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Genetic Diversity and Population Structure of maize, *Zea mays*, in Both Landraces and Cultivar Lines

Man Kyu Huh*† and In Sup Lee**

*Department of Biology Education Pusan National University, Pusan 609-735
**Department of Biology, Kyungsung University, Pusan 608-736

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Abstract Enzyme electrophoresis was used to estimate genetic diversity and population structure of maize, *Zea mays* L. (Graminales) in Korea. In nine populations, fourteen of the 24 loci (58.3%) showed detectable polymorphism. Genetic diversity (0.205) was higher than average values for species with similar life history traits. Although our data are relatively small and the landraces not direct ancestors of cultivar, apparently the domestication process has eroded the levels of genetic variation of maize. The recent cultivars were found to have fewer alleles per locus (1.42 vs. 1.56), fewer alleles per polymorphic locus (2.27 vs. 2.33), lower percent polymorphic locus (33.3% vs. 41.7%), and lower diversity (0.159 vs. 0.185) than landraces. These genetic diversity parameters indicated that the cultivar populations were genetically depauperate relative to landlaces. The *GST* value of nine populations was 0.239. Nearly 76% of the total genetic diversity in *Zea mays* was apportioned within populations. The indirect estimate of gene flow based on mean *GST* was moderate (*Nm*=0.80).

Key words: genetic diversity, population structure, cultivar, landrace, maize, *Zea mays*

Introduction

The amount and distribution of genetic variation within species is a subject of considerable interest because of its evolutionary importance [12]. Many plant species have been surveyed using electrophoresis, prompting several attempts to elucidate relationships between ecological features of plants and the amount and patterns of genetic variation [10, 18,20].

Isozyme electrophoresis has had a positive influence on a large number of biological disciples [3,11,15]. Hamrick and Godt [12] noted considerable heterogeneity among species for levels of within population variation. A significant proportion of the variation was associated with the life history and ecological characteristics of the species. Species that were widespread, long-lived, primarily outcrossed by wind-pollination, and characteristic of the later stages of succession, maintained higher levels of intrapopulational variation than the species with other combination of these traits [12,13]. Maize or corn (also outside the United States and Canada), *Zea mays* L. is one of important crops for providing the current and future needs of the world. Maize is native to tropical America and is the only cereal that was domesticated in the America [5]. This species is cultivated in developing countries and of this approximately 50 million hectares are in the tropics, mainly at low elevations [6]. As a species it contains enormous genetic variation. The potential yield of maize is large than that of either wheat or rice and we can expect maize to assume a proportionally larger and more important role in world food production.

The purposes of this study were: 1) to estimate total genetic diversity maintained in maize populations; 2) to analyze the genetic structure of Korean populations; and 3) to compare our estimates with those for species having very similar life history traits and wild maize species. In addition, our basic questions were: 1) has the domestication process eroded the levels of genetic variation of the cultivated populations as has been shown in many cultivated species? and 2) is it possible to detect the revealing pedigree relationships between lines using the isozyme markers?

Materials and Methods

Sampling procedure

Zea mays L. (Graminales) was collected from nine populations in Korea (Table 1). The landraces and cultivars were composed of five and four populations, respectively. One leaf per plant was collected during the period from 2000 to 2001. The landraces were from the collection of the Laboratory of Genetics, Kyungsung Univ. in Korea. Thirty-five to 65 plants were randomly selected from each population. All

†Corresponding author
Phone: 82-51-510-2698, Fax: 82-51-510-2698
E-mail: mkhuh200@yahoo.co.kr

Table 1. The code of populations and localities of landraces and cultivars investigated for isozyme analysis

Code	Localities	Cultivated types
P1	Anei-myen, Hamyang-gun	Landrace
P2	Ueigyang-myen, Kechang-gun	Landrace
P3	Meongseok-myen, Chinju-city	Landrace
P4	Dosan-myen, Tongreng-gun	Landrace
P5	Chilbuk-myen, Haman-gun	Landrace
C1	Gijang-myen, Busan-city	Cultivar: Suwon 19
C2	Habuk-myen, Yangsan-gun	Cultivar: Suwon 19
C3	Ungchon-myen, Ulsan-city	Cultivar: Suwon 19
C4	Gonmeong-myen, Sacheon-city	Cultivar: Suwon 19

samples were placed on ice until isozyme extraction.

