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Allozyme variation in *Paraixeris*: a test for the diploid hybrid origin of *Paraixeris koidzumiana* (Compositae)

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Abstract. Variation in isozyme patterns of four Korean *Paraixeris* species was used to test the hypothesis that *P. koidzumiana* is a hybrid derivative of *P. chelidoniifolia* and *P. denticulata,* or alternatively, of the progenitor *P. chelidoniifolia-P. sonchifolia* pair. Eleven loci from eight enzymes were examined for 29 populations of *P. koidzumiana*, *P. denticulata*, *P. chelidoniifolia*, and *P. sonchifolia*. Although recent RAPD and morphological data supported the hybrid origin of *P. koidzumiana*, isozyme evidence did not. No evidence of additivity was found in *P. koidzumiana* at the loci that significantly differentiate *P. sonchifolia* from other species. In addition, *P. koidzumiana* populations did not combine the marker alleles differentiating *P. denticulata* from *P. chelidoniifolia*. Recent divergence between *P. koidzumiana* and *P. chelidoniifolia* is strongly supported by the high genetic identity value and sharing of the same high frequency alleles at most loci. Our isozyme data largely support a conclusion that the unidentified individuals found in species sympatry are hybrid derivatives.

Keywords: Hybridization; Isozyme; *Paraixeris koidzumiana*.

Introduction

Natural hybridization is common in flowering plants and provides an important source of increasing genetic variation, creating novel lineages via stabilization of the hybrid derivatives and breakdown or reinforcement of isolating barriers (Levin, 1979; Rieseberg and Ellstrand, 1993; Wolfe and Elisens, 1993; Arnold, 1992, 1997; Morrell and Rieseberg, 1998; Nielsen, 2000). However, due to sterility or limited fertility in hybrid individuals, and the lack of molecular markers to detect parental taxa, diploid hybridization has been assigned only minor evolutionary importance (Arnold, 1992; Wolfe et al., 1998). Several recent studies of plant speciation suggest that new diploid species might arise rapidly through hybridization between genetically divergent species (Ungerer et al., 1998). In this case, genetic additivity of allozyme data has been useful in determining parental species (Gallez and Gottlieb, 1982). However, the detection of ancient hybrid origin may be difficult due to the parental taxa and its derivatives having had a chance to accumulate new genetic variation (Morrell and Rieseberg, 1998). Although many cases of recombinational speciation in the Far East Asian *Paraixeris* group have been proposed based on cytological and morphological studies (Ono, 1946, 1950, 1951), few have been tested with molecular markers.

The Korean endemic *Paraixeris koidzumiana* Kitam. (Kitamura, 1942; Pak, 1991) is a diploid (2n = 10) herbaceous biennial, restricted to Mt. Chiri, while the closely related *P. chelidoniifolia, P. denticulata*, and *P. sonchifolia* are also diploid, but widely distributed in Korea, Japan, and Manchuria (Pak and Kawano, 1990). Since Kitamura (1942) described *P. koidzumiana* from the specimen of Koidzumi collected from Mt. Chiri, Lee (1979) proposed that *P. koidzumiana* originated from hybridization between *P. sonchifolia* and *P. chelidoniifolia*. This hybrid origin was originally proposed because of the intermediate leaf morphology between the two species. *Paraixeris koidzumiana* resembles *P. chelidoniifolia* in having pinnately divided leaves, but is similar to *P. sonchifolia* in having margined petioles that clasp at the base. However, a cytological study by Pak (1991) did not give support to *P. sonchifolia* being one of the parental species of *P. koidzumiana*. Additionally, the difference between the flowering periods of *P. sonchifolia* and *P. chelidoniifolia* argues against their hybridization (Tae et al., 2001). A recent molecular study using RAPD data supports the hybrid origin of *P. koidzumiana* from *P. denticulata* (Houtt.) Nakai and *P. chelidoniifolia* (Tae et al., 2001). In addition, at Mt. Chiri, where the above three species grow sympatrically, plants were discovered with leaves and inner-involucre numbers that appeared to be intermediate between *P. chelidoniifolia* and *P. denticulata*. Canonical variate analysis on the morphological characters suggested that the individuals were hybrids derived from *P. chelidoniifolia* and *P. denticulata* (Pak, unpublished data). The objective of the study was to determine, using isozyme analysis, whether *P. koidzumiana* and putative hybrid populations were derived through hybridization among *P. chelidoniifolia*, *P. denticulata*, and *P. sonchifolia*.

