1 Analysis of the Genetic Structure and Diversity of Upland 2 Cotton Groups in Different Planting Areas Based on SNP 3 Markers

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12 **Abstract:** Genetic diversity, kinship and population genetic structure analyses of
13 *Gossypium hirsutum* germplasm can provide a better understanding of the origin and
14 evolution of *G. hirsutum* biodiversity. In this study, 1313331 SNP molecular markers
15 were used to construct a phylogenetic tree of each sample using MEGAX, to perform
16 population structure analysis by ADMIXTURE software and principal component
17 analysis (PCA) by EIGENSOFT software, and to estimate relatedness using SPAGeDi.
18 ADMIXTURE software divided the experimental cotton population into 16 subgroups,
19 and the *Gossypium hirsutum* samples could be roughly clustered according to source
20 place, but there were some overlapping characteristics among samples. The
21 experimental cotton population was divided into six groups according to source to
22 calculate the genetic diversity index (H), and the obtained value (0.306) was close to
23 that for germplasm collected by others in China. Cluster 4 had a relatively high
24 genetic diversity level (0.390). The degrees of genetic differentiation within the
25 experimental cotton population groups were low (the population differentiation
26 indexes ranged from 0.02368 to 0.10664). The genetic distance among cotton

27 accessions varied from 0.000332651 to 0.562664014, with an average of 0.25240429.
28 The results of this study may provide a basis for mining elite alleles and using them
29 for subsequent association analysis.

30 **Keywords:** *Gossypium hirsutum*; SNP marker; Genetic structure; Genetic diversity

31 Introduction

32 Cotton is one of the most important economic crops and a significant component of
33 Chinese economy; moreover, it is widely cultivated worldwide and has a long history.
34 It is the main textile material in the global textile industry, accounting for more than
35 60% of raw domestic textile materials and more than 50% of the total sales of
36 fiber-based products in international consumer markets. Cotton is the foremost source
37 of natural fiber, and cottonseed oil is considered a very-good-quality dietary fiber oil
38 that accounts for approximately 10% of the global production of animal and plant oils.
39 Cotton kernels are rich in protein and an important source of protein for humans;
40 kernel powder from low-phenol cotton is also used as a major additive in high-grade
41 foods. Cotton stalks can act as combustion materials and, after crushing, can provide
42 crude feed for the livestock industry and can be used as feedstock in some industrial
43 aspects (Liu.2015). With the increase in textile industrial development and
44 improvements in the scientific and mechanistic levels of crop cultivation in China, the
45 country has gradually become the largest cotton producer in the world, as well as the
46 largest cotton consumer, with an annual planting of up to 80 million mu, providing the
47 motivation for economic development and to meet the needs of the people.

48 In accordance with its botanical classification, cotton is a dicotyledon that
49 belongs to the clade Angiospermae, order Malvales, family Malvaceae, and genus
50 *Gossypium*. Beasley divided diploid cotton species into five groups, a to e, based on
51 kinship and local environmental conditions, to lay a foundation for subsequent studies
52 on the classification of cotton species (Beasley *et al.*1940). In 1978, Fryxell (1979)
53 divided the genus *Gossypium* into four cultivated species and 39 other wild species

54 based on previous works, and this classification remained partially controversial, even
55 though it was mostly recognized. Afterwards, Fryxell (1992) summarized the genus
56 *Gossypium* into 50 species. A few years ago, botanists again discovered two new
57 tetraploid cotton species, *G. ekmanianumsi* and *G. stephensi* (Grover *et al.* 2015; Gallagher *et al.* 2017). Currently, the genus *Gossypium* can be divided into a
58 total of 52 species, including seven allotetraploid species and 45 diploid species.

60 At present, some researchers use sequence-related amplified polymorphisms
61 (SRAPs), simple sequence repeats (SSRs), amplified fragment length polymorphisms
62 (AFLPs), single-nucleotide polymorphisms (SNPs) and other molecular markers to
63 study cotton genetic diversity. Dong (2007) used SSR markers to evaluate the
64 diversity of 96 germplasm resources of upland cotton, sea island cotton, Asian cotton
65 and grass cotton and found that the main phenotypic traits of cotton were significantly
66 different between germplasms and within species. An extremely significant finding
67 was that the phenotypic diversity index of upland cotton germplasm was the highest.
68 Wu *et al.* (2001) studied 36 varieties using ISSR (inter-simple sequence repeat)
69 technology and showed that the hereditary basis of upland cotton cultivars is
70 relatively narrow. Liu *et al.* (2003) used RAPD marker technology to analyze the
71 genetic diversity of 166 representative cotton varieties (or lines) in China since the
72 founding of the People's Republic and showed that the genetic range of selfing upland
73 cotton varieties in China was narrower than that of imported varieties. The hereditary
74 basis of hybrid upland cotton is narrower than that of the conventional variety; that of
75 the upland cotton varieties generated after the 1980s is narrower than that of the
76 varieties from the 1970s; that of the Yangtze River cotton varieties is narrower than
77 that of the Huanghuai cotton varieties; and that of the northwest inland cotton
78 varieties is narrower than that of the Yangtze River cotton varieties. Multani *et al.*
79 (1995) used RAPD technology to analyze 14 Australian cotton varieties and found
80 that their genetic relationships were relatively close. Cotton also shows a certain
81 degree of differentiation at the molecular level. Gao *et al.* (2010) used SSR
82 technology to analyze the genetic diversity of tetraploid cotton species in China. The