Enzyme electrophoresis

Homogenization, starch gel electrophoresis and enzyme assay procedures followed the methods of Soltis et al. [29]. Approximately 300 to 500 mg leaf tissue were ground with a cold mortar and pestle in 300 to 500 μL of extraction buffer (0.05 mL 0.1% 2-mercaptoethanol, 0.001 M EDTA, 0.001 M KCl, 0.01 M magnesium chloride hexahydrate, 1 g 4% w/v PVP, 0.1 M Tris-HCl buffer of pH 8.0). Electrophoresis was performed using 11.0% starch gels and a total of twelve enzyme systems were assayed for this study: acid phosphatase (ACP), esterase (EST), fluorescent esterase (FE), leucine aminopeptidase (LAP), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), peroxidase (PER), 6-phosphogluconate dehydrogenase (PGD), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), and shikimate dehydrogenase (SKD).

For enzymes resolved in more than one zone of activity, the most anodal isozyme was arbitrarily designated 1 and subsequent isozymes were sequentially assigned higher numbers. Likewise, alleles were designated sequentially with the most anodally migrating allozyme designated 'a' and progressively slower forms 'b', 'c', and so on.

Data analysis

To provide information on genetic diversity of populations used in this study, we calculated the following genetic standard measures [14]: mean number of alleles per locus (A), effective number of alleles per locus (A_E), mean heterozygosity per population under Hardy-Weinberg expectations (the genetic diversity, H_E), observed heterozygosity (H_O), and proportion of percentage polymorphic loci (P) where a locus was considered polymorphic if the frequency of the common allele <95%. Subscript refers to population ($_P$) level parameter. Nei's gene diversity formulae (H_T, H_S, D_{ST}, and G_{ST}) were used to evaluate the distribution of genetic diversity within and among populations [24]. The G_{ST} coefficient, in particular, estimates relative population differentiation. Nei's genetic identity (I) was calculated for each pairwise combination of populations [25]. We used the PC-SAS program [27] to conduct a cluster analysis on genetic distances via the unweighted pairwise groups method arithmetic average (UPGMA).

The correlations to the coancestry coefficient (f) were determined. For a given f values of two populations, the genetic similarity (GS) was calculated according to Melchinger et al. [23].

The genetic structure within and among populations was also evaluated using Wright's [30] F-statistics: F_{IT}, F_{IS}, and F_{ST}. The F_{IT} and F_{IS} coefficients measure excesses of homozygotes or heterozygotes relative to the panmictic expectations within the entire samples and within populations, respectively. Deviations from Hardy-Weinberg expectations were examined for each population by calculating Wrights fixation index deviations [31] and testing for significant deviations from the expected values ($F = 0$) by chi-square tests [19]. Two indirect estimates of gene flow were calculated. One estimate of *Nm* (the number of migrants per generation) was based on G_{ST} [32] and the other estimate was based on the average frequency of "rare" alleles found in only one population [1]. The absolute population differentiation (Dm) was calculated using Neis statistics [24]. Correlation between geographical and genetic distance was tested using Mantel's test as advocated by Smouse et al. [28].

Results

Genetic diversity

In maize thirteen of the 24 loci (58.3%) were polymorphic in at least one population. The remaining eleven loci (*Acp-2*, *Est-3*, *Fe-1*, *Idh-1*, *Lap*, *Me-1*, *Me-2*, *Per-1*, *Per-2*, *Pgd-1*, and *Pgd-2*) were monomorphic in all populations. An average of 38.0% of the loci (P_P) was polymorphic within populations, with individual population values ranging from 29.2% to 45.8% (Table 2). The average number of alleles per locus (A) was 1.50 across populations, ranging from 1.38 for the population with the lowest mean number of alleles to 1.71 for the population with the highest mean. The effective number of alleles per locus (A_E) was similar at the species (1.37) and the population level (1.32). The mean genetic diversity within population (H_{EP}) was 0.173. Population P3 had the highest expected diversity (0.202), while population C3 had the lowest (0.140). Genetic diversity at the population level ($H_{EP} = 0.106$) was low.

In landrace maize, P, A, H_{OP}, and H_{EP} were 41.7%, 1.56, 0.116 and 0.185, respectively. Cultivar maize thus showed lower levels of genetic diversity than landraces by Wilcoxons signed-rank test ($p < 0.05$).