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Materials and Methods

A total of 361 individuals from 29 populations, representing four *Paraixeris* species and four hybrid populations previously used in Canonical variate analysis by Pak (unpublished data), were examined for isozyme variation (Table 1). Voucher specimens were deposited at the Herbarium of Kyung-Nam University (KNUH). Soluble enzymes were isolated from the fresh leaf tissue of field-collected plants, ground in an extracting buffer containing 0.1 M tris-HCl, pH 7.5, 1 mM EDTA (tetrasodium salt), 10 mM $MgCl_2$, 10 mM KCl, 14 mM 2-mercaptoethanol, and 5-10 mg/ml solid polyvinylpolypyrrolidone (Gottlieb, 1981). Leaf extracts were centrifuged in 1.5 ml tubes and the supernatant absorbed onto wicks of Whatman 17 MM chromatography paper.

Eight enzymes were resolved on 12.5% starch gels utilizing two buffer systems. System I had an electrode buffer of 0.065 M L-histidine and 0.007 M citric acid, adjusted to pH 6.5 with NaOH. This gel buffer was a 1:3 dilution of the electrode buffer. System II consisted of an electrode buffer with 0.18 M tris, 0.1 M boric acid, and 0.004 M EDTA, pH 8.6. System I was used to resolve malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6PGD), glyceraldehyde 3-phosphate dehydrogenase (GA3PD), malic enzyme (ME), and phosphoglucomutase (PGM). System II was used to resolve the enzyme systems alcohol dehydrogenase (ADH), phosphoglucose isomerase (PGI), and triosephosphate isomerase (TPI). Enzyme-activity staining and agarose overlays generally followed the protocols of Soltis et al. (1983). Isozymes and allozymes were numbered sequentially and lettered alphabetically, beginning with the most anodal form. The BIOSYS-1 program (Swofford and Selander, 1981) was used to calculate mean number of alleles per locus (A), percentage of polymorphic loci (P), mean observed heterozygosity (H_o), and mean expected heterozygosity (H_e) within the populations studied. For the analysis of population differentiation Wright's (1965) F statistics were calculated. The F statistics include F_{IS}, an index of inbreeding, F_{IT}, the overall inbreeding coefficient, and F_{ST}, a measure of the genetic differentiation over subpopulations (Wright, 1965). A UPGMA phenogram was produced by input of Nei's (1978) genetic identity values into the BIOSYS-1 program. We investigated genetic variation among populations using principle components analysis (PCA) of allelic data matrix, carried out using NYSYS-pc (Rohlf, 1992).

Results

Eleven loci, coding for eight enzymes, were scored for 29 populations of four species of *Paraixeris* (Tables 1, 2). The number of isozymes detected in Korean *Paraixeris* groups (2n = 10) was similar to that reported for other diploid species (Weeden and Wendel, 1989).

Allele frequencies for 11 polymorphic loci were summarized for four species of *Paraixeris* and their hybrid populations (Table 2). The distribution of the highest-frequency alleles was consistent among four species in ADH-1 and GA3PD-1. *Paraixeris sonchifolia* had five high frequency or fixed alleles (GA3PD-2^d, ME-1^d, PGI-1^a, PGM-1^c, and TPI-1^c) which were either not found or had a low frequency in *P. koidzumiana*, *P. chelidoniifolia*, and *P. denticulata*. *Paraixeris koidzumiana* and *P. chelidoniifolia* had the same allele in highest frequency at most of the loci. ADH-2^c was the highest frequency allele in *P. chelidoniifolia*, but was absent from *P. denticulata* and was a low frequency allele in *P. sonchifolia*. *Paraixeris denticulata* and *P. sonchifolia* shared a diagnostic allele ADH-2^b, which is a low frequency allele found only in a population of *P. chelidoniifolia*.

The alleles observed in *P. koidzumiana* were not a combination of those observed in *P. sonchifolia* and *P. chelidoniifolia*. None of the five unique to high frequency alleles to *P. sonchifolia* were found in *P. koidzumiana*

Table 1. Collection data for 29 populations representing four species and four hybrid populations of *Paraixeris* examined for electrophoretic variation. The number of individuals examined is in parentheses.

Paraixeris denticulata (Houtt.) Nakai. DEN01 = Jiri Mt., Umjung-dong 650 m, Pak & Park 01 (15); DEN02 = Jiri Mt., Umjung-dong 800 m, Pak & Park 02 (29); DEN03 = Jiri Mt., Bykso-ryung 1,000 m, Pak & Park 04 (24); DEN04 = Jiri Mt., Bykso-ryung 1,000 m, Pak & Park 06 (16); DEN05 = Jiri Mt., Bykso-ryung 1,050 m, Pak & Park 10 (7); DEN06 = Chungsong, Jinbo, Park 20 (12); DEN07 = Jungsun, Park 21 (22); DEN08 = Chungsong, Park 22 (13); DEN09 = Pochun, Pak 23 (8); DEN10 = Gaji Mt. Unmun-ryung, Pak 26 (7); DEN11 = Koje-do, Hakdong, Park 29 (15); DEN12 = Koje, Tongryung, Park 30 (10); DEN13 = Andong, Park 19 (2).

