criteria (%P₉₅), heterozygosity observed from electrophoresis (Ho), heterozygosity expected from allele frequencies (He), deviations from expected Hardy-Weinberg proportions (F), Wright's (1965) F-statistics, Rogers' (1972) genetic similarity and Nei's (1972) genetic distance were calculated using BIOSYS-1 (Swofford and Selander, 1989).

F-statistics for each species were calculated to ascertain the degree of genetic differentiation among populations of each species. For each species, the level of gene flow among the regional populations (*N_m*) was calculated. Chi-square contingency tests were used to determine if *F_{IS}* and *F_{ST}* values differed significantly from zero (Waples, 1987).

In addition, to survey the temporal genetic variation on *R. nigromaculata* populations from 1978 to 1997, the present data were compared with the previous

data (Yang, 1983). At this time, He ($1 - \sum A_i^2$, where *A_i* is the mean frequency of the *i*th allele at the locus) was used as the parameter of genetic diversity (Spellerberg, 1991). In order to compare the temporal changes in allele frequencies of *Rana nigromaculata* populations from 1978 to 1997, Non-higherarchical F-statistics (using combined across loci) by input of allele frequency (*F_{XY}*: Wright, 1978) were used. Calculations of *F_{XY}* values were made using the BIOSYS-1 program.

Results

Genic variation, population structure and gene flow within each species

Of the 28 presumptive loci scored (see Appendix II), 4 loci (*Alat-1*, *Alat-2*, *Ck*, and *Gp-1*) were monomorphic across all populations of *R. nigromaculata* and the remaining 24 loci were polymorphic at the %P₉₅ criterion level. In *R. plancyi*, only 9 loci (*Pgm*, *Sdh-1*, *Sdh-2*, *Mdh*, *Gp-1*, *Got-1*, *Mpi*, *Ipo*, and *Xdh-2*) were polymorphic.

Based on allelic frequencies listed in Appendix II, the degrees of genic variation on 28 populations of these two species were calculated (Table 2). Although *R. nigromaculata* is broadly distributed in South Korea, this species shows a similar level of genic variability (%P=14.3, Ho=0.042, He=0.043) with that of *R. plancyi* (%P=15.2, Ho=0.052, He=0.048) which occupies a relatively restricted area in South Korea.

Table 3. Summary of F-statistics at the 24 variable loci found within the *Rana nigromaculata* populations

Locus	F _{IS} ^a	F _{IT}	F _{ST} ^b
Lap	-.017	-.001	.016
Pgm	-.034	-.001	.032*
Gdh	-.043	-.003	.039**
ldh	.018	.140	.124***
Sdh-1	-.048	-.009	.037
Sdh-2	.092*	.148	.062***
Me-1	.059	.266	.220***
Me-2	-.091*	-.008	.076***
Mdh	-.078	-.009	.065***
Gp-2	-.053	-.002	.048***
Gp-3	-.091*	-.003	.080***
Got-1	-.097*	.118	.196***
Got-2	-.025	-.004	.021
Aco	.013	.101	.089***
Ldh-1	-.048	-.006	.040***
Ldh-2	-.037	-.005	.031***
Mpi	-.113***	-.000	.102***
Adh	-.029	-.002	.026
Ipo	-.019	-.002	.016
Pept	-.043	-.003	.039**
aGpd	.125***	.190	.074***
Xdh-1	-.053	-.004	.047***
Xdh-2	-.043	-.003	.039**
Fum	-.078	-.004	.069***
Mean	-.001	.149	.149

Significance levels for chi-square tests H₀: F_{IS}=0, and H₀: F_{ST}=0; *=*P*<0.05; **=*P*<0.01; ***=*P*<0.001.

^a Chi-square=F_{IS}²N(k-1), df=[k(k-1)]/2 (Waples, 1987), where N is the total number of individuals and k is the number of alleles at each locus.

^b Chi-square=2NF_{ST}(k-1), df=(k-1)(s-1) (Waples, 1987), where N and k are given above, and s is the total number of populations sampled.