Total genetic diversity values (H_T) varied between 0.066 (*Fe-2*) and 0.606 (*Mdh-1*) with an average over all polymorphic loci of 0.304 for landrace maize (Table 3) and 0.194 for cultivar maize (Table 4). The interlocus variation of population genetic diversity (H_S) was low: values ranged from 0.057 (*Fe-2*) to 0.500 (*Pgd-1*) with an average over all polymorphic loci of 0.270 for landraces and 0.167 for

Table 2. Percentage of polymorphic loci (*P*), mean number of alleles per locus (*A*) and polymorphic locus (*A*p), effective number of alleles per locus (*Ae*), observed heterozygosity (*Hop*), Hardy-Weinberg expected heterozygosity or genetic diversity (*Hep*) of landraces and cultivar maize

Pop.	N	*P*	*A*p	*A*	*Ae*	*Hop* (SD)	*Hep* (SD)
Landrace polulations							
P1	45	45.83	2.27	1.58	1.34	0.127 (0.011)	0.191 (0.041)
P2	65	33.33	2.25	1.42	1.32	0.100 (0.010)	0.167 (0.043)
P3	37	45.83	2.27	1.58	1.37	0.120 (0.011)	0.202 (0.044)
P4	38	37.50	2.33	1.50	1.31	0.109 (0.010)	0.168 (0.042)
P5	45	45.83	2.55	1.71	1.35	0.122 (0.011)	0.196 (0.042)
Mean		41.67	2.33	1.56	1.34	0.116 (0.005)	0.185 (0.019)
Cultivar polulations							
C1	39	29.17	2.29	1.38	1.27	0.092 (0.010)	0.146 (0.040)
C2	44	33.33	2.13	1.38	1.29	0.099 (0.010)	0.157 (0.041)
C3	37	25.00	2.50	1.38	1.28	0.071 (0.009)	0.140 (0.042)
C4	35	45.83	2.18	1.54	1.35	0.112 (0.010)	0.193 (0.042)
Mean		33.33	2.27	1.42	1.30	0.093 (0.005)	0.159 (0.021)
Total mean		37.96	2.31	1.50	1.32	0.106	0.173
Species		58.33	2.71	2.00	1.37	-	0.205

N: Number of individuals in the sample.

Table 3. Total genetic diversity (H_T), genetic diversity within population (H_S), deviations of genotype frequencies from Hardy-Weinberg expectations over all populations (F_{IT}) and within individual populations (F_{IS}), and proportion of total genetic diversity partitioned among populations (G_{ST}) of landraces

Locus	H_T	H_S	D_{ST}	D_m	F_{IS}	F_{IT}	G_{ST}
Fe-2	0.066	0.057	0.009	0.011	0.403	0.483	0.133
Mdh-1	0.606	0.486	0.120	0.150	0.385	0.507	0.198
Mdh-2	0.294	0.287	0.009	0.011	0.510	0.525	0.030
Mdh-3	0.233	0.177	0.056	0.070	0.351	0.508	0.241
Acp-1	0.316	0.293	0.024	0.030	0.238	0.296	0.075
Pgm-1	0.341	0.308	0.033	0.041	0.351	0.414	0.097
Pgm-2	0.072	0.061	0.011	0.014	0.528	0.601	0.154
Skd	0.293	0.270	0.022	0.028	0.525	0.561	0.075
Idh-2	0.248	0.236	0.013	0.016	0.147	0.190	0.051
Per-3	0.497	0.367	0.130	0.162	0.362	0.529	0.261
Est-1	0.494	0.471	0.023	0.028	0.489	0.512	0.046
Est-2	0.212	0.193	0.019	0.024	0.436	0.487	0.089
Pgd-1	0.500	0.500	0.000	0.000	0.333	0.333	0.000
Pgi	0.085	0.079	0.062	0.008	0.442	0.483	0.073
Mean	0.304	0.270	0.034	0.042	0.393	0.459	0.109

Table 4. Total genetic diversity (H_T), genetic diversity within population (H_S), deviations of genotype frequencies from Hardy-Weinberg expectations over all populations (F_{IT}) and within individual populations (F_{IS}), and proportion of total genetic diversity partitioned among populations (G_{ST}) of cultivar maize

Locus	H_T	H_S	D_{ST}	D_m	F_{IS}	F_{IT}	G_{ST}
Mdh-1	0.404	0.304	0.100	0.114	0.572	0.678	0.247
Mdh-2	0.224	0.195	0.030	0.034	0.637	0.685	0.132
Mdh-3	0.317	0.273	0.044	0.050	0.337	0.429	0.138
Acp-1	0.072	0.062	0.011	0.012	0.363	0.456	0.146
Pgm-1	0.028	0.025	0.003	0.003	0.435	0.493	0.101
Pgm-2	0.014	0.013	0.001	0.001	0.470	0.497	0.050
Skd	0.206	0.164	0.042	0.048	0.390	0.515	0.204
Idh-2	0.098	0.082	0.016	0.018	0.173	0.309	0.164
Per-3	0.339	0.286	0.053	0.060	0.426	0.516	0.156
Est-1	0.182	0.152	0.030	0.034	0.411	0.510	0.170
Est-2	0.080	0.071	0.009	0.010	0.346	0.416	0.107
Pgd-1	0.500	0.500	0.000	0.000	0.333	0.333	0.000
Pgi	0.560	0.047	0.009	0.010	0.546	0.617	0.157
Mean	0.194	0.167	0.027	0.030	0.419	0.496	0.136

cultivar maize.