Paraixeris koidzumiana Kitam. KOI14 = Jiri Mt., Umjung-dong 800 m, Pak & Park 03 (16); KOI15 = Jiri Mt., Bykso-ryung 1,100 m, Pak & Park 07 (19); KOI16 = Jiri Mt., Bykso-ryung 1,050 m, Pak & Park 11 (29); KOI17 = Jiri Mt., Bykso-ryung, near the helicopter stop, Pak & Park 13 (14); KOI18 = Jiri Mt., Bykso-ryung, helicopter stop, Pak & Park 16 (3); KOI19 = Jiri Mt., Bykso-ryung refuge, Pak & Park 18 (11).

Paraixeris chelidoniifolia (Makino) Nakai. CHE20 = Jiri Mt., Bykso-ryung 1,100 m, Pak & Park 08 (15); CHE21 = Jiri Mt., Bykso-ryung, near the helicopter stop, Pak & Park 14 (10); CHE22 = Chungdo-gun, Gagi Mt., Salbawi, Park 23 (11); CHE23 = Chungdo-gun, Gajee Mt., Salbawi, Pak 25 (13); CHE24 = Youngduk-gun, Oke stream, Park 32 (14).

Paraixeris sonchifolia (Bunge) Maxim. SON25 = Kyung Nam, Sarang island, Park 31 (8).

Hybrid populations. HYB26 = Jiri Mt., Bykso-ryung 1,000 m, Pak & Park 05 (2); HYB27 = Jiri Mt., Bykso-ryung 1,050 m, Pak & Park 12 (4); HYB28 = Jiri Mt., Bykso-ryung, helicopter stop, Pak & Park 15 (4); HYB29 = Chungdo-gun, Gagi Mt., Unmon-ryung, Pak 28 (8).

Table 2. Summary of allele-frequency data for 11 polymorphic loci among 29 populations of four species and their hybrid populations in *Paraixeris* (Compositae).

Locus/Allele	*P. denticulata*									
	1	2	3	4	5	6	7	8	9	10
ADH-1										
a	0.321	0.196	0.152	0.077	0.667	—	0.500	0.250	0.688	—
b	0.679	0.587	0.826	0.538	0.333	1.000	0.441	0.750	0.313	0.875
c	—	0.217	0.022	0.385	—	—	0.059	—	—	0.125
ADH-2										
a	—	0.017	—	—	—	—	0.370	0.083	—	—
b	1.000	0.983	1.000	1.000	1.000	1.000	0.630	0.917	1.000	1.000
c	—	—	—	—	—	—	—	—	—	—
GA3PD-1										
a	0.056	—	—	0.100	—	0.042	0.023	—	—	0.500
b	0.944	0.933	0.770	0.800	0.667	0.958	0.955	1.000	1.000	0.500
c	—	0.067	0.230	0.100	0.333	—	0.023	—	—	—
GA3PD-2										
a	—	0.029	0.025	—	—	—	0.185	—	—	—
b	0.583	0.471	0.500	0.567	0.583	0.500	0.259	0.500	0.500	0.375
c	0.417	0.500	0.475	0.433	0.417	0.500	0.500	0.500	0.500	0.625
d	—	—	—	—	—	—	0.056	—	—	—
MDH-2										
a	—	0.018	0.050	—	—	0.167	0.357	—	—	—
b	—	0.625	0.100	—	0.286	0.833	0.643	0.269	1.000	0.333
c	0.464	0.357	0.850	0.607	0.714	—	—	0.654	—	0.667
d	0.500	—	—	0.393	—	—	—	0.077	—	—
e	0.036	—	—	—	—	—	—	—	—	—
f	—	—	—	—	—	—	—	—	—	—
ME-1										
a	—	0.067	—	0.133	—	—	—	—	—	—
b	0.800	0.883	0.708	0.867	1.000	1.000	1.000	0.923	1.000	0.857
c	0.200	0.050	0.292	—	—	—	—	0.077	—	0.143
d	—	—	—	—	—	—	—	—	—	—
6PGD-1										
a	0.100	0.685	0.174	0.179	0.417	—	0.325	—	0.188	0.214
b	0.900	0.315	0.826	0.750	0.583	0.917	0.650	0.615	0.813	0.786
c	—	—	—	—	0.083	0.025	0.385	0.385	—	—
d	—	—	—	0.071	—	—	—	—	—	—
PGI-1										
a	—	0.100	—	—	0.071	—	0.087	0.083	—	—
b	0.967	0.900	0.979	0.969	0.929	0.800	0.609	0.542	0.875	0.786
c	0.033	—	0.021	0.031	—	0.200	0.304	0.375	0.125	0.214
PGM-1										
a	0.167	0.074	—	—	0.071	—	—	—	0.188	—
b	0.833	0.926	1.000	1.000	0.929	1.000	0.909	0.739	0.813	0.917
c	—	—	—	—	—	—	0.091	0.261	—	0.083
TPI-1										
a	—	—	—	—	—	0.042	0.023	—	—	—
b	1.000	0.983	1.000	0.969	1.000	0.917	0.977	0.950	1.000	1.000
c	—	0.017	—	0.031	—	0.042	—	0.050	—	—
TPI-2										
a	—	—	—	—	—	—	0.045	—	—	—
b	1.000	1.000	0.938	0.938	1.000	1.000	0.886	0.775	0.722	0.929
c	—	—	0.063	0.063	—	—	0.045	0.225	0.278	—
d	—	—	—	—	—	—	0.023	—	—	0.071

