

Demography of ethnic minorities and its effects on the genetic diversity of rice landraces in Guizhou Province, China

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Research Article

Keywords: landraces, traditional culture, genetic diversity, haplotype, conservation

Posted Date: April 25th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1534025/v1>

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Abstract

Ethnic minorities living in Guizhou Province have a long history of rice farming and traditional culture, and they produce numerous of rice landraces, which are rich in genetic variation. Therefore, studying the genetic structure and diversity of rice landraces in Guizhou has become a hot topic. However, the influence of ethnic minorities and their traditional farming system and culture on rice landraces remains unclear. In this study, we analyzed the genetic diversity and population structure in 598 rice landraces in six eco-rice farming zones using simple sequence repeat (SSR) markers and the nucleotide variation between two similar populations collected during different periods using single nucleotide polymorphism (SNP) haplotype analysis of six unlinked nuclear loci. The results were as follows: 1) The genetic diversity level of rice landraces in six different eco-rice farming zones of Guizhou Province was high ($He = 0.7659$), and the genetic background was rich. Qianxinan Miao and Buyi Autonomous Prefecture is the genetic diversity center of rice landraces in Guizhou, with the highest He (0.7549) and PIC (0.7278). 2) The neighbor-joining (N-J) tree and the STRUCTURE model showed the same genetic structure, and the population structure of *japonica* was correlated with geographical origin. Under the influence of the domestication of the Buyi, Miao and Dong people, rice landraces in the III and IV eco-rice farming zones were isolated into a subgroup, forming a special ecological type of landrace. 3) Nucleotide variation analysis was performed on similar rice landraces collected in 1980 and 2015. The results showed that after 35 years of domestication, the original dominant haplotypes could be preserved well, and the frequency of the most favorable haplotypes gradually increased, while the frequency of a few unfavorable haplotypes gradually decreased to adapt to the corresponding environment and traditional culture. This study can promote the protection and sustainable utilization of rice landraces and provide more valuable germplasm materials and information for rice breeding and basic research.

Introduction

Guizhou Province is located in southwest China and has a complex terrain and diverse climate. There are more than fifty ethnic minorities living in Guizhou Province, each of which has a different traditional culture and lives in different ecological environments (Suo and Xiao 2021). Therefore, Guizhou has rich and diverse crop genetic resources in its long history, which have become the material basis for generations of people of all ethnic groups (Zheng et al. 2016; Yang 2017). Because the traditional culture, living customs and farming culture of various ethnic minorities are different, they have created many unique crop genetic resources in the long term. Some crop genetic resources have obvious regional and irreplaceable characteristics (Liu et al. 2022a) and are often accompanied by strong adaptability, such as drought resistance, cold resistance and disease and insect resistance (Pusadee et al. 2009), which are widely considered by crop breeding and basic researchers.

The traditional cultural and farming methods of ethnic minorities will have a profound impact on the evolution of local varieties (Wang et al. 2018a); therefore, understanding the traditional cultural background of ethnic minorities is an important prerequisite for the protection and sustainable utilization of these crop genetic resources. At present, there have been many studies on the relationship between ethnic minorities and their traditional culture, traditional farming methods and crop genetic resources. Negri (2005) found that the diversity of local varieties in Europe was closely related to local traditional culture, and the richness of traditional culture was one of the important factors affecting the preservation and maintenance of crop variety diversity (Cromwell and Oosterhout 2000). Asian rice farmers continue to plant thousands of varieties of rice

with different taste qualities to meet different traditional food cultures (Bertuso et al. 2000). Influenced by Chinese traditional waxy rice culture, people living in East and Southeast Asia prefer to eat waxy food, resulting in the directional accumulation of waxy genes in crops and eventually forming numerous waxy germplasm resources (Fuller and Rowlands, 2011). In China, studies on the role of ethnic minorities in crop domestication started late and were mainly in southwestern China (Wang et al. 2016). Xu et al. (2012) investigated crop genetic resources in different areas inhabited by ethnic minorities in Yunnan Province and found that the loss of rice landraces is serious, while the rice landraces still planted mainly meet the traditional cultural and dietary habits of local ethnic minorities (Wang et al. 2018a; Liu et al. 2022b). In addition, the genetic diversity of local varieties planted by different ethnic minorities varies greatly (Dong et al., 2013; Dong et al., 2020), which is not only related to different ecological conditions but also mainly caused by the differences in the traditional culture, traditional dietary customs and traditional agricultural farming methods of each ethnic group (Khan et al. 2020).

Guizhou Province has rich rice germplasm resources (Zhu et al. 2007), among which rice landraces are the main component (Ruan et al. 2007). Rice landraces are the product of long-term natural and artificial selection during the domestication and production of rice. Compared with bred varieties, rice landraces have a complex genetic background and rich genetic diversity and heterogeneity (Cui et al. 2016); they have strong adaptability to the environment and are an excellent gene source for important traits, such as high quality, resistance to disease and insects, and resistance to stress (Cui et al. 2020). The *indica-japonica* differentiation of rice landraces was obvious in Guizhou Province; the genetic structure of the *indica* subpopulation is related to rice maturity, while that of the *japonica* subpopulation is related to water conditions (Zhang et al. 2006). Part of *japonica* in Guizhou Province is a very old rice variety (Xian et al. 2013), which is a variant of a certain quality formed in the process of continuous human domestication (Li 2008). Over the years, scholars have performed much research on the investigation and collection (Wang et al. 2018a), phenotypic identification (Lei et al. 2021) and genetic diversity evaluation (Liu et al. 2022a) of rice landraces in Guizhou Province. They also conducted considerable investigation, collection, identification and protection of the characteristic rice resources in Guizhou, such as “HE” resources, “red rice” resources and “dry rice” resources (Ruan et al. 2015). However, the overall utilization of rice resources in Guizhou Province is low (Zhang et al. 2006), especially for rice landraces with ethnic traditional cultural characteristics planted in the living areas of ethnic minorities. There is still a lack of systematic research, and the domestication effect of ethnic minorities on these rice landraces is not clear.

All ethnic minorities in Guizhou Province have their own traditional cultures and living customs, which protect rice landraces (Zheng and Gao 2016). However, with the development of the economy, science and technology, as well as cultural exchanges among different ethnic groups, the traditional culture and living customs of some ethnic minorities are gradually disappearing (Xue 2019). Local rice landraces adapted to the disappearing traditional culture and living customs have also been lost. In this study, rice landraces distributed in six eco-rice farming zones in Guizhou were selected. Modern molecular marker techniques were used to explore the domestication characteristics of rice landraces by ethnic minorities. In addition, two populations of similar accessions in southeastern Guizhou from 1980 and 2015 were selected to further explore the effects of short-term selection and domestication of ethnic minorities on rice genetic diversity and important haplotypes. This study is expected to promote the protection and sustainable utilization of rice landraces and provide more valuable germplasm materials and scientific support for rice breeding and basic research.

Materials And Methods

Plant materials

A total of 640 materials were used for the population genetic study, including 598 rice landraces and 42 wild rice, all from the national gene bank (collected in 1980). A total of 598 rice landraces were distributed in 72 counties of six eco-rice farming zones in Guizhou Province (five accessions were selected in each county on average; due to the diversity of rice landraces in areas inhabited by ethnic minorities, eight to nine materials were selected for these counties). In this study, rice landraces were divided into seven groups according to six eco-rice farming zones. As the Ⅹ eco-rice farming zone was inhabited by ethnic minorities with a large population belonging to two different ethnic autonomous prefectures regarding geographical administrative divisions, the accessions were divided into two parts (Ⅹ-1 and Ⅹ-2). Table 1 and Fig. 1 show the detailed information of 640 accessions.