The values of Rogers' genetic similarities among regional populations of the same species were high (*R. nigromaculata*: S>0.950, *R. plancyi*: S>0.956, Appendix III). In *R. nigromaculata*, the weighted mean F_{ST} values were low (0.149), but significant, for 19 of 24 polymorphic loci, and Wright's F_{IS}, a measure of potential inbreeding within species (Wright, 1951, 1965), was significantly greater than zero at 6 loci (Table 3). The weighted mean F_{ST} values of *R. plancyi* were also very low (0.096), but significant for 5 of 9 polymorphic loci, and Wright's F_{IS} was significantly greater than zero at 2 loci (Table 4). Mean F_{ST} for all loci implies that only 14.9% (*R. nigromaculata*) and 9.6% (*R. plancyi*) of the total genetic variance are distributed among populations within each species.

The estimated numbers of immigrants per generation (*Nm*) for *R. nigromaculata* and *R. plancyi* are 1.427 and 2.354, respectively. Although these values are commonly consistent with moderately high rates of gene flow and agrees with low levels of overall genic heterogeneity among populations of each species, the rate of gene flow among *R. plancyi* populations is higher than that of *R. nigromaculata* populations.

Interspecific genetic differentiation

There were no significant allelic differences among populations within each species, whereas fixed allelic differences between two species at 6 loci (*Ck*, *Gp-2*, *Adh*, *Pept*, *aGpd*, and *Fum*) were detected and these could serve as genetic markers to discriminate *R. nigromaculata* and *R. plancyi*. In addition, statistically

Table 4. Summary of F-statistics at the 9 variable loci found within the *Rana plancyi* populations

Locus	F _{IS} ^a	F _{IT}	F _{ST} ^b
Pgm	.124	.261	.156***
Sdh-1	-.111	-.026	.077**
Sdh-2	-.071	-.017	.051
Mdh	-.362**	-.319	.031***
Gp-1	-.200	-.043	.130**
Got-1	-.167	-.123	.037
Mpi	-.143	-.065	.069*
Ipo	-.053	-.013	.038
Xdh-2	.467***	.632	.310***
Mean	-.133	-.023	.096

Significance levels for chi-square tests H₀: F_{IS}=0, and H₀: F_{ST}=0; *=*P*<0.05; **=*P*<0.01; ***=*P*<0.001.

^a Chi-square=F_{IS}²N(k-1), df=[k(k-1)]/2 (Waples, 1987), where N is the total number of individuals and k is the number of alleles at each locus.

^b Chi-square=2NF_{ST}(k-1), df=(k-1)(s-1) (Waples, 1987), where N and k are given above, and s is the total number of populations sampled.

significant allelic differences at the other six loci (*Sdh-1*, *Mdh*, *Got-2*, *Ldh-1*, *Mpi*, and *Xdh-1*) were detected. The average genetic similarities between two species were notably lower (S=0.553, Appendix III) and no evidence of gene flow between these two species at sympatry was found.

Temporal genetic variation in the Korean *R. nigromaculata*

In order to survey the temporal genetic variation on *R. nigromaculata* populations, previous data (Yang, 1983) were compared with the present data at 8 localities (Tables 5, 6, 7). Of the 28 loci examined in the present study, 13 loci (*Pgm*, *Gdh*, *ldh*, *Sdh-2*, *Mdh*, *Got-1*, *Got-2*, *Ldh-1*, *Ldh-2*, *Mpi*, *Ipo*, *aGpd*, and *Fum*) were consistent with the previous report. Of these 13 loci, only one locus (*Gdh*) was monomorphic across 8 populations of *R. nigromaculata* and the rest 12 loci were polymorphic at the %P₉₉ criterion level (Table 5). From 1978 to 1997, there were some minor allele frequency changes (Table 5) but no significant differences on the overall average genetic diversity (Table 6) and genetic heterogeneity (Table 7) were detected.