Table 2. (Continued)

Locus/Allele	*P. denticulata*			*P. koidzumiana*						*P. chelidoniifolia*	
	11	12	13	14	15	16	17	18	19	20	21
ADH-1											
a	—	0.375	0.333	—	—	0.071	—	—	—	0.091	0.500
b	—	0.500	0.667	1.000	1.000	0.893	0.222	1.000	1.000	0.909	0.500
c	1.000	0.125	—	—	—	0.036	0.778	—	—	—	—
ADH-2											
a	—	—	0.028	—	—	—	—	—	—	0.000	—
b	1.000	1.000	0.972	—	—	0.206	—	—	—	0.000	—
c	—	—	—	1.000	1.000	0.794	1.000	1.000	1.000	1.000	1.000
GA3PD-1											
a	0.045	—	—	—	—	—	—	—	—	—	0.100
b	0.955	1.000	1.000	1.000	1.000	0.792	1.000	1.000	1.000	0.500	0.867
c	—	—	—	—	—	0.208	—	—	—	0.500	0.033
GA3PD-2											
a	—	—	—	—	—	—	—	—	—	—	—
b	0.500	0.625	0.500	0.500	0.500	0.500	0.500	0.450	—	0.500	0.500
c	0.500	0.375	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
d	—	—	—	—	—	—	—	0.050	0.500	—	—
MDH-2											
a	—	—	0.333	—	—	0.071	—	0.111	—	—	—
b	0.523	0.688	0.625	—	0.313	0.357	0.192	0.444	0.333	—	0.167
c	0.477	0.313	0.042	—	0.594	0.571	0.538	0.444	0.667	0.364	0.700
d	—	—	—	—	0.094	—	0.269	—	—	0.636	0.133
e	—	—	—	0.500	—	—	—	—	—	—	—
f	—	—	—	0.500	—	—	—	—	—	—	—
ME-1											
a	—	—	—	—	—	0.263	—	—	—	0.545	—
b	0.909	0.875	1.000	1.000	0.813	0.500	1.000	1.000	1.000	0.409	1.000
c	0.091	0.125	—	—	0.188	0.237	—	—	—	0.045	—
d	—	—	—	—	—	—	—	—	—	—	—
6PGD-1											
a	0.136	—-	0.375	—	—	0.056	—	—	—	—	—
b	0.841	1.000	0.625	—	0.750	0.472	0.017	—	—	1.000	0.067
c	0.023	—	—	1.000	0.250	0.472	0.879	1.000	1.000	—	0.867
d	—	—	—	—	—	—	0.103	—	—	—	0.067
PGI-1											
a	—	—	—	—	—	—	—	—	—	—	0.100
b	0.977	0.875	0.813	1.000	1.000	1.000	1.000	1.000	1.000	0.944	0.900
c	0.023	0.125	0.188	—	—	—	—	—	—	0.056	—
PGM-1											
a	—	—	—	—	—	0.158	0.574	0.200	0.333	—	—
b	0.955	1.000	1.000	1.000	1.000	0.816	0.426	0.800	0.667	1.000	1.000
c	0.045	—	—	—	—	0.026	—	—	—	—	—
TPI-1											
a	—	—	—	—	—	—	—	—	—	—	—
b	1.000	1.000	1.000	0.500	1.000	0.974	1.000	1.000	1.000	1.000	1.000
c	—	—	—	0.500	—	0.026	—	—	—	—	—
TPI-2											
a	—	—	0.050	—	—	—	—	—	—	—	—
b	0.977	0.778	0.850	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
c	0.023	0.056	0.050	—	—	—	—	—	—	—	—
d	—	0.167	0.050	—	—	—	—	—	—	—	—

Table 2. (Continued)