Table 1
Geographic distribution of 640 accessions

Category	Geographic distribution	Population	Quantity
Rice landraces	Ⅹ Eco-rice farming zone in central Guizhou	Ⅹ	190
	Ⅹ Eco-rice farming zone in eastern Guizhou	Ⅹ	45
	Ⅹ Eco-rice farming zone in southwestern of Guizhou	Ⅹ	85
	Ⅹ-1 Eco-rice farming zone in southern Guizhou	Ⅹ-1	84
	Ⅹ-2 Eco-rice farming zone in southeastern Guizhou	Ⅹ-2	142
	Ⅹ Eco-rice farming zone in northern Guizhou	Ⅹ	23
	Ⅹ Eco-rice farming zone in northwestern Guizhou	Ⅹ	29
Wild rice	Guangdong, Guangxi and Hunan provinces	WR	42
Total			640

Another part of the accessions (164 rice landraces) in this study were collected by our team during an ethnobiological survey in southeast Guizhou in 2015 (Wang et al. 2018a). These accessions were from the same region with the same genetic background as those of Ⅹ-2 and were used as control accessions for the short-term domestication of ethnic minorities. The names of all accessions are shown in Supplementary Table 1 and Supplementary Table 2.

DNA extraction and SSR molecular marker assays

All research accessions were planted in Sanya, Hainan. Total genomic DNA was extracted manually from fresh young leaves using a modified CTAB procedure (Doyle and Dickson 1987). DNA concentration was determined using a Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific), and DNA integrity was checked by electrophoresis on 1% agarose gels. 36 SSRs (Supplementary Table 3), evenly distributed throughout the rice genome, was used for genotypic identification. PCR amplifications were carried out using the following thermal cycling program: a pre-denaturation step at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 50–60°C for

30 s, and 72°C for 1 min, with a final extension at 72°C for 5 min. PCR products were sequenced by TSINGKE on a 3730XL DNA Sequencer (Applied Biosystems Inc.). Fragment lengths were analyzed using Gene Marker V1.6 (Soft Gene), and the data were retained for later analysis.

Population structure and differentiation and haplotype analysis

We estimated the genetic diversity and population structure of 640 accessions by software Power Marker V3.25 (Liu and Muse, 2005) and STRUCTURE 2.3.4 (Pritchard et al. 2000; Falush et al. 2003) respectively. Nine standalone runs were designed for each *k* value (from 2 to 10) with the following parameters: burn-in length, 100,000; run length, 100,000. Neighbor-joining (N-J) cluster analysis was carried out using Mega6.0 (Tamura et al. 2013) and Power Marker V3.25.

Six unlinked nuclear loci across the rice chromosomes (*SKC1*, *SAP8*, *Pid3*, *Xa23*, *GS5*, *Ehd1*) were used in this study to compare the nucleotide variation and haplotype of rice landraces collected in 1980 and 2015 (Supplementary Table 4). The DNA sequences of parts of six genes were obtained from the NCBI database. PCR amplification was carried out using the following thermal cycling program: pre-denaturation at 94° for 5 min, followed by 35 cycles of 94°C for 30 s, 55–62°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were Sanger sequenced, ClustalX1.83 (Thompson et al. 1997) was then used to remove redundant fragments at either end, align sequences, and filter erroneously aligned nucleotides. We then used Multi Domain Analysis in DnaSPv5.0 (Rozas, 2009), using Network4.5 (Bandelt et al. 1999) to construct the haplotype network.

Results

Statistical analysis of total genetic diversity

Using 36 selected SSR markers, we performed fluorescence-labeled SSR genotyping using capillary electrophoresis on 598 rice landraces from Guizhou Province and 42 wild rice as contrast accessions (Table 2). A total of 846 alleles were detected, and the number of alleles varied from 6 (RM495) to 55 (RM206), with an average of 23.5. Alleles of RM206, RM592, RM228 and RM257 were abundant, with 55, 48, 42 and 42, respectively. The genetic diversity index (*He*) of 36 pairs of SSR loci varied from 0.5257 (RM495) to 0.9503 (RM206), with an average of 0.7824. The heterozygosity (*Ho*) ranged from 0.0578 to 0.3875, with an average of 0.1648, and the polymorphic information content (PIC) ranged from 0.4274 to 0.9482, with an average value of 0.7586. Different indices showed that the SSR primers had many differences, indicating high genetic diversity.

Table 2
Genetic index summary of 640 accessions at 36 SSR loci

Marker	Chr	<i>Na</i>	<i>He</i>	<i>Ho</i>	PIC	Marker	Chr	<i>Na</i>	<i>He</i>	<i>Ho</i>	PIC
RM44	8	19	0.8558	0.0734	0.8411	RM228	10	42	0.8683	0.2375	0.8571
RM287	11	13	0.8007	0.1219	0.7764	RM21	11	22	0.6786	0.1516	0.6579
RM336	7	21	0.8491	0.1641	0.8351	RM449	1	24	0.8075	0.1453	0.784
RM135	3	11	0.601	0.0969	0.5213	RM235	12	21	0.7832	0.1188	0.7668
RM249	5	25	0.5939	0.1109	0.5446	RM247	12	35	0.8527	0.1578	0.8435
RM18	7	26	0.8456	0.1734	0.8278	RM17	12	17	0.6742	0.1234	0.6284
RM180	7	16	0.5399	0.0578	0.5141	RM253	6	19	0.8712	0.1031	0.8587
RM251	3	25	0.7917	0.1063	0.7785	RM335	4	25	0.8596	0.1094	0.8484
RM430	5	32	0.8039	0.1141	0.7887	RM223	8	20	0.8261	0.125	0.8083
RM206	11	55	0.9503	0.2844	0.9482	RM280	4	24	0.6499	0.1188	0.6224
RM592	5	48	0.9387	0.0891	0.9359	RM258	10	13	0.5771	0.1094	0.5357
RM276	6	27	0.8681	0.1031	0.8557	RM241	4	27	0.8844	0.1516	0.8753
RM208	2	12	0.5779	0.2656	0.5519	RM333	10	28	0.9298	0.3297	0.9253
RM213	2	16	0.7778	0.2891	0.7456	RM584	6	16	0.7438	0.1359	0.7006
RM219	9	39	0.9093	0.3063	0.9027	RM525	2	15	0.8162	0.2781	0.7972
RM220	1	25	0.8509	0.1984	0.8351	RM6838	8	11	0.7279	0.0953	0.685
RM495	1	6	0.5257	0.0672	0.4274	RM285	9	10	0.7935	0.3031	0.7658
RM231	2	19	0.8297	0.1297	0.8092	RM257	9	42	0.9137	0.3875	0.9097
Chr = chromosome, <i>Na</i> = observed number of alleles, <i>He</i> = genetic diversity; <i>Ho</i> = Heterozygosity;											
PIC = polymorphic information content											

Wild rice had the highest *He*, *Ho* and PIC, which were 0.8526, 0.5833 and 0.8374, respectively (Table 3). The genetic diversity of rice landraces in the six eco-rice farming zones of Guizhou was similar, with minor variation ranging from 0.6578 to 0.7416. The population of the 2 eco-rice farming zone had a low *Ho* (0.1047) but the highest *f* (0.8582), which was located in southeastern Guizhou Province. The administrative division is named Qiandongnan Miao and Dong Autonomous Prefecture (QDN). This finding indicates that the population has high homogeneity and minor genetic variation within the population. Through ethnobiological investigation by our team (Wang et al. 2018a), we found that this type of special eco-type rice landrace is the traditional variety cultivated and bred for a long time by local Dong people in the QDN, which is called Kam Sweet Rice locally with distinctive minority cultural characteristics.