Discussion

Goldman and Barton (1992) suggested that we would learn more about evolution by investigating patterns of genetic variation within species rather than by comparing different species. That is because patterns of genetic variation reflect the population structure of a species, which can influence the likely mechanisms of divergence and speciation.

In this study, Korean *R. plancyi* which occupies a restricted area shows a relatively low level of genic variability (%P=15.2, H₀=0.052, H_e=0.048) in comparison to other amphibian species (average %P=33.6, average H=0.082; Selander, 1976), and *R. nigromaculata* which is broadly distributed in Korea and shows a relatively low level of genic variability (%P=14.3, H₀=0.042, H_e=0.043). In general, amphibian species with

Table 5. Allele frequencies of 8 populations of *Rana nigromaculata* at 13 of the same loci between the previous data (Yang, 1983: '78) and this study ('97).

Locus	Chindo Isl.		Cheju Isl.		Namhae Isl.		Kyodong Isl.		Yangyang		Kyongju		Kwangju		Sunchon	
	'78 (20) ¹	'97 (30)	'78 (33)	'97 (27)	'78 (15)	'97 (30)	'78 (15)	'97 (20)	'78 (14)	'97 (17)	'78 (20)	'97 (30)	'78 (10)	'97 (24)	'78 (20)	'97 (30)
<i>Pgm</i>	b(1.00)	b(1.00)	b(0.82) c(0.18)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	a(0.02) b(0.98)	b(0.97) c(0.03)
<i>Gdh</i>	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)
<i>Idh</i>	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(0.97) c(0.03)	b(1.00)	b(0.97) c(0.03)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(0.17) c(0.83)	b(1.00)	b(1.00)	b(1.00)	b(1.00)
<i>Sdh-2</i>	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(0.97) c(0.03)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(0.07) c(0.93)	b(1.00)	b(1.00)	b(1.00)	b(1.00)
<i>Mdh</i>	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(0.91) c(0.09)	b(1.00)	b(0.97) c(0.03)	b(1.00)	b(1.00)	b(1.00)	a(0.02) b(0.98)
<i>Got-1</i>	b(1.00)	b(1.00)	b(1.00)	a(0.02) b(0.98)	b(1.00)	b(1.00)	b(1.00)	a(0.02) b(0.98)	a(0.07) b(0.93)	a(0.27) b(0.74)	b(1.00)	a(0.28) b(0.72)	b(1.00)	a(0.02) b(0.98)	b(1.00)	a(0.02) b(0.87) c(0.11)
<i>Got-2</i>	b(1.00)	b(1.00)	b(1.00)	b(0.98) c(0.02)	b(1.00)	b(1.00)	b(1.00)	b(0.98) c(0.02)	b(1.00)	b(1.00)	a(0.02) b(0.98)	b(0.98) c(0.02)	b(1.00)	b(1.00)	b(0.98)	b(1.00) c(0.02)
<i>Ldh-1</i>	c(1.00)	c(1.00)	c(1.00)	c(1.00)	b(0.07) c(0.93)	c(1.00)	a(0.03) c(0.97)	c(1.00)	c(1.00)	c(1.00)	c(1.00)	c(1.00)	c(1.00)	a(0.04) c(0.96)	c(1.00)	b(0.03) c(0.97)
<i>Ldh-2</i>	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	a(0.03) b(0.97)	b(1.00)	b(1.00)	b(1.00)	a(0.02) b(0.98)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	a(0.03) b(0.97)
<i>Mpi</i>	b(0.83) c(0.17)	a(0.02) b(0.70) c(0.28)	b(1.00)	a(0.02) b(0.98)	b(1.00)	b(1.00)	b(1.00)	a(0.02) b(0.98)	b(0.96) c(0.04)	a(0.03) b(0.97)	a(0.05) b(0.85) c(0.10)	a(0.17) b(0.78) c(0.05)	a(0.04) b(0.93) c(0.03)	a(0.04) b(0.96)	b(0.98) c(0.02)	b(0.93) c(0.07)
<i>Ipo</i>	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	a(0.02) b(0.98)	b(1.00)	b(1.00)
<i>α-Gpd</i>	b(0.90) c(0.10)	b(0.95) c(0.05)	b(1.00)	b(1.00)	b(0.73) c(0.27)	b(0.97) c(0.03)	b(0.93) c(0.07)	b(0.75) c(0.25)	b(1.00)	a(0.06) b(0.94)	b(0.90) c(0.10)	b(0.85) c(0.15)	b(0.86) c(0.14)	b(0.79) c(0.21)	b(0.78) c(0.22)	b(0.93) c(0.07)
<i>Fum</i>	a(0.42) b(0.58)	b(1.00)	a(0.21) b(0.79)	b(1.00)	a(0.07) b(0.93)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	a(0.02) b(0.98)