The comparison of banding patterns between landraces and cultivar indicated that 8.3% of the alleles were not shared by the two species. Four alleles were found specific to landraces, whereas only one allele was specific to cultivars.

Genetic structure

For landraces, Wright's *F* coefficients showed significant deficiencies of heterozygotes for most loci both at the population level (F_{IS} = 0.393) and at in sample as a whole (F_{IT} = 0.459) (Table 3). For cultivars significant positive F_{IS} (0.419) and *F*IT (0.496) values were also observed for most polymorphic loci (Table 4).

On a per locus basis, the proportion of total genetic variation found among populations (G_{ST}) was 0.109 for landraces, indicating for landraces most of the genetic variance (89%) resided within populations. The mean G_{ST} of cultivar populations was 0.136. The absolute population differentiation (*D*m) was 0.042 for landraces and 0.030 for cultivars. The correlation coefficients between genetic distance and geographical distance using Mantel's test for all populations were 0.25 (r^2 = 0.06) for landraces and 0.31 (r^2 = 0.10) for cultivars. Most of the variation in genetic distance seemed to be caused by unknown factors other than geographic distance.

The values of genetic distance (D) were below 0.157 in most except in pairs involving populations P1 and C4. Genetic identity (I) values among pairs of populations of maize ranged from 0.855 to 0.990. Prediction of GS based

on f values and by linear regression was shown Fig. 2. For both groups, linear regression of landrace populations was lower than that of cultivar populations. But, no significant difference for the slope as well as the intercept was found between both groups. The indirect estimate of gene flow based on mean G_{ST} was low (Nm = 0.80), and the estimated gene flow based on private alleles was 0.45.

Discussion

One of the goals of population genetics is to produce phyologenetic relationships that best reflect the evolutionary events among taxa. Crops such as maize undergo environmental adaptation and agronomic selection that may lead to parallel or convert evolution [21,22]. Isozymatic data were analyzed by both phylogenetic and phenetic methods in an attempt to reveal latent occurrence of these evolutionary phenomena.

The phylogenetic tree shown in Fig. 1 clearly distinguishes two major clades, the landraces and cultivar groups. The correlation between genetic distance and geographical distance is relatively high in landraces and low in cultivar groups. In the landrace groups, the positions of the populations in the tree almost completely match the corresponding geographical positions. In the cultivar population clades, two populations from the eastern part of the Gyeongsangnam- do area form a group (C1 and C3). The correlation between genetic distance and geographic distance was high (r = 0.31, p <0.05), for the landrace populations, indicating that geographically close populations tended to be genetically similar, whereas it was low (r = 0.25, p < 0.05) for the cultivar populations.

Fig. 1. A dendrogram showing the genetic relationships among nine Korean populations of maize, based on allozyme variation.

The crop materials with which we are concerned are the landraces, grown by peasant cultivars, now so rapidly being replaced by modern improved varieties. In these, there are special conditions operating which will affect the level at which total isolation occurs and populations arise possessing different alleles. Even although growing conditions may be landraces there has been sufficient amelioration and uniformity of cultural methods that selection can be uniform over quite wide ranges. This will act against divergence. But equally, different cultural traditions can select for different genotypes in regions that are environmentally similar: this seems to have occurred in the differentiation of *Gossypium hirsutum* where quite different types of cotton have evolved in Central America in association with different ethnic groups rather than different environments [17]. In maize, very distinctive varieties are maintained side by side in a state of uniformity by Indians in Central America [22]. But even where there is uniformity of cultural techniques this cannot overcome basic differences in environment due to altitude, exposure, rainfall or soil, all of which will have strong selective effects [2].

In most traditional cultures it was common practice for individual farmers to retain their own seed or to get seeds from neighbours: this prevents long distance movement of genotypes [16]. But equally, in times of disturbance or migration, it was normal for groups to take their seed with them.

For the allozyme variations of two cultivators, one of the most striking features of this study was the most of genetic variation (85%) resided within populations. The mean G_{ST} of Korean maize populations was 0.151.