Table 3
Results of genetic diversity analysis of 640 accessions

Population	<i>N</i>	AMF	<i>A</i>	<i>He</i>	<i>Ho</i>	PIC	<i>f</i>
□	190	0.3921	14.9167	0.7416	0.1345	0.7107	0.8195
□	45	0.4864	9.5556	0.6578	0.1562	0.6283	0.7672
□	85	0.3717	12.8611	0.7549	0.1359	0.7278	0.8219
▣-1	84	0.4646	11.5	0.6823	0.1558	0.6562	0.7741
▣-2	142	0.3836	13.1667	0.7334	0.1047	0.7033	0.8582
□	23	0.3768	7.7222	0.7396	0.1389	0.7015	0.8196
□	29	0.4095	9.25	0.725	0.1964	0.6959	0.7373
WR	42	0.2414	15.6944	0.8526	0.5833	0.8374	0.3266
Total	640	0.3519	23.5	0.7824	0.1648	0.7586	0.7897
<i>N</i> = sample size, <i>A</i> = allele number, AMF = major allele frequency, <i>He</i> = gene diversity, <i>Ho</i> = heterozygosity, <i>f</i> = inbreeding coefficient							

Population genetic analysis based on SSR marker data

To assess the population structure of rice landraces in different eco-rice farming zones of Guizhou, we constructed a neighbor-joining (N-J) tree using SSR data for the 640 accessions (Fig. 2). The N-J tree was divided into three groups: Group I consisted of wild rice, Group II contained the *japonica* rice landrace population, and Group III represented the *indica* rice landrace population. The population structure of *indica* was not directly related to the geographical origin of rice landraces, and all the accessions were mixed and clustered together, indicating that the genetic background of *indica* rice landraces in Guizhou was relatively complex. However, *Japonica* was further divided into three subgroups. Subgroup 1 is from the ▣-2 eco-rice farming zone in southeastern Guizhou (QDN); Subgroup 2 is from the □ and ▣-1 eco-rice farming zone in southwestern Guizhou, and the administrative division is named Qiannan Buyi and Miao Autonomous Prefecture (QN) and Qianxinan Buyi and Miao Autonomous Prefecture (QXN). The rice landraces of Subgroup 2 were cultivated and domesticated by the local Buyi and Miao people for a long time, which was similar to subgroup 1 with distinctive ethnic cultural characteristics. Subgroup 3 is from the □, □, □ and □ Eco-rice farming zones, and all the accessions are clustered together. The minority population in these areas is relatively small, and the traditional culture and agricultural farming methods are greatly influenced by modern society.

To further study the population structure of the 640 rice landraces, a Bayesian analysis was performed using STRUCTURE V. 2.3 (Falush et al. 2003). According to a method proposed by Evanno et al. (2005) to detect natural population clustering, we determined the number of subgroups within each population. From Fig. 3, when $K = 2$ and $K = 8$, there are two distinct peaks in the value of ΔK , so the population structure is best divided into two or eight subgroups. When $K = 2$, the population was divided into two subgroups (*japonica* and *indica*), and at $K = 8$, the result was consistent with N-J. *Indica* were not further separated according to the regional

classification rule; *japonica*-rice landraces from QDN (IV-2) and QN (Ⅷ) QXN (Ⅷ-1) were clearly distinguished, and other accessions were mixed from other regions (Fig. 4).

Relationship between ethnic minority demography and rice population structure

According to the results of N-J clustering and STRUCTURE analysis, the *japonica* rice landraces in eco-rice farming zones III and IV were significantly different from those in other areas regarding population structure. The analysis suggests that this result is related to the population structure of the III and IV eco-rice farming zones. QN, QXN and QDN are three special ethnic autonomous prefectures in Guizhou Province. Autonomous prefectures are a type of administrative division of China, with the same administrative status as prefecture-level cities. They are the main areas inhabited by ethnic minorities. The ethnic minority villages and population of ethnic minorities in these three regions are high, mainly Dong, Miao and Buyi (Wei et al. 2021) (Fig. 5). The Dong, Miao and Buyi people are the main population in these three regions and are called the “Rice minority”; among them, Buyi is one of the earliest rice-growing ethnic groups in China (Yang 2016). Therefore, the rice landraces were domesticated into special ecotype varieties with the same genetic background under the traditional culture of ethnic minorities, traditional cultivation methods and traditional dietary customs. In conclusion, the population structure of ethnic minorities will affect the population structure of rice and then affect its domestication and genetic background.

Notably, the II eco-rice farming zone (TR city) also has a large population of ethnic minorities with 29 ethnic minorities (Jia 2021). Due to the integration of the modern culture of many ethnic groups, no unique rice culture has been formed in TR. In addition, the TR area is mainly mountainous throughout the territory, which is not suitable for developing rice farming. The Tujia people, which have the highest proportion of minority population in TR, are called the “Mountain minority” (Yin 2011). Moreover, the Tujia people do not have their own ethnic language and are greatly influenced by modern culture, while the Buyi and Dong people have their own ethnic languages and retain their traditional culture and farming methods.

QDN = Qiandongnan Miao and Dong Autonomous Prefecture, QN = Qiannan Buyi and Miao Autonomous Prefecture, QXN = Qianxinan Buyi and Miao Autonomous Prefecture, TR = Tongren city, BJ = Bijie city, ZY = Zunyi city, AS = Anshun city, LPS = Liupanshui city, GY = Guiyang city

Diachronic analysis of genetic diversity in rice landraces under artificial selection and domestication

To further study the short-term domestication of ethnic minorities on rice landraces under the background of traditional culture and special farming practices, we compared the dynamic changes in genetic diversity and domestication gene haplotypes in two rice landrace populations collected in 1980 and 2015 in the QDN (they are the same ecotype rice, which called Kam sweet rice by local Dong people). The genetic diversity results showed that the main allele frequency (MAF), gene diversity index (H_e), observed heterozygosity rate (H_o) and polymorphic information content (PIC) of rice landraces collected in 1980 were higher than those in 2015 (Fig. 6), indicating that some alleles disappeared and genetic diversity and heterozygosity decreased during a 35-year period of artificial selection and domestication. AMOVA showed that the proportion of genetic variation

between the two populations in different periods accounted for 3.43%, and the genetic variation within the population accounted for 89.35%. Therefore, the genetic variation mainly came from within the population.

The results of ethnobiological investigation showed that most of the rice landraces in the QDN were planted in mountainous areas with poor soil and had excellent characteristics of salt and alkali resistance, cold resistance and disease and insect resistance(Wang et al. 2018a). Therefore, six related nonlinked nuclear gene loci, *SKC1*, *GS5*, *Pid3*, *SAP8*, *Ehd1* and *Xa23*, were used for sequencing in this study. The sequence length of each locus was 4268 bp, with a range of 482–670 bp. DnaSP V. 5.0 was used to estimate the haplotypes and genetic diversity at different loci in 1980 and 2015, respectively (Table 4). There was no significant difference in mean nucleotide variation between the 1980 population ($\pi = 0.00299$, $\theta_w = 0.00204$) and 2015 population ($\pi = 0.00129$, $\theta_w = 0.00168$) ($P > 0.05$, for both π and θ_w). In addition, there were a large number of shared polymorphisms at each gene locus between the 1980 population and the 2015 population (Table 5).

Neutral detection included Tajima's D value, Fu and Li's D* value and the F value of each locus (Table 4). With the exception of a few loci, the neutral test values of most genes were not significant. In 2015, the D value (2.43142*) of the *Pid3* gene was significantly positive, indicating that there were numerous moderate frequency polymorphisms in rice landraces at this locus, indicating that Dong people were subjected to positive selection in the domestication process of rice blast resistance. The D (-1.89378*) value of the *GS5* gene was significantly negative, indicating that there were numerous low-frequency polymorphisms, i.e., rare alleles at this gene locus indicated that Dong people were strongly positively selected during the domestication of rice grain size. Four-gamete testing revealed that the Rm ranged from 0 to 3 in landraces from 1980, with an average of 1.2, and from 0 to 1 in landraces from 2015, with an average of 0.3, indicating that there was low heterozygosity in the rice landraces due to high levels of inbreeding over 35 years.