¹Number of specimen

wide geographical distributions tend to maintain higher levels of allozyme diversity than species with more restricted ranges in Korea (Yang et al., 1997a, b, c), and low genic variability over a broad geographic range probably indicates the area was recently colonized by a small number of individuals that had low genic variation (e.g., Highton and Webster, 1976; Larson et al., 1984; Martin and Simon, 1990; Highton and Hedges, 1995). Therefore, it is assumed that the low level of variability in Korean *R. nigromaculata* might be due to the result of recent colonization from a small number

Table 6. Temporal genetic diversity (He) on 8 populations of *Rana nigromaculata* from 1978 ('78) to 1997 ('97). Numbers in parenthesis indicate total number of compared loci. (13) indicates that these 13 loci are the same with the loci used in 1978

Population	No of specimen		He				ΔHe	
	'78	'97	'78 (16)*	'78 (13)	'97 (13)	'97 (28)	(13)	(16, 28)
Chindo Isl.	20	30	.063	.074	.041	.027	-.033	-.036
Cheju Isl.	33	27	.047	.049	.006	.010	-.043	-.037
Namhae Isl.	15	30	.060	.054	.005	.021	-.049	-.039
Kyodong Isl.	15	20	.037	.030	.041	.048	.011	.011
Yangyang	14	17	.029	.015	.057	.041	.042	.012
Kyongju	20	30	.051	.042	.119	.077	.077	.026
Kwangju	20	30	.028	.029	.045	.052	.016	.024
Sunchon	20	30	.034	.038	.058	.047	.020	.013
Average	20	27	.044	.041	.047	.040	.006	.004

* Number of locus

of individuals that had low genetic variability.

Throughout this paper F_{ST} was used as an estimate of gene flow, based on the relationship between F_{ST} and Nm , the product of effective population size and average number of immigrants (Wright, 1951): $Nm = [(1/F_{ST}) - 1]/4$. In view of the inherent inaccuracies in estimating Nm (Slatkin and Barton, 1989; Whitlock, 1992), F_{ST} values were used as a qualitative indication of the magnitude of gene flow. Following Porter's (1990) general guide: $F_{ST} < 0.2$ ($Nm > 1$), gene flow is moderate (important in promoting genetic similarity); $0.2 < F_{ST} < 0.33$ ($0.5 < Nm < 1$), gene flow is weak, but would permit exchange of alleles; $F_{ST} > 0.33$ ($Nm < 0.5$), gene flow is unimportant and populations are more or less completely isolated. In this study, Wright's F-statistics ($F_{ST} < 0.149$) and Rogers' genetic similarity coefficients ($S > 0.950$) indicate no significant genetic differentiation among regional populations in *R. nigromaculata* and *R. plancyi*. However, the level of genetic differentiation (F_{ST}) found within Korean populations of *R. nigromaculata* were considerably high, relative to the value within *R. plancyi* populations. Populations of *R. nigromaculata* exhibited relatively higher genetic differentiation ($F_{ST} = 0.149$) and lower levels of gene flow ($Nm = 1.427$) among 24 populations, whereas *R. plancyi* had lower levels of differentiation ($F_{ST} = 0.096$) and higher levels of gene flow ($Nm = 2.354$)