Locus/Allele	*P. chelidoniifolia*			*P. sonchifolia*	Hybrid populations			
	22	23	24	25	26	27	28	29
ADH-1								
a	—	—	—	—	—	0.125	—	—
b	1.000	1.000	0.875	1.000	1.000	0.625	1.000	1.000
c	—	—	0.125	—	—	0.250	—	—
ADH-2								
a	—	—	—	—	—	—	—	—
b	—	0.045	—	0.813	1.000	0.800	1.000	0.140
c	1.000	0.955	1.000	0.188	—	0.200	—	0.860
GA3PD-1								
a	—	—	0.071	—	—	—	—	—
b	1.000	1.000	0.929	0.700	0.500	0.500	1.000	1.000
c	—	—	—	0.300	0.500	0.500	—	—
GA3PD-2								
a	—	—	—	—	—	—	—	—
b	0.500	0.500	0.500	—	0.500	0.500	0.750	0.500
c	0.500	0.500	0.500	0.500	0.500	0.500	0.250	0.500
d	—	—	—	0.500	—	—	—	—
MDH-2								
a	1.000	—	0.176	0.250	—	—	—	—
b	—	0.909	0.647	0.125	—	0.800	—	0.500
c	—	0.091	0.176	0.625	0.500	0.200	0.125	0.500
d	—	—	—	—	0.500	—	0.857	—
e	—	—	—	—	—	—	—	—
f	—	—	—	—	—	—	—	—
ME-1								
a	—	—	—	—	—-	0.100	—	—
b	0.900	1.000	1.000	0.500	1.000	0.900	1.000	1.000
c	0.100	—	—	—	—	—	—	—
d	—	—	—	0.500	—	—	—	—
6PGD-1								
a	—	—-	0.350	0.136	0.500	0.375	—	0.071
b	1.000	0.045	0.650	0.682	0.500	0.625	—	0.679
c	—	0.955	—	0.182	—	—	0.500	0.250
d	—	—	—	—	—	—	0.500	—
PGI-1								
a	—	—	—	1.000	—	—-	0.125	—
b	1.000	1.000	0.875	—	1.000	1.000	0.875	0.789
c	—	—	0.125	—	—	—	—	0.211
PGM-1								
a	—	—	—	—	—	0.100	0.375	—
b	1.000	1.000	1.000	0.500	1.000	0.900	0.625	1.000
c	—-	—	—	0.500	—	—	—	—
TPI-1								
a	—	—	—	—	—	—	—	—
b	1.000	1.000	1.000	—	1.000	1.000	1.000	1.000
c	—	—	—	1.000	—-	—	—	—
TPI-2								
a	—	—	—	—	—	—	—	—
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.750
c	—	—	—	—	—	—	—	0.250
d	—	—	—	—	—	—	—	—

whereas most of the high frequency alleles in *P. koidzumiana* were found in *P. chelidoniifolia*. The diagnostic allele, ADH-2[b] in *P. denticulata* and *P. sonchifolia*, was found in only a few individuals of a population of *P. koidzumiana* (Table 2). The low frequency, but unique alleles of *P. denticulata*, GA3PD-2[a], PGI-1[a,c] and TPI-2[c,d] were not found in *P. koidzumiana*.

Genetic identity values among populations within species were 0.933 for *P. denticulata*, 0.915 for *P. koidzumiana*, and 0.866 for *P. chelidoniifolia* (Table 3). Nei's (1978) genetic identities between species show *P. koidzumiana* to be more genetically similar to *P. chelidoniifolia* (0.882) than to *P. denticulata* (0.764) or *P. sonchifolia* (0.561).

Table 3. Mean values for Nei's (1978) genetic identity coefficients for pairwise comparisons of populations within and among four species in *Paraixeris* and their four hybrid populations.

	1	2	3	4	5	6	7	8
1. *P. denticulata*	0.933							
2. *P. koidzumiana*	0.764	0.915						
3. *P. chelidoniifolia*	0.787	0.882	0.866					
4. *P. sonchifolia*	0.629	0.561	0.549	—				
5. Hybrid pop. 26	0.935	0.792	0.815	0.667	—			
6. Hybrid pop. 27	0.956	0.815	0.848	0.621	0.991	—		
7. Hybrid pop. 28	0.833	0.787	0.754	0.577	0.926	0.834	—	
8. Hybrid pop. 29	0.862	0.914	0.922	0.608	0.868	0.903	0.789	—