Table 4
Summary of nucleotide polymorphisms and neutrality tests

Group	Gene	S	h	Hd	π	θ_w	D	D*	F*	Rm
1980	<i>SKC1</i>	5	6	0.536	0.00171	0.00147	0.33442	-0.10579	0.0488	0
	<i>GS5</i>	12	7	0.511	0.00464	0.00349	0.83449	-1.22227	-0.56291	2
	<i>Pid3</i>	10	8	0.69	0.00472	0.00270	1.80688	-0.14217	0.66169	3
	<i>SAP8</i>	3	4	0.461	0.00071	0.00080	0.00080	0.0008	0.0008	0
	<i>Ehd1</i>	5	4	0.487	0.00392	0.00186	2.21023*	1.00485	1.666	1
	<i>Xa23</i>	7	9	0.764	0.00223	0.00194	0.33333	0.24508	0.32723	1
	Average	7	6	0.575	0.00299	0.00204	0.92002	-0.03658	0.35694	1.2
2015	<i>SKC1</i>	4	4	0.059	0.00017	0.00115	-1.54948	-1.64515	-1.9091	0
	<i>GS5</i>	12	5	0.081	0.00081	0.0034	-1.89378*	-1.96452	-2.31825*	0
	<i>Pid3</i>	7	5	0.534	0.00391	0.00185	2.43142*	1.15386	1.89957*	1
	<i>SAP8</i>	1	2	0.047	0.00007	0.00026	-0.76387	0.46303	0.10017	0
	<i>Ehd1</i>	5	4	0.081	0.00065	0.00182	-1.26868	-0.13207	-0.60925	0
	<i>Xa23</i>	6	7	0.660	0.00213	0.00162	0.64574	0.06034	0.30913	1
	Average	6	5	0.244	0.00129	0.00168	-0.39978	-0.34409	-0.42129	0.3

S: Number of polymorphic (segregating) sites; h: Number of haplotypes; Hd: Haplotype (gene) diversity; π : Nucleotide diversity; Theta-w: Watterson's parameter for silent sites; D: Tajima's D; D* and F*: Fu and Li's D* and Fu and Li's F*, respectively; Rm: Minimum number of recombination events. P**<0.05, P*<0.02.

Table 5
Polymorphisms between rice landraces in 1980 and 2015 at all genes

Gene	Kam Sweet Rice in 1980	Kam Sweet Rice in 2015	Shared
<i>SKC1</i>	2	1	3
<i>GS5</i>	5	5	7
<i>Pid3</i>	3	0	7
<i>SAP8</i>	2	0	1
<i>Ehd1</i>	0	0	5
<i>Xa23</i>	1	0	5
<i>Average</i>	2.2	1.0	4.7

Phylogenetic analysis of haplotypes showed that there were 6, 8, 10, 4, 5 and 9 haplotypes at the *SKC1*, *GS5*, *Pid3*, *SAP8*, *Ehd1* and *Xa23* gene loci, respectively, and a total of 42 haplotypes (Fig. 7). The haplotype numbers of rice landraces from 1980 and 2015 in these networks were 5, 4; 7, 5; 8, 5; 4, 2; 4, 4 and 9, 7, respectively. A total of 73.0% of the haplotypes were retained in the rice landraces collected in 2015, and most of them were dominant haplotypes. Moreover, four rare haplotypes (Hap5 of *Ehd1*, Hap8 and Hap10 of *Pid3* and Hap8 of *GS5*) were newly developed, suggesting that short-term selective domestication of minorities can better maintain the original dominant haplotype. The frequency of gene haplotypes also changed in the two populations at different periods. In most dominant haplotypes, the frequency of the 2015 population was higher than that of the 1980 population, such as Hap1 of *Ehd1* (80.95%), Hap1 (41.27%) and Hap3 (40.00%) of *Pid3*, Hap1 (80.95%) of *GS5*, Hap1 of *SAP8* (82.86%), Hap1 of *SKC1* (81.9%), Hap1 of *GS5* (80.95%), Hap1 of *SAP8* (82.86%), Hap1 of *SKC1* (81.9%), and Hap1 (42.54%) and Hap2 (27.94%) of *Xa23*. Four haplotypes (Hap2 of *SAP8*, Hap2 of *Ehd1*, Hap4 of *Pid3* and Hap2 of *GS5*) were common haplotypes (frequency > 25%) in 1980, with a range of 28.57%-32.65%. By 2015, they had become rare haplotypes (frequency < 3%), with a range of 2.38%-2.98%, indicating that the haplotypes of these genes may have been selected by nature and humans after short-term domestication. The frequency of the most favorable haplotypes gradually increased, while that of a small number of unfavorable haplotypes gradually decreased.

Discussion

It is important to evaluate the genetic diversity of rice landraces

Genetic diversity is an important basis for rice breeding and genetic improvement and is very important for rice production (Cui et al. 2020; Liu et al. 2022a). Southwest China is considered to be one of the origins and diversity centers of cultivated rice in Asia (Zhang et al. 2006), so many scholars have studied the genetic diversity of rice landraces in this area by SSR markers. Yang et al. (2008) analyzed the genetic diversity of 63 rice landraces in Yunnan and showed that the genetic diversity index (*He*) was 0.7187. Chen et al. (2019) increased the accession number of Yunnan rice landraces to 908, and genetic diversity analysis showed that the gene diversity index (*He*) was 0.7307. Both rice populations with fewer accessions and more accessions

revealed the high genetic diversity of rice landraces in Yunnan. Guizhou Province, which borders Yunnan Province, also has rich rice landrace resources. In this study, a total of 846 alleles were detected using 36 pairs of SSR primers covering 12 pairs of chromosomes in rice. The number of alleles per pair ranged from 6 to 55, with an average of 23.5 alleles per pair of primers. The genetic diversity index (He) of 598 rice landraces in Guizhou was 0.7659, which was higher than that in previous research, including 537 *indica* and *japonica* rice varieties in Guizhou ($He=0.6960$) (Zhang et al. 2007) and 147 red rice varieties in Guizhou ($He=0.3549$) (Yang et al. 2021). This finding indicated that the genetic diversity of rice landraces in six different eco-rice farming zones in Guizhou was high and that the genetic background was rich, which was of great significance in breeding new rice varieties and broadening the genetic basis of cultivated rice.

In this study, the average alleles per locus of rice landraces from the III eco-rice farming zone (QXN) was 12.8611, and this region had the highest He (0.7549) and PIC (0.7278), consistent with the research results of Zhang et al. (2006), indicating that QXN is the genetic diversity center of rice landraces in Guizhou. In the future, intensive research on rice landraces in QXN will make more effective use of these excellent rice resources to explore new genes and breed new varieties. Moreover, the rice landraces in the IV-2 eco-rice farming zone (QDN) had the lowest observed heterozygosity rate (0.1047). Due to long-term breeding by local Dong people, the heterozygosity rate within the population is very low, and the variety has relatively single parents, which may lead to genetic vulnerability.

Synergistic evolution of rice landraces and traditional cultural environments

The diversity and population structure of rice landraces are affected by many factors, including not only environmental and genetic factors but also farmers' traditional farming activities and traditional culture (Wang et al. 2016; Song et al. 2019). On the one hand, varieties of rice landraces were planted in the specific living environment of ethnic minorities, forming the corresponding cultural diversity (Pei 2011); on the other hand, the cultural diversity of ethnic minorities will also have a profound impact on rice landrace resources and even lead to their evolution. These diverse cultures often contain rich life and production experience, thus realizing the harmonious unity of human, environment and resource utilization and protection (Gao et al. 2016). Therefore, we believe that rice landrace resources have evolved in coordination with the traditional culture and farming activities of ethnic minorities.

The differentiation of *indica* and *japonica* is the main trend of genetic differentiation of Asian cultivated rice, but the intraspecific structure or classification of *indica* and *japonica* has been the focus of much attention (Wang et al. 2018b). In this study, the genetic background of *indica* in Guizhou Province was complex and did not show the same clustering rule as the geographical origin, indicating that the genetic basis in *indica* was broad and might have different sources. This finding is consistent with the research of Xian et al. (2013), which showed that *indica* has been introduced several times in Guizhou historically, gradually replacing local glutinous rice to boost grain yields and meet local people's basic needs (Yang et al., 2004). In contrast, the population structure of *japonica* in Guizhou was correlated with geographical origin, especially the rice landraces in the Ⅱ and Ⅱ-1 eco-rice farming zones, which were individual subgroups. We found that this was related to the fact that these *japonica* rice were planted in the areas inhabited by the Dong, Miao and Buyi ethnic groups, who have made a living from rice cultivation for generations and formed corresponding rice culture. The characteristics favored by local people and those adapted to traditional culture are constantly

preserved under natural and artificial selection so that most of the traditional cultivated *japonica* rice resources are handed down from generation to generation and become a special ecological type of landrace. Although the genetic background of these landraces is relatively simple, due to careful domestication and artificial selection, they often have good resistance to diseases, insects and stress, so they are ideal materials for breeding and need to strengthen protection and research.