83 results showed that wild lines of broad-leaved cotton and upland cotton have the
84 closest genetic relationship, and the genetic relationship between Darwin cotton and
85 sea island cotton was very close. Brown cotton was also relatively closely related to
86 these types of cotton. Wu (2012) analyzed the diversity of 168 sea island cotton
87 samples by morphological observation combined with SRAP technology, which
88 showed that the genetic basis of Chinese sea island cotton is narrow and that the level
89 of diversity is low. Kuang *et al.* (2011) used 36 pairs of primers with high
90 polymorphism to analyze the genetic diversity of 32 main cultivated varieties selected
91 in 2008 in China. Through cluster analysis, they found that the genetic differences
92 among cotton varieties in the Yangtze River Basin were the largest, the differences in
93 the Xinjiang cotton area were the second largest, and the differences in the Yellow
94 River basin were the smallest. The genetic diversity of hybrids is richer than that of
95 conventional species. Chen *et al.* (2006) analyzed the diversity of 43 upland cotton
96 basic germplasms in China and found that their genetic diversity level showed a
97 downward trend, and the diversity level of cotton areas in the Yangtze River and
98 Yellow River basins was lower than that of foreign areas where basic germplasms are
99 grown. Thus, the genetic backgrounds of the breeds are relatively narrow.

100 Population genetics is a discipline in which mathematical and statistical methods
101 are applied to study gene and genotype frequencies in populations and the effects of
102 selection and mutations that influence these frequencies in order to study the
103 relationships of processes such as migration and genetic drift with genetic structure,
104 thereby exploring evolution. Analysis of genetic differences using molecular markers
105 is an essential approach in population genetics. Population genetics studies are often
106 performed by using SNP markers.

107 Before performing GWAS analysis, clarifying the genetic background and
108 population structure of the tested material is fundamental. Therefore, the work carried
109 out in this paper will lay a foundation for further association analysis.

111 1 Materials and methods

112 1.1 Experimental materials

113 This study used 273 domestic and foreign resources of upland cotton varieties
114 (Appendix 1) as research materials, and all materials were provided by the Economic
115 Crop Research Institute of the Xinjiang Academy of Agricultural Sciences. Middle
116 intact leaves were collected from individual plants grown in the field.

117 1.2 Extraction and sequencing of cotton DNA

118 The genomic DNA of cotton leaves was extracted by the modified CTAB method and
119 then tested. Enzymatic 3' A processing, attachment of a dual-index (Kozich *et al.*2013)
120 sequencing adapter, PCR amplification, purification, mixing, and sequencing on an
121 Illumina sequencing platform were performed. Japanese rice was used as a
122 sequencing control to evaluate the accuracy of the enzymatic experiment. The
123 dual-index adapter was used to identify the original data obtained by sequencing and
124 obtain the reads from each sample. After filtering the adapters from the sequencing
125 reads, sequencing quality and data volume assessments were performed. The
126 digestion efficiency of HaeIII+SspI-HF® was evaluated through Nipponbare rice data
127 to judge the accuracy and effectiveness of the experimental process.

128 1.3 Development of cotton snp markers

129 The sequenced reads obtained by simplified sequencing needed to be realigned to the
130 reference genome to perform subsequent variation analysis. Using Zhejiang
131 University Cotton v2.1
132 (download:https://www.cottongen.org/data/download/genome_tetraploid/TM-1) as
133 the reference genome, BWA 0.7.15 (Li *et al.*2009) was used to compare sequenced
134 reads to the reference genome. Using GATK 4.0 (McKenna *et al.*2010) and SAMtools
135 1.9 (Li *et al.*2009b) methods, SNPs and SNP marker intersections were identified to
136 create the final reliable SNP marker dataset. SnpEff 4.0 (Cingolani *et al.*2012)
137 software was used to obtain the locations of the variable sites (intergenic zones, gene

138 zones, or CDS zones) in the reference genome and the effects of the variations
139 (synonymous mutations, nonsynonymous mutations, etc.).