Measures of genetic variation within and among populations of four species and four hybrid populations are presented in Table 4. Among the four species, *P. denticulata* and *P. sonchifolia* showed higher values of *A* (1.80-1.90), P (62.91-63.60), H_o (0.251-0.428) and H_e (0.242-0.314) than *P. koidzumiana* or *P. chelidoniifolia* (Table 4). Most of the putative hybrid populations represented levels of isozyme polymorphism greater than those found in their parental species (Table 4). Genetic variation within and between populations was measured using F-statistics (Table 5). A UPGMA phenogram based on genetic identity values depicts the close genetic relationship among populations of *P. chelidoniifolia* and *P. koidzumiana* whereas the population of *P. sonchifolia* was clearly separated from the remaining species (Figure 1). Three putative hybrid populations (HYB26, HYB27 and HYB28) form a cluster with *P. denticulata* populations whereas hybrid population 29 is nested in the *P. koidzumiana* and *P. chelidoniifolia* populations. Principal components analyses (PCA) based on genetic data showed the populations of *P. koidzumiana* to be most similar to *P. chelidoniifolia* and *P. sonchifolia* to be distinct from all other species (Figure 2). The putative hybrid populations, HYB26 and HYB27 were most similar or overlapping to the populations of *P. denticulata*.

Table 4. Average values for mean number of alleles per locus (*A*), proportion of polymorphic loci (*P*), mean observed propotion of heterozygous loci (H_o), and mean expected proportion of heterozygous loci (H_e) in 29 populations of *Paraixeris* species and their hybrid populations.

Species	*A*	*P*	H_o	H_e
P. denticulata	1.90	62.91	0.251	0.242
P. koidzumiana	1.58	39.42	0.199	0.193
P. chelidoniifolia	1.52	39.94	0.156	0.151
P. sonchifolia	1.80	63.60	0.428	0.314
Hybrid pop. 26	1.40	36.40	0.364	0.242
Hybrid pop. 27	1.80	72.70	0.368	0.320
Hybrid pop. 28	1.50	45.50	0.159	0.185
Hybrid pop. 29	1.60	54.50	0.272	0.227

Table 5. Summary of F statistics for analysis of genetic structure of three Korean *Paraixeris* species.

Species	F_{IS}	F_{IT}	F_{ST}
P. denticulata	-0.091	0.134	0.206
P. chelidoniifolia	-0.080	0.354	0.402
P. koidzumiana	-0.128	0.197	0.288

Discussion

Hybrid Origin of P. koidzumiana

Five species of *Paraixeris* have been reported from far eastern Asia (Kitamura, 1955) and, aside from *P. yoshinoi*, four of these occur in Korea. *Paraixeris koidzumiana*, an endemic species to Korea, is found in sunny and moist habitats, mainly at low elevations on Jiri Mountain. It is sympatric with *P. chelidoniifolia* and *P. denticulata* (Tae et al., 2001). Based on the leaf, bract, and flower morphology, *P. sonchifolia* and *P. chelidoniifolia* were proposed as the putative parents of *P. koidzumiana* (Lee, 1979). Recent cytological data strongly suggested the exclusion of *P. sonchifolia* as a putative parent of *P. koidzumiana* based on a unique satellite at the chromosome of *P. sonchifolia* (Pak and Kawano, 1990; Pak, 1991). Additionally, flowering periods strongly support the exclusion of *P. sonchifolia* as a parental species. *Paraixeris sonchifolia* blooms in late spring to early summer, but the remaining species bloom between September and November.

Molecular analysis using RAPD markers provided support for the hybrid origin of *P. koidzumiana* (Tae et al., 2001). The specific RAPD markers from *P. chelidoniifolia* and *P. denticulata* also occur in *P. koidzumiana*. Considering the floret numbers, *P. koidzumiana* lies between *P. chelidoniifolia* and *P. denticulata*, suggesting a hybrid origin (Tae et al., 2001).

Although the RAPD and morphological data suggested a hybrid origin for *P. koidzumiana*, our isozyme evidence did not support this hypothesis. The alleles observed in *P. koidzumiana* were not a combination of those observed in *P. sonchifolia* and *P. chelidoniifolia* or those in *P. chelidoniifolia* and *P. denticulata*. No evidence of additivity was found in *P. koidzumiana* at five isozyme loci that significantly differentiate *P. sonchifolia* from the remaining species. With one exception, the populations of *P. koidzumiana* did not combine the marker alleles that differentiate *P. denticulata* from *P. chelidoniifolia*. Although isozymes and RADPs are equally useful in identi-

fying parental species of putative hybrids by a comparison of electrophoretic profiles of the hybrid and its parents, the invalid assessment of band homology has been considered one of the major problems with RAPDs in systematic studies (Dowling et al., 1996; Edwards, 1998; Såstad et al., 1999).