Traditional rice landraces are the basis for the life of ethnic minorities, as well as the focus and spiritual support of their lives. The production of ethnic minorities revolves around landraces throughout the year and at the same time constructs the basic connotation of daily life practice (Yang 2016). Population structure assessment of rice landraces is important for gene conservation and selection of superior varieties. The single population structure is closely related to the life history, ecological characteristics, narrow distribution range and traditional culture of local minorities; therefore, it is necessary for us to have a deeper understanding of the development process of rice landraces under the influence of ethnic minority traditional culture and management mode to reasonably protect and utilize these characteristic landraces.

Effective genetic diversity conservation mechanism of rice landraces

The conservation approaches of rice landraces include *in situ* conservation (on-farm conservation) and *ex situ* conservation (gene bank conservation), which have different advantages and disadvantages, respectively. Many studies have shown that *in situ* conservation can not only maintain the continuous evolution of landraces in the original habitat but also include the protection and utilization of landrace resources by farmers' participation and selection, traditional cultural customs and farming methods and effectively increase the genetic background and genetic diversity (Cui et al. 2016; Wang et al. 2016). The results of this study showed that some loci, such as *Pid3* (blast resistance) and *GS5* (grain size), were positively selected after continuous selection and domestication by ethnic minorities, improving rice quality and resistance. Simultaneously, the short-term selection domestication of minorities maintained the original dominant haplotypes 35 years ago, and the frequency of most favorable haplotypes gradually increased. However, the results of this study showed that the short-term selection and domestication of ethnic minorities reduced the genetic diversity of rice landraces; that is, *in situ* conservation (collected in 2015) did not increase the genetic diversity of rice, and some alleles were lost and heterozygosity decreased. This result can be explained in two ways. On the one hand, it may be that the Dong people, in their specific traditional diet customs, living customs, and ethnic culture of internal demand, driven by the constant directional domestication and improvement of local varieties, breeding and selecting distinguished quality rice varieties, elimination of low yield, poor quality varieties, cause the single rice genetic background and population structure, while genetic diversity and haplotype richness decrease. On the other hand, due to limited conditions, this study only selected the genetic diversity of similar landraces with an interval of 35 years for comparison. Thirty-five years is a very short period in the long process of crop domestication, which cannot fully explain the role of the domestication and artificial selection of ethnic minorities.

In conclusion, we believe that *in situ* conservation and *ex situ* conservation are equally important. Although *ex situ* conservation cannot maintain the dynamic evolution process of crop genetic resources in the original environment, it can maintain the genetic integrity and diversity of crops and will not be lost over time (Schwartz 2017). The two approaches complement each other. In addition, although the diversity of rice landraces in this

study decreased during 35 years of domestication, the original dominant haplotypes can be better preserved, and the frequency of the most favorable haplotypes gradually increased, while the frequency of a few unfavorable haplotypes gradually decreased to adapt to the corresponding environment and traditional culture. Furthermore, farmers are the guardians of the rice landraces, and they continue to cultivate and protect many ancient local varieties. The initiative of farmers should be fully mobilized regarding the protection and utilization of rice resources (Zhu et al. 2004). Simultaneously, as rice landraces are important carriers of traditional culture and farming methods of ethnic minorities, the traditional farming culture of ethnic minorities should also be protected to promote the protection of rice landraces.

Conclusions

The results revealed that, the genetic diversity of rice landraces in six different eco-rice farming zones of Guizhou was high, and with rich genetic background. Among which, Qianxinan (QXN), where Buyi and Miao live, is the genetic diversity center of local rice landraces in Guizhou. The *japonica* rice landraces in Guizhou showed a group division related to geographical origin. In the areas inhabited by Buyi, Miao and Dong ethnic groups, the rice landraces were clustered into a subgroup, indicating that they were affected by the domestication of traditional culture and traditional farming methods of ethnic minorities. Nucleotide variation analysis of the same ecotype landraces collected in 1980 and 2015 showed that the original dominant haplotypes of rice population could be better preserved after 35 years of short-term domestication. Moreover, the frequency of most favorable haplotypes gradually increased, while the frequency of a few unfavorable haplotypes gradually decreased, so as to adapt to the corresponding environment and traditional culture. This study can promote the protection and sustainable utilization of rice landraces and provide more valuable germplasm resource and information for rice breeding and basic research.

Declarations

Funding

This work was supported by the National Key Research and Development Program of China (2021YFD1200500), the National Natural Science Foundation of China (31901487), CAAS Science and Technology Innovation Program, National Crop Germplasm Resources Center (NCGRC-2021-2), the Program of Protection of Crop Germplasm Resources (19200385-1), the Third National General Survey and Collection Action of Crop Germplasm Resource (19210859, 19210860), the National Natural Science Foundation of China (32171669), and the biodiversity investigation, observation, and assessment program (2019-2023) of Ministry of Ecology and Environment of China.

Conflict of Interest

All the authors declare that there are no conflicts of interest to disclose.

Authors' contributions

Chunhui Liu and Yanjie Wang as the principal researcher conducted the field research, Yanjie Wang conceived and designed the experiments, Chunhui Liu analyzed data analysis, drafted the manuscript. Dr. Dayuan Xue and Dr. Longzhi Han as supervisors initiated and provided oversight to the study and input in its planning and provided the ethic and cultural advice based on their rich experience and knowledge. Aixia Jiao, Xiaoding Ma, Di Cui, Xiaobing Li, Bing Han, Huicha Chen, Renchao Ruan carefully revised the manuscript. All authors reviewed and approved the final manuscript.

Data availability statement

All data supporting the findings of this study are available within the manuscript and within its supplementary materials.