140 1.4 Analysis of cotton genetic evolution

141 1.4.1 Phylogenetic analysis

142 A phylogenetic tree is used to indicate the evolutionary relationships between species
143 that are considered to have a common ancestor and to describe the classification and
144 evolutionary relationships between species. In the analysis, according to the genetic
145 data of the population, the distance of the genetic relationship between the materials
146 was inferred, a distance matrix was constructed, and a phylogenetic tree was created
147 based on the distance matrix. MEGAX 7.0.14 software was used to construct the
148 phylogenetic tree of each sample based on the neighbor-joining method and the
149 p-distance model, and bootstrapping was repeated 1,000 times.

150 1.4.2 PCA

151 Principal component analysis is a statistical method that is performed by transforming
152 a set of correlated variables into a set of linearly uncorrelated variables; the
153 transformed set of variables is called the principal components. In population genetics,
154 different materials are clustered into different subgroups based on the degree of SNP
155 differentiation (or degree of divergence) between materials, and the results can be
156 used for mutual verification with the results of other clustering methods. Based on the
157 SNPs identified, EIGENSOFT 7.2.1 (Alkes *et al.*2006) software was used to perform
158 principal component analysis to cluster the samples. Through PCA, we could
159 determine which samples were relatively closely related and which samples were
160 relatively distantly related, facilitating evolutionary analysis.

161 1.4.3 Analysis of genetic structure

162 Population structure, also known as group stratification, refers to the existence of
163 subgroups with different gene frequencies in the studied group. The materials in the
164 same subgroup are closely related, and the subgroups are relatively distantly related.

165 Group structure analysis can reveal the number of ancestors of the studied group and
166 the blood origin of each sample. The group cluster analysis method is currently
167 widely used, as it helps to understand the evolution of materials. Based on the SNPs
168 identified, ADMIXTURE V250 (Alexander *et al.* 2009) software was used to analyze
169 the group structure of the research materials. For the research group, the number of
170 subgroups (K value) was preset to 1-20 for clustering, the clustering results were
171 cross-validated, and the optimal number of clusters was determined according to the
172 lowest cross-validation error rate.

173 1.4.4 Genetic relationship analysis

174 Estimation of the affinity (relative kinship) between natural groups can be performed
175 using SPAGeDi 1.3 (Hardy *et al.* 2002) software. The kinship itself is the relative
176 value that defines the genetic similarity between two specific materials and that
177 between any material and itself, so when the kinship value between the two materials
178 is less than 0, it is directly defined as 0.

179 1.4.5 Analysis of genetic diversity

180 Population genetic parameters and the population index (F_{st}) were calculated using
181 VCFtools software (<https://vcftools.github.io/index.html>). According to Wright, when
182 the group differentiation index (F_{st}) equals 0 or 1, there is no differentiation between
183 subgroups or complete differentiation between subgroups, respectively. However,
184 values of $0 < F_{st} < 0.05$, $0.05 \leq F_{st} < 0.15$, $0.15 \leq F_{st} < 0.25$, or $0.25 \leq F_{st} < 1$
185 indicate weak, moderate, strong or very strong genetic differentiation between
186 subgroups, respectively.

187 2 Results and analysis

188 2.1 Genotype analysis of the cotton population

189 In the experiment, the digestion efficiency of HaeIII+SspI-HF® was 97.02%, and a
190 total of 2,161.76 Mb reads were obtained. A total of 1,313,331 SNPs were obtained

191 from the population through comparison with the cotton genome. The average Q30 of
192 the sequences was 96.87%, and the average GC content was 36.50%. The Nipponbare
193 rice sequencing used to evaluate the accuracy of the experimental database yielded
194 2.12 Mb reads of data(Appendix 2).

195 Plotting the distribution of SNPs on chromosomes revealed that SNPs were present in
196 high density at both ends of the chromosomes and at low density in the
197 juxtacentromeric region. The densities of SNPs on chromosomes A07, A13, D01, D05,
198 and D09 were relatively high (Figure 1). Chromosome ends are enriched in functional
199 genes, and the mid-centromere and near-centromere regions are mostly repetitive
200 sequences. The density distribution of SNPs on chromosomes was in accordance with
201 that expected.