On the other hand, the observed levels of gene flow

Table 7. Summary of variance components and F-statistics (overall genetic heterogeneity) combined across 12 common variable loci in 1978 and 1997

Comparison		Variance component		F _{xy}	
X	Y	1978	1997	1978	1997
Locality	Species	.07431	.07273	.118	.108
Locality	Total	.06328	.06216	.103	.093

among *R. nigromaculata* populations ($Nm=1.427$), which have a broad geographic range, is relatively very high in comparison to other Korean amphibian species, which are broadly distributed in South Korea; *Rana amurensis* 0.805, *Rana rugosa* 0.338, *Kaloula borealis* 0.487, *Hyla japonica* 1.750, and *Rana dybowskii* 2.836 (Yang et al., unpublished data). In the study, *H. japonica* and *R. dybowskii* have a relatively wide migration range. In this study, the high level of gene flow is probably the result of the high dispersal abilities of *R. nigromaculata*.

A survey of the temporal genetic variation on *R. nigromaculata* showed that there was no significant change on the overall average genetic diversity from 1978 (average $H_e=0.044$) to 1997 (average $H_e=0.040$) in spite of the considerable decrease in some island populations (Table 6). Wright's F-statistics also indicated no significant temporal changes of over all genetic differentiation among populations of *R. nigromaculata* from 1978 ($F_{ST}=0.118$) to 1997 ($F_{ST}=0.108$). These results suggest that the environmental change appears to have had little influence on the genetic composition of *R. nigromaculata* in the study areas during the past 20 years. Although speculative, it seems likely that the observed pattern of low level of temporal variation is the result of the high dispersal ability of this species.

Genetic variation, as measured by average heterozygosity and allelic diversity, is expected to be lower in island populations than in mainland populations of similar size (Berry, 1986; Browne, 1977). Both isolation by physical barriers to dispersal and isolation by distance among subpopulations may reduce gene flow thereby increasing inbreeding and genetic drift (Ashley and Wills, 1987; Wayne et al., 1991). The overall decrease of temporal variability on all island populations with the exception of Kyodong Island in this study is probably due to inbreeding and genetic drift. In general, the importance of genetic consequences for small population size is the impact of genetic drift (Gray, 1996). In practice, some rare alleles of these island populations are deleted from 1978 to 1997 (See Table 5). Moreover, the Ulreung island population which had a typical small population size, colonized from several mainland populations in the 1920's (Yang, 1983), is extinct in our 1997 surveys.

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Appendix I. Buffer systems and enzymes for the analysis of horizontal starch gel electrophoresis of *Rana nigromaculata* and *R. plancyi*

Buffer system	E. C. No.*	Enzyme	Condition
Continuous tris citrate II (pH 8.0)	2.6.1.2	Alanine aminotransferase (<i>Alat-1,2</i>)	100V/3hrs
	1.1.1.42	Isocitrate dehydrogenase (<i>Idh</i>)	
	2.7.5.1	Phosphoglucumutase (<i>Pgm</i>)	
	1.4.1.2	Glutamate dehydrogenase (<i>Gdh</i>)	
	1.1.1.37	Malate dehydrogenase (<i>Mdh</i>)	
	1.1.1.14	Sorbitol dehydrogenase (<i>Sdh-1,2</i>)	
	3.4.11.1	Leucine amino-peptidase (<i>Lap</i>)	
	2.7.3.2	Creatine kinase (<i>Ck</i>)	
	1.1.1.40	Malic enzyme (<i>Me-1,2</i>)	
LiOH (pH 8.1)	N. S.**	General protein (<i>Gp-1,2,3</i>)	250V/3hrs
	2.6.1.1	Glutamate oxaloacetate transaminase (<i>Got-1,2</i>)	
Discontinuous tris citrate (pH 8.2)	4.2.1.3	Aconitase (<i>Aco</i>)	200V/3hrs
	1.1.1.1	Alcohol dehydrogenase (<i>Adh</i>)	
	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>)	
Tris maleic EDTA (pH 7.4)	3.4.11.11	Peptidase (<i>Pept</i>)	100V/4hrs
	1.1.1.204	Xanthine dehydrogenase (<i>Xdh-1,2</i>)	
	4.2.1.2	Fumarase (<i>Fum</i>)	
	1.1.99.5	α -Glycerolphosphate dehydrogenase (<i>αGpd</i>)	

* E. C. No. : Enzyme commission number, ** N. S.: Non specific.