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Figures

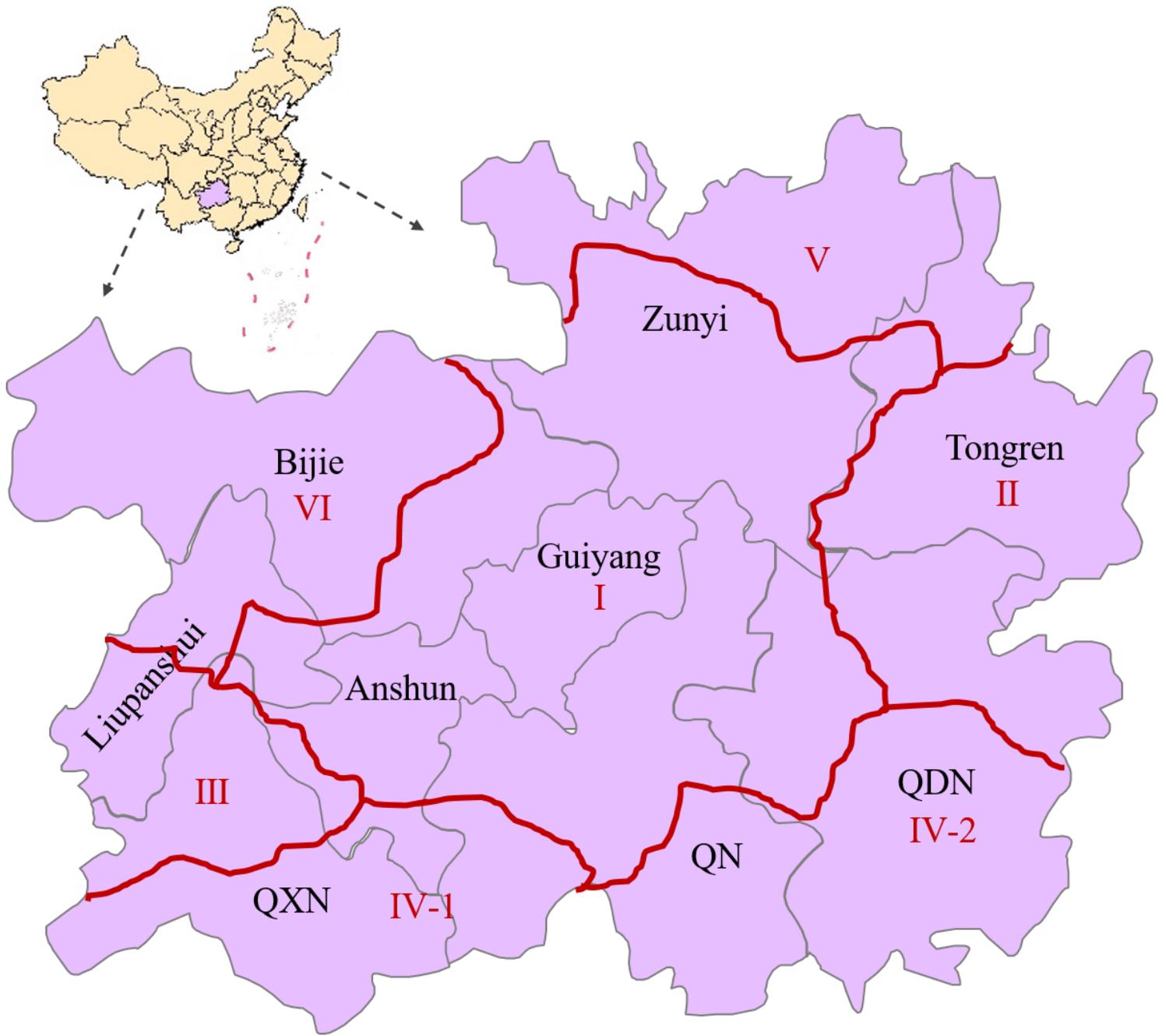


Figure 1

Geographical distribution of 598 accessions

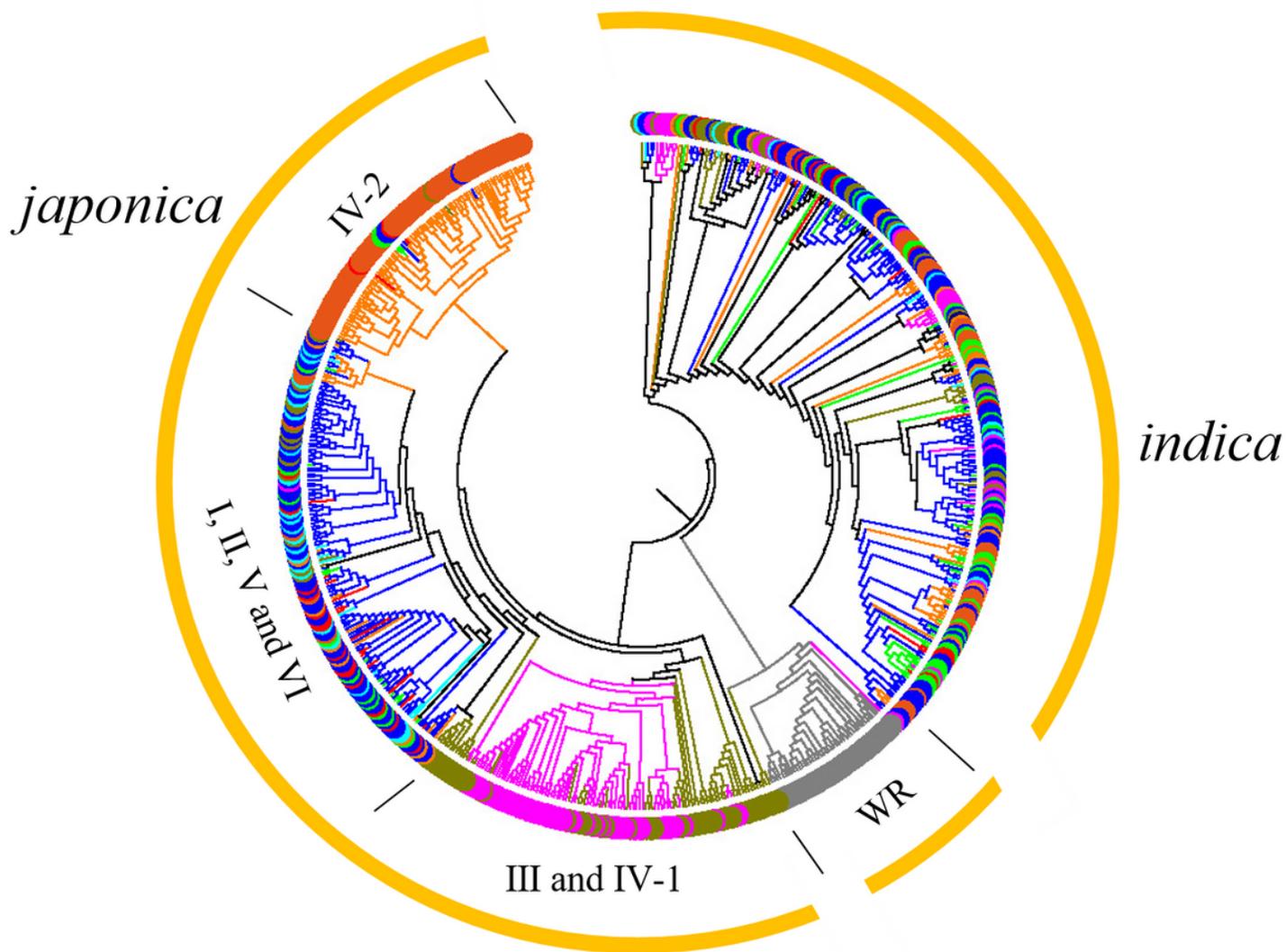


Figure 2

N-J cluster analysis based on SSR markers for 640 accessions

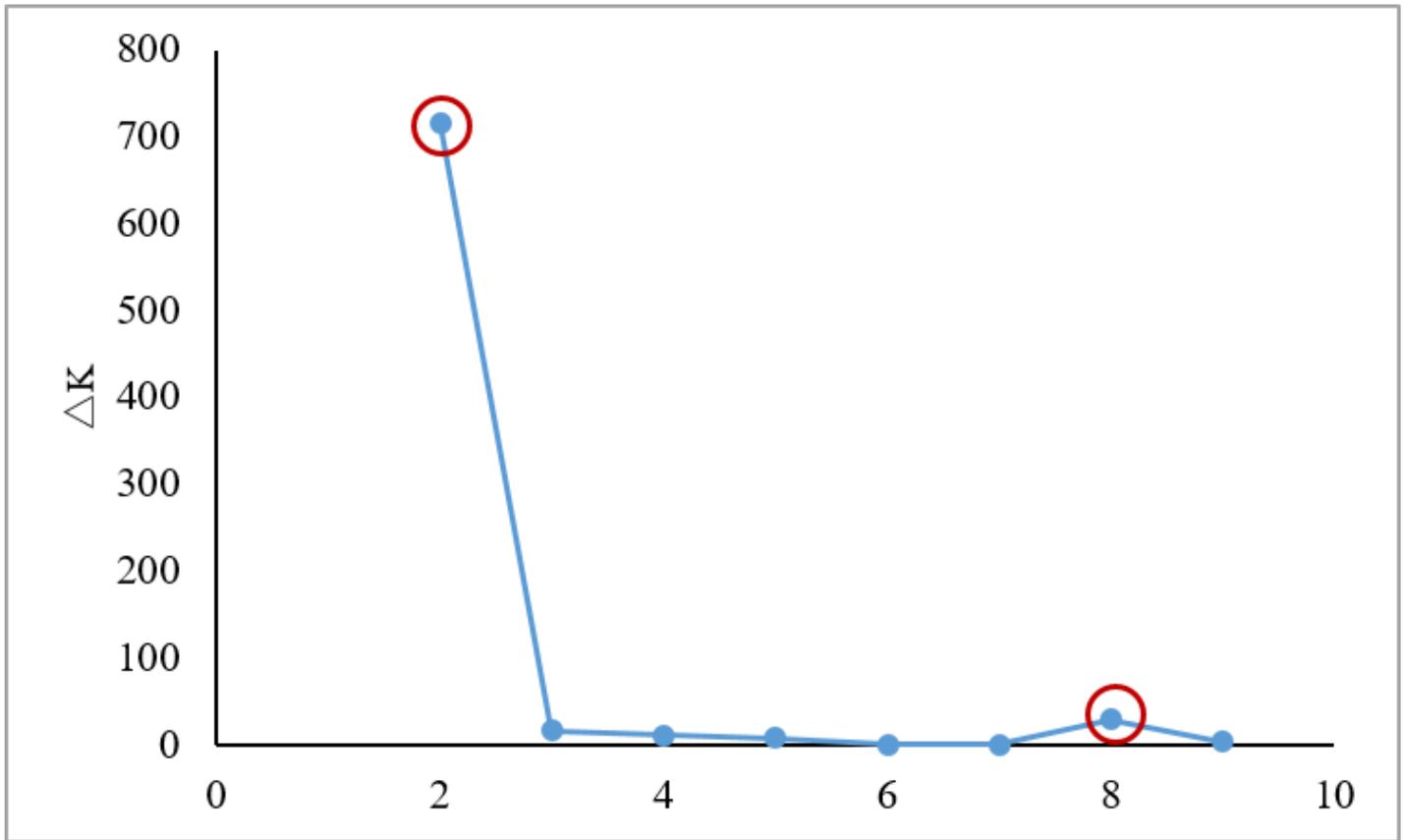