Comparing within species identity values (I = 0.915 - 0.866), the high genetic identity value, 0.882, between *P. koidzumiana* and *P. chelidoniifolia* indicates that either relatively little genetic differentiation has occurred between them or that *P. koidzumiana* is a recent derivative of *P. chelidoniifolia* (Rieseberg et al., 1990; Soltis and Bloom, 1991; Wolfe and Elisens, 1993). *Paraixeris koidzumiana* is restricted to a small geographic area covering the national park at Jiri Mountain while *P. chelidoniifolia* is relatively widespread and commonly occurs in wet and open mountain areas and roadsides. The UPGMA and PCA

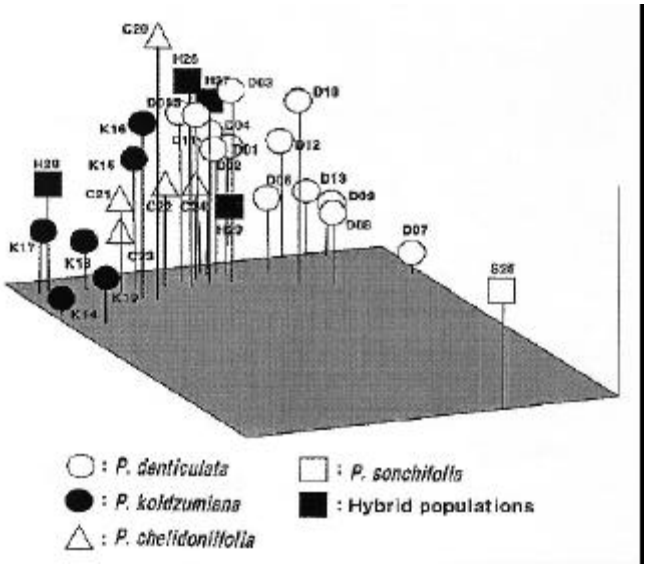

Figure 2. Three-dimensional model of numbered populations (Table 1) derived from principal components analysis of gene frequency data in Korean *Paraixeris*.

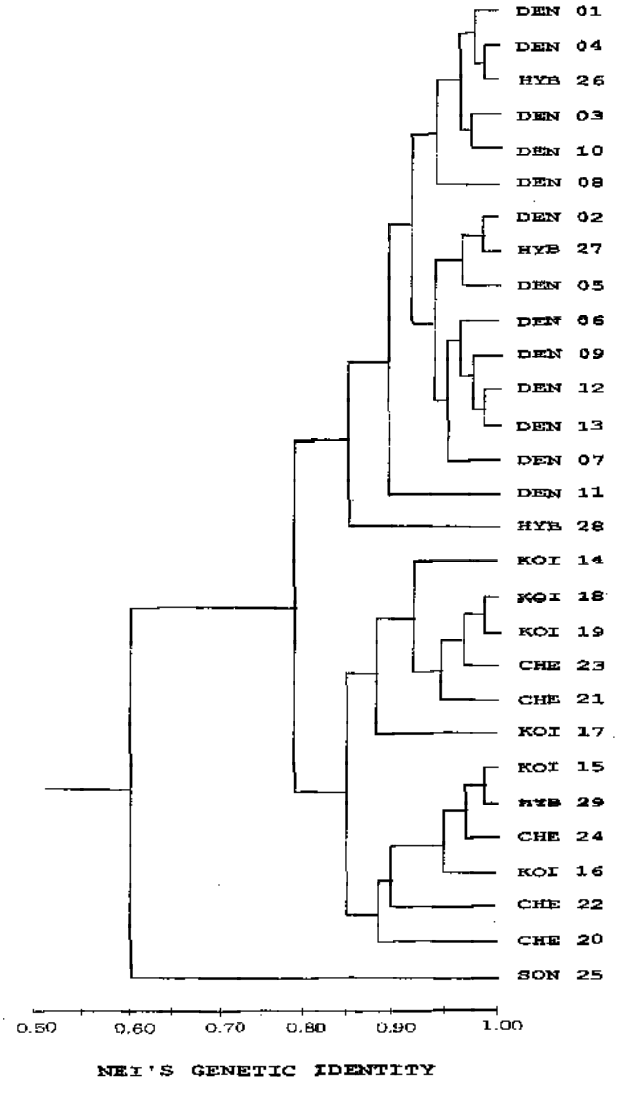

Figure 1. UPGMA phenogram derived from Nei's genetic identity value among 29 populations representing four Korean *Paraixeris* species and four hybrid populations. Species abbreviation and population numbers refer to Table 1.

results are consistent with a recent divergence in the above two species. The phenogram illustrated a close genetic relationship between *P. chelidoniifolia* and *P. koidzumiana* and that the populations of *P. koidzumiana* and *P. chelidoniifolia* are intermixed (Figure 1). These data suggest that *P. koidzumiana* and *P. chelidoniifolia* may be a recent divergent species pair rather than a stabilized hybrid derivative as proposed by Lee (1979) and Tae et al. (2001). However, the hypothesis of progenitor-derivative species pair seems to be unreliable because a significant number of low frequency alleles occurring in *P. koidzumiana* were not found in *P. chelidoniifolia*.