Although the genetic diversity parameters of cultivars are lower than those of landraces, both have relatively high levels of allozyme variation as compared to the similar species. The percentage of polymorphic loci at the species level was 58.3%. The value is higher than wind-pollinated outcrossing species (49.7%), annual herbaceous (50.7%), and temperate-zone species (48.5%). The same patterns are observed for other measures of genetic variation. However, Korean maize has relatively high levels of allozyme variation as compared to the other wild maize species. In Korean cultivars, P, A, and He were 33.3, 1.42, and 0.159, respectively. In wild maize, *Z. mays* subsp. *mays*, the values of corresponding parameters were 91%, 7.1, and 0.182, respectively (Table 5) [7]. The same is found for the other wild maize species, *Z. mays* var. *parviglumis*: 100%, 6.6, and 0.261, respectively [7].

In cultivars, the observed high, significant, and positive F_{IS} value indicates that homozygotes were significantly in ex-

Table 5. Measures of genetic variability for all previously studied *Zea mays* species

Species	Country	A	P	H_T	H_{ES}	Nei's *I*	Data source
Zea mays subsp. *Mays*	Mexico	7.1	50.0	0.251	0.182	0.95(0.87~0.99)	Doebley et al., 1985
Zea mays var. *parviglumis*	America	6.6	69.0	0.311	0.261	0.91(0.82~0.98)	Doebley et al., 1984
Zea mays	Korea	2.0	58.3	0.222	0.205	0.97(0.93~0.99)	In this study
F-test		***	ns	*	ns	ns	

ns: not significant, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

cess. A deficit of heterozygotes was found for maize, indicating that inbreeding may occur, or that plants are intermating and dispersing over a smaller scale than that sampled (the Wahlund effect). The range of F_{IS} values (Table 3, 0.126 to 0.637) of the inbreeding coefficient was substantially greater than expected, suggesting that unknown evolutionary forces differ in their impacts upon 14 loci.

Highly significant ($p < 0.05$) positive correlations above 0.43 were found between f and both population groups (Fig. 2). Similar findings for maize lines were obtained with four AFLP (amplified fragment length polymorphism) primer combinations [21]. In a comparable study of a wide range of dent germplasm, the closest correlations between f and GS estimates were obtained with RFLPs and AFLP markers [26]. Therefore, molecular markers including isozymes in reflecting pedigree relationships between lines and produce a similar ranking of GS estimates.

The origin of all cultivar maize lines are entirely obscure. Since most of ancient crops cultivated in Korea are considered to originate from China, the landraces may be also from China. Although our data are relatively small and the landraces not direct ancestors of cultivars, apparently the domestication process has eroded the levels of genetic variation of maize [7,9].

Maize is a crop of economic importance and native strains have been replaced by hybrids and improved varieties [4]. Conclusionally, in this study we can split Korean maize into racial groups, the landraces and the more recent introduced cultivar maize. Among Asian countries, China is a major producer of maize. The landraces may be also from China and cultivars are directly introduced from North America. Both lines are perhaps considered as the races of the different sources. High-yielding hybrids are replacing local varieties, including improved cultivars (mostly in rice fields for commercial production), but landraces and locally adapted cultivars of maize can be found in several regions (mostly grown in dry fields for self-consumption). We have found that isozymes can be used for identification of two maize groups.

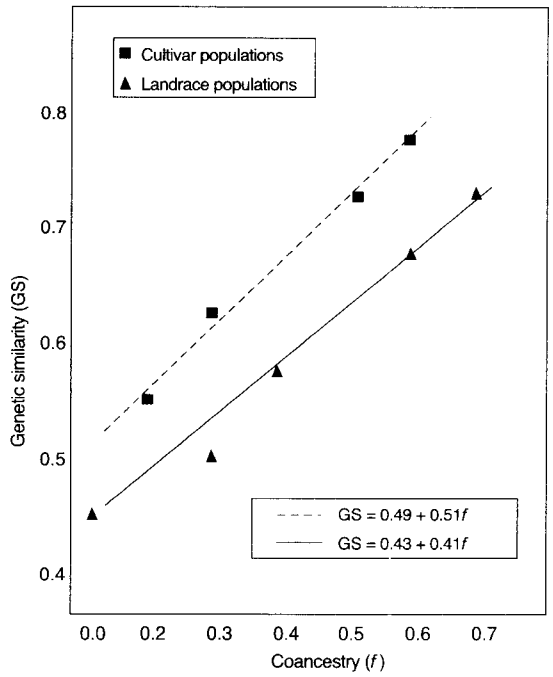

Fig. 2. Genetic similarity (GS) based on isozyme loci as a function of coancestry for related ($f > 0$) pairs of maize populations in Korea.

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