Figure 3

ΔK value in structure analysis based on the model

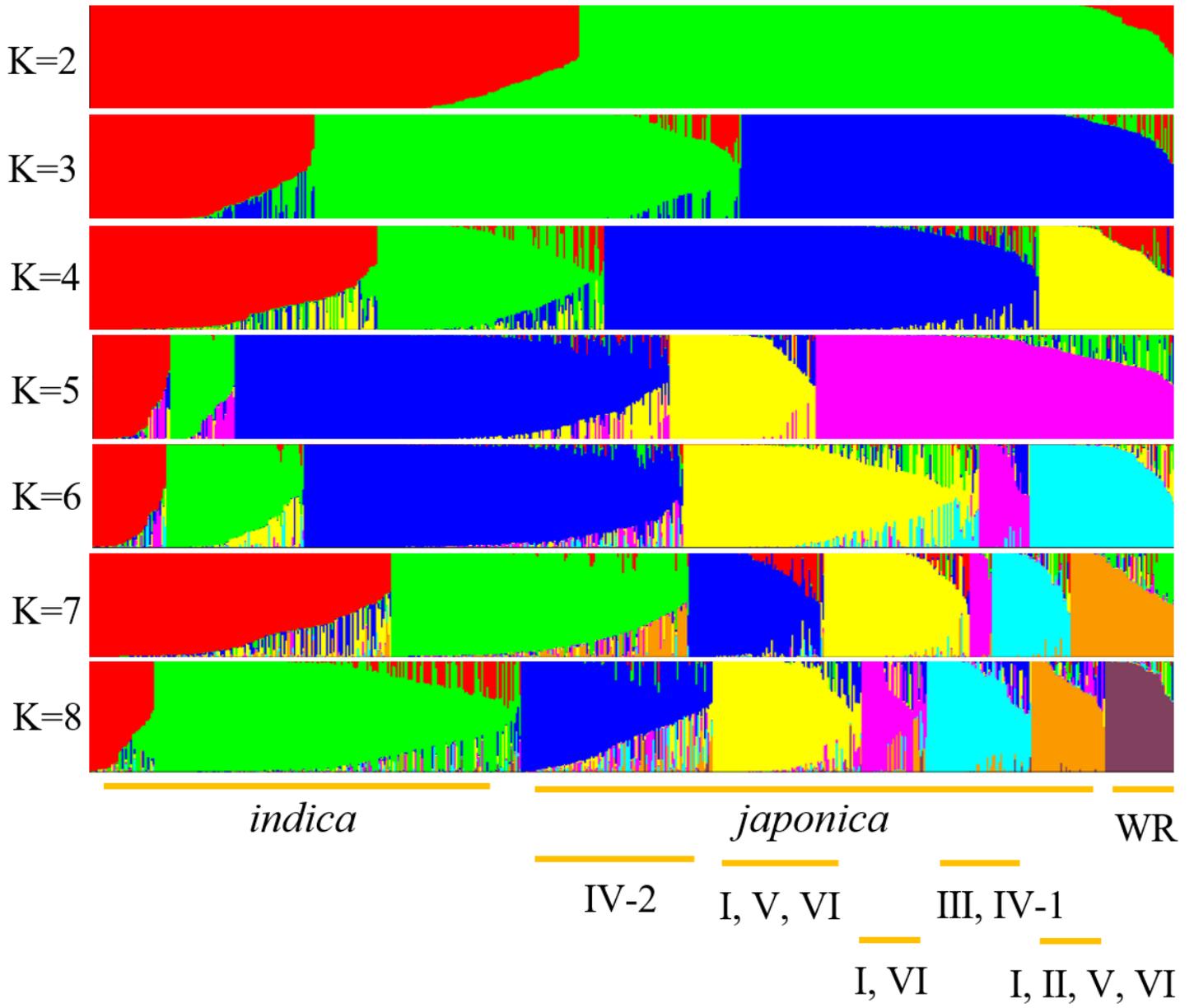


Figure 4

Population structure analysis for 640 accessions

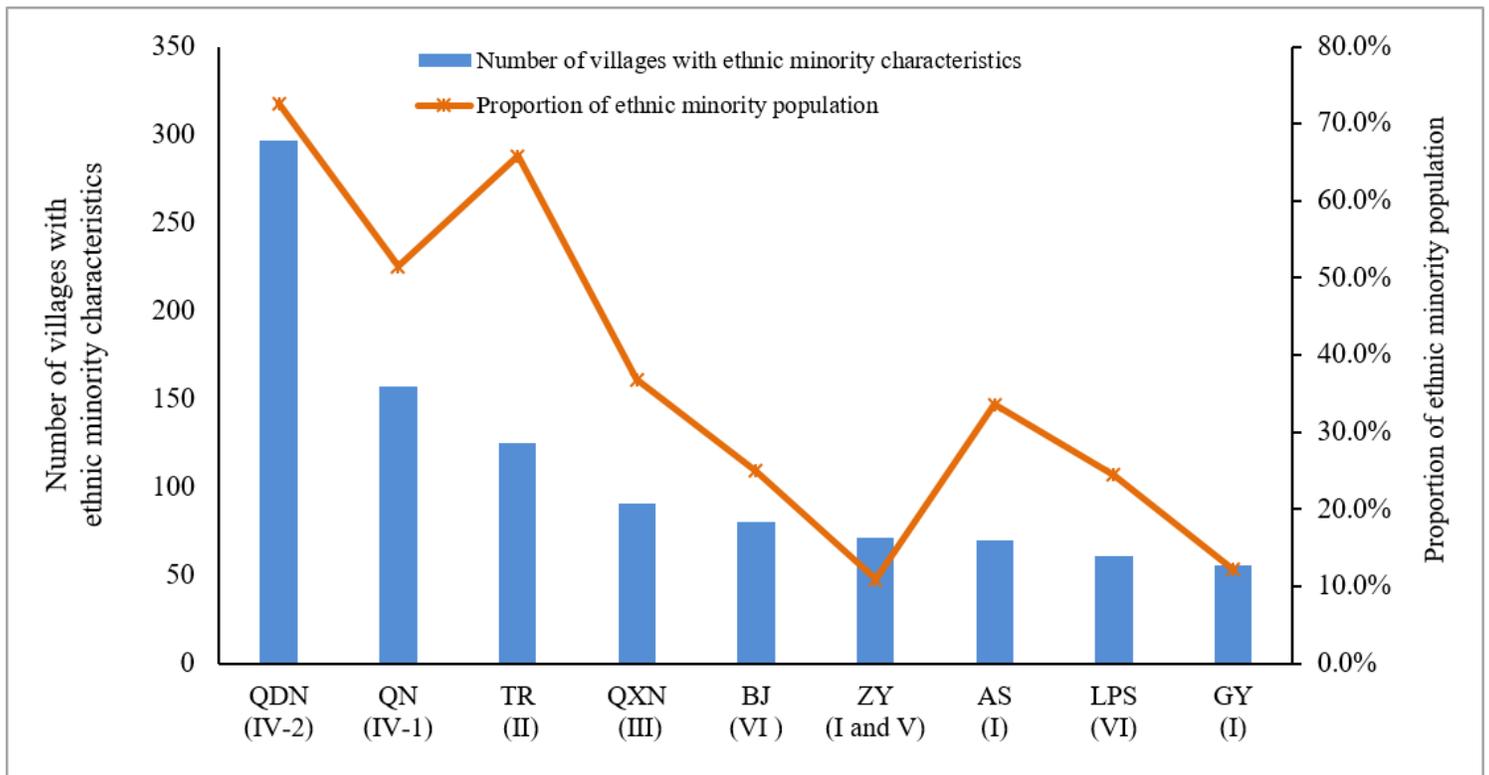


Figure 5

Number of minority villages and proportion of minority population in each city of Guizhou