Hybridization among Species-Pairs in Korean Paraixeris

Previous canonical variate analysis using morphological data, pollen stain-ability analysis, and pollen morphology (Pak, unpublished data) strongly suggest that individuals from populations 26, 27 and 28 found at roadsides at Bykso-ryung, were most likely hybrids of *P. denticulata* and *P. chelidoniifolia*. A low percentage of pollen stain ability (26.2%) in aniline blue and intermediate pollen grain size between the two parental species indicate that these individuals are hybrids. Our isozyme results revealed that the three populations shared a high genetic identity with *P. denticulata*. Population 27 had the most marker alleles of *P. denticulata*, but shared a marker allele (ADH-2^c= 0.2) with *P. chelidoniifolia*, indicating that this population may also have experienced past introgression in the direction from *P. chelidoniifolia* to *P. denticulata*. The most common alleles from the presumed hybrid population 29 were shared by both *P. koidzumiana* and *P. chelidoniifolia*. In addition, the Gagi Mountain population showed high genetic identities with these two species. The cluster analysis using electrophoretic results

showed that the population of Gagi Mountain actually clustered most closely with the populations of *P. koidzumiana* and *P. chelidoniifolia*.

Hybrid populations usually display levels of isozyme polymorphism greater than their putative parents (Morrell and Rieseberg, 1998). The proposed hybrid populations, 27 and 29, revealed greater values of genetic variation compared with the putative parental species (Table 4). Thus, hybridization among Korean *Paraixeris* species seems to be commonplace where the species are sympatric.

Genetic Variation in Korean Paraixeris

The mean values of *A* and *P* for four species of Korean *Paraixeris* were similar to those calculated by Hamrick and Godt (1989) for animal pollinated out-crossing species with annual habit. Comparing the genetic variation data from the Bonin Islands' *Crepidiastrum* species (Ito and Ono, 1990; $P = 22.34$, $A = 1.24$, $H_e = 0.056$), the most closely related species to *Paraixeris*, Korean *Paraixeris* species show significantly higher values of genetic variation. This may be due to the relatively widespread distribution of *Paraixeris* species across Korea, Japan, and Manchuria. However, the *Crepidiastrum* species of the Bonin Islands should have experienced a bottleneck effect due to the small population size and restricted habitats (Ito and Ono, 1990).

The widespread species *P. denticulata* and *P. sonchifolia* have a higher genetic variation than the restricted, endemic species *P. koidzumiana*. These data are consistent with the previous hypothesis that the geographic range had a significant effect on genetic variation at species and population level (Frankel et al., 1995). A geographically restricted species such as *P. koidzumiana* may have a chance to experience high levels of inbreeding, genetic drift, and strong directional selection toward genetic uniformity (Karron, 1991).

The relatively low levels of genetic variation and the high genetic differentiation among populations ($F_{ST} = 0.402$) of *P. chelidoniifolia* are exceptional. Unknown recent history of the populations and some of the attributes presented by *P. chelidoniifolia*, such as extensive clonal growth and lack of efficient seed dispersal mechanisms, should result in low genetic variation and a high genetic divergence among populations.

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菊苣（*Paraixeris*）同功異構酶的變化： *Paraixeris koidzumiana*二倍體雜交種源的分析

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Paraixeris koidzumiana 是以 *P. chelidoniifolia* 和 *P. denticulate* 或 *P. chelidoniifolia- P. sonchifolia* 配對祖先的雜交物種，以這四種韓國菊苣的同功異構瓷型態的變化當做是試驗的假說。從 *P. koidzumiana, P. denticulate, P. chelidoniifolia* 和 *P. sonchifolia* 的 29 個樣品檢視八個酵素其在十一個基因座中。雖然最近 RAPD 和型態學的資料支持種源為 *P. koidzumiana*，但是在同功異構瓷的證據卻不是這樣。沒有證據顯示在 *P. koidzumiana* 和 *P. sonc hifolia* 的基因座有差異，且 *P. koidzumiana* 中沒有增加不同的標誌對偶基因與 *P. denticulate* 和 *P. chelidoniifolia*。近來有強烈證據顯示 *P. chelidoniifolia* 和 *P. koidzumiana* 的相異，因為有高度的遺傳一致性和大部分的基因座中有高頻率的對偶基因。我們的同功異構瓷的資料支持這個結論是未被認出且獨特的發現，在同一地區物種內並存但不會雜交而成混合種可認為是雜交的衍生。

關鍵詞：雜交；同功異構瓷；*Paraixeris koidzumiana*。