Appendix II. Allele frequencies of 28 populations of *Rana nigromaculata* and *R. plancyi*

Locus	Allele	<i>Rana nigromaculata</i>																								<i>R. plancyi</i>					
		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		
<i>Ck</i>	a																														
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
<i>Lap</i>	a																.017		.017												
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.983	1.000	.983	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
<i>Pgm</i>	a																											.250	.286		
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.750	.714	
	c																									.967	.033	1.000	1.000		
<i>Gdh</i>	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.978	.950	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
	b																				.022	.050									
<i>Idh</i>	a																.167	.042	.017												
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.833	.958	.983	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
<i>Sdh-1</i>	a		.050			.017					.017	.067	.033																		
	b	1.000	.925	.975	1.000	1.000	.983	1.000	1.000	.979	1.000	1.000	.983	.933	.967	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
	c		.025	.025						.021																		.100	.900	1.000	1.000

* Population numbers shown in Table 1

Appendix III. Rogers' (1972) genetic similarity coefficients (S, above diagonal) and Nei's (1972) distance (D, below diagonal) based on allele frequencies of 28 genetic loci among 28 populations of *Rana nigromaculata* and *R. plancyi*

Populations	<i>Rana nigromaculata</i>																								<i>Rana plancyi</i>			
	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
1*	-	.979	.980	.990	.976	.969	.984	.983	.980	.970	.970	.973	.974	.972	.975	.951	.951	.974	.977	.952	.963	.963	.969	.960	.567	.554	.542	.555
2	.002	-	.976	.982	.974	.962	.984	.984	.978	.964	.967	.971	.969	.969	.972	.950	.951	.977	.970	.947	.962	.961	.960	.954	.569	.556	.544	.557
3	.002	.003	-	.984	.983	.972	.988	.982	.976	.973	.971	.977	.975	.976	.978	.952	.953	.970	.983	.957	.963	.965	.969	.964	.561	.548	.538	.549
4	.000	.002	.001	-	.981	.971	.986	.986	.976	.972	.976	.974	.975	.972	.976	.952	.952	.977	.980	.956	.968	.970	.970	.963	.563	.550	.538	.551
5	.003	.004	.002	.002	-	.967	.980	.979	.974	.965	.975	.979	.965	.974	.980	.951	.949	.973	.975	.951	.967	.970	.962	.958	.552	.539	.529	.540
6	.006	.009	.006	.006	.008	-	.970	.973	.966	.971	.957	.969	.968	.971	.967	.956	.957	.962	.979	.966	.957	.959	.972	.981	.566	.548	.538	.552
7	.001	.001	.001	.001	.002	.006	-	.990	.982	.975	.970	.982	.973	.979	.986	.962	.962	.979	.982	.955	.964	.964	.971	.962	.566	.553	.541	.554
8	.002	.002	.001	.001	.002	.006	.001	-	.975	.976	.971	.981	.970	.978	.981	.958	.958	.984	.981	.957	.966	.970	.969	.962	.568	.555	.542	.556