QDN= Qiandongnan Miao and Dong Autonomous Prefecture, QN=Qiannan Buyi and Miao Autonomous Prefecture, QXN=Qianxinan Buyi and Miao Autonomous Prefecture, TR=Tongren city, BJ=Bijie city, ZY=Zunyi city, AS=Anshun city, LPS=Liupanshui city, GY= Guiyang city

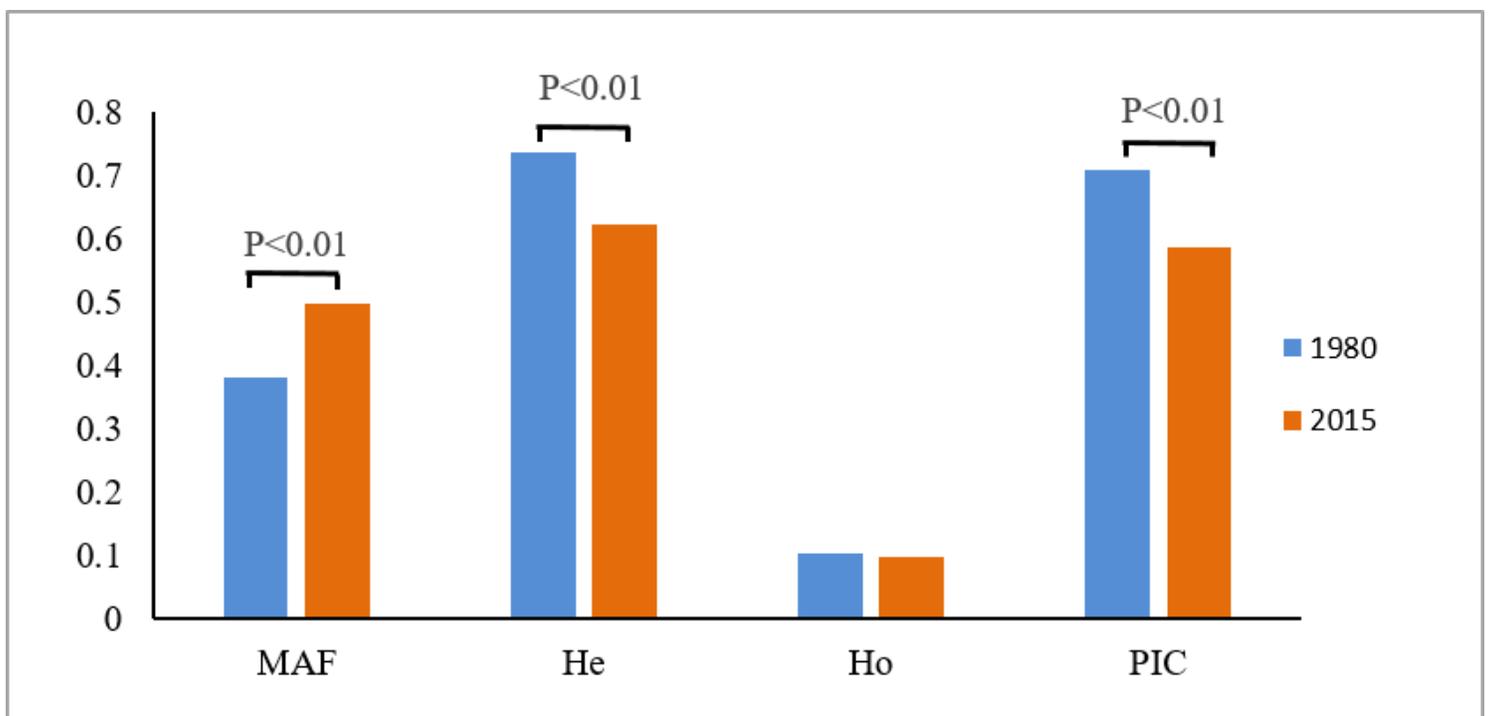


Figure 6

Comparison of diversity between the two populations in 1980 and 2015

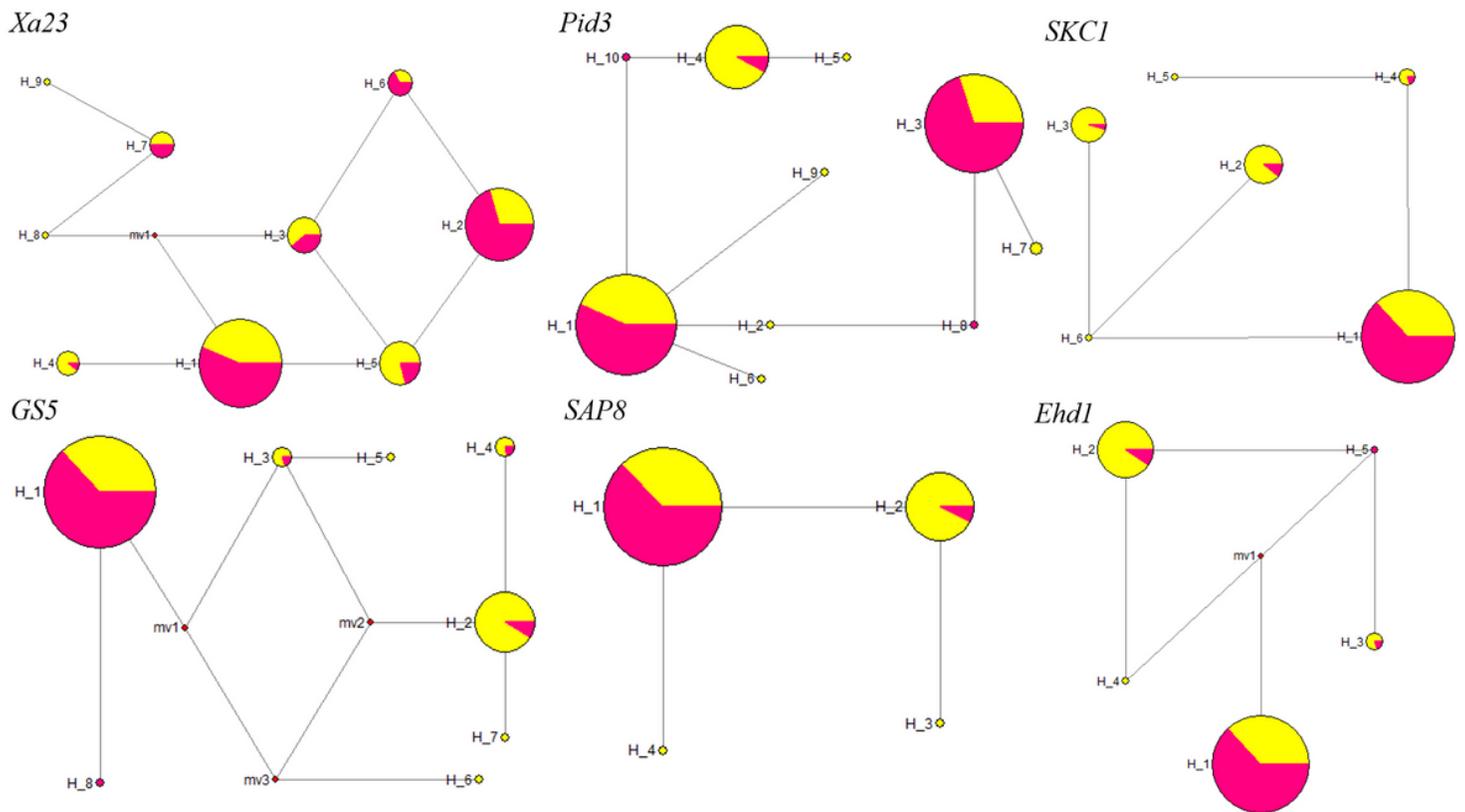


Figure 7

Haplotype networks of six loci in accessions from 1980 and 2015

Supplementary Files

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