9	.002	.002	.003	.003	.005	.007	.001	.003	-	.967	.959	.977	.970	.980	.981	.961	.961	.969	.977	.951	.953	.952	.968	.958	.572	.559	.548	.560
10	.008	.010	.005	.009	.011	.007	.006	.006	.006	-	.956	.970	.980	.978	.976	.960	.966	.967	.981	.968	.949	.958	.972	.968	.579	.567	.554	.567
11	.012	.011	.013	.009	.008	.023	.013	.010	.019	.030	-	.960	.959	.956	.959	.935	.936	.969	.965	.942	.978	.975	.952	.949	.544	.531	.519	.532
12	.004	.004	.002	.004	.003	.006	.002	.003	.002	.006	.017	-	.967	.984	.989	.964	.963	.971	.977	.957	.958	.961	.969	.962	.565	.551	.540	.553
13	.006	.008	.004	.007	.010	.007	.005	.007	.004	.004	.029	.005	-	.976	.970	.955	.956	.960	.979	.963	.951	.952	.973	.971	.572	.559	.547	.560
14	.004	.005	.003	.005	.005	.005	.003	.004	.002	.003	.024	.002	.003	-	.988	.972	.969	.973	.985	.964	.953	.959	.973	.965	.572	.559	.546	.560
15	.004	.004	.002	.004	.003	.007	.001	.003	.001	.004	.019	.001	.005	.001	-	.969	.967	.974	.979	.959	.954	.960	.967	.960	.565	.553	.540	.553
16	.009	.010	.008	.010	.011	.008	.007	.008	.006	.007	.030	.005	.007	.004	.005	-	.982	.957	.963	.970	.949	.946	.956	.956	.569	.549	.534	.553
17	.013	.012	.011	.013	.014	.010	.010	.010	.008	.008	.034	.007	.010	.005	.007	.002	-	.957	.962	.980	.948	.952	.956	.959	.575	.556	.540	.559
18	.004	.002	.004	.003	.003	.010	.002	.001	.005	.010	.008	.005	.012	.007	.005	.010	.012	-	.970	.952	.966	.973	.960	.952	.565	.552	.539	.553
19	.003	.005	.002	.003	.005	.004	.002	.003	.002	.003	.021	.003	.002	.001	.002	.005	.007	.007	-	.969	.962	.963	.979	.974	.571	.558	.544	.559
20	.013	.015	.011	.013	.015	.008	.012	.012	.011	.009	.035	.010	.009	.007	.010	.003	.002	.015	.007	-	.951	.956	.960	.970	.572	.553	.537	.556
21	.010	.009	.010	.008	.007	.017	.010	.008	.015	.024	.004	.012	.023	.018	.015	.019	.022	.006	.015	.022	-	.975	.951	.952	.559	.536	.524	.543
22	.008	.008	.008	.006	.005	.014	.008	.006	.013	.018	.005	.010	.020	.013	.011	.017	.018	.004	.013	.020	.005	-	.951	.949	.560	.548	.534	.547
23	.005	.007	.004	.006	.009	.003	.005	.006	.003	.004	.028	.005	.002	.002	.004	.006	.008	.011	.001	.007	.021	.018	-	.973	.571	.556	.547	.560
24	.010	.013	.009	.011	.014	.002	.010	.011	.008	.007	.034	.008	.006	.006	.009	.007	.008	.016	.005	.006	.024	.022	.003	-	.575	.556	.545	.561
25	.543	.541	.550	.552	.565	.551	.544	.545	.531	.527	.595	.546	.538	.537	.542	.542	.535	.549	.542	.542	.571	.563	.539	.540	-	.977	.956	.971
26	.571	.569	.578	.580	.593	.581	.571	.573	.559	.554	.624	.575	.564	.565	.570	.574	.568	.577	.570	.576	.602	.591	.568	.571	.003	-	.961	.970
27	.580	.578	.586	.589	.603	.590	.581	.583	.568	.564	.634	.585	.573	.575	.580	.586	.580	.588	.580	.588	.613	.603	.576	.580	.011	.009	-	.976
28	.562	.560	.570	.571	.585	.572	.563	.565	.550	.548	.615	.567	.556	.558	.562	.565	.559	.570	.562	.566	.592	.585	.558	.561	.006	.006	.006	-

* Population numbers shown in Table 4