202

203 **Figure 1** Distribution of SNPs on chromosomes. The abscissa is the length of the
204 chromosome. Each band represents a chromosome. The genome is divided according
205 to the size of 1Mb. The more SNP markers in each window, the darker the color, and
206 the fewer SNP markers, and the lighter the color. ; The darker the color in the figure is
207 the area where the SNP markers are concentrated.

208 2.2 Genetic evolutionary analysis

Phylogenetic trees were drawn based on the SNP markers, where most of the materials on the branches were from the inland cotton area of northwestern China, but they also contained materials from Central Asia(Figure 2).

212

Figure 2 Phylogenetic tree.Each branch in the picture is a sample, and each source has a color. 1: Northwest Inland, 2: Yellow River Basin, 3: Yangtze River Basin, 4: Extra-early Cotton Area, 5: Central Asia, 6: United States and other sources.

Based on principal component analysis of 1,313,331 SNP molecular markers from the population, the first three components explained 11.15%, 8.26%, and 6.47% of the variation, respectively. The results of the first three components were used to draw a PCA plot in the R environment, which is shown in Figure 3. The cotton population showed a certain distribution gap, but most varieties were clustered together, with no obvious differences among the 273 cotton materials. In terms of group stratification, the varieties from the United States and Central Asia were close to the early Chinese land cotton varieties, which corresponds to the early introduction

224 of Chinese land cotton varieties mainly from the United States and the Soviet Union
225 and breeding with the introduced materials as the parents.

226

227 **Figure 3** PCA three-dimensional clustering map. In the figure, the samples are
228 gathered into three dimensions by PCA analysis, PC1 represents the first principal
229 component, PC2 represents the second principal component; PC3 represents the third
230 principal component. A point represents a sample, and a color represents a grouping 1:
231 Northwest Inland, 2: Yellow River Basin, 3: Yangtze River Basin, 4: Extra-early
232 Cotton Area, 5: Central Asia, 6: United States and other sources.

233 For the group genetic structure analysis of the 273 cotton varieties through
234 1,313,331 SNP molecular markers, the K value range for the number of subgroups
235 was set to 1-20, and the cross-validation error (CV error) was calculated under the
236 different K values. As shown in Figure 4, when K increased from 1 to 6, the CV error
237 value decreased rapidly; when K increased from 6 to 11, the CV error value gradually
238 decreased and tended to flatten; when K increased from 11 to 16, the CV error value
239 showed a volatile, downward trend; and when K was greater than 16, the CV error
240 value increased to a certain extent. Thus, when K was equal to 16, the CV error value
241 is the smallest, and the 273 cotton variety groups could therefore be divided into 16
242 subgroups :Subgroup 1~Subgroup 16(Figure 4).

243 Depending on the Q value, each individual in the 16 subgroups was classified
244 into the maximum-Q value subgroup (Table 1). The 16 subgroups were 9, 16, 52, 4,

10, 13, 8, 30, 13, 9, 26, 18, 8, 31, 5, and 21. Group structure analysis is more consistent with PCA and systematic evolutionary tree analysis, with independent tests on the subgroups and the source of the materials. The results of these analyses showed that the division of the subgroups was significantly correlated with the material source, indicating that the genetic background of the resources was relatively homogeneous.

Table 1 Specific species grouping

Grouping	Variety number	Quantity
Q1	1,29,37,43,44,46,139,226,234	9
Q2	2,36,57,67,68,71,72,73,127,146,152,221,235,247,257,268	16
Q3	10,13,25,58,59,63,66,75,78,80,82,83,87,89,94,96,99,119,153,154,155,157,158,162,168,172,173,176,177,178,179,180,181,182,183,185,186,187,206,209,212,213,219,223,246,258,259,260,262,265,267,270	52
Q4	32,53,133,134	4
Q5	4,8,11,76,77,130,142,149,164,269	10
Q6	5,7,27,28,52,111,124,128,131,132,137,141,143	13
Q7	55,56,61,64,147,165,241,243	8
Q8	6,15,31,34,65,81,86,91,92,97,100,101,102,104,108,109,113,116,120,121,122,123,163,190,198,199,205,230,231,233	30
Q9	14,24,26,39,42,69,138,189,210,216,217,236,254	13
Q10	18,40,41,48,49,229,232,250,251	9
Q11	20,23,35,38,47,50,70,90,95,107,112,114,117,118,125,135,136,140,148,193,196,202,224,228,263,264	26
Q12	12,16,17,19,21,22,30,33,60,62,88,160,161,188,191,195,197,245	18
Q13	145,237,238,239,242,244,252,255	8
Q14	3,45,54,74,84,85,98,129,144,150,151,156,159,175,192,194,203,208,215,222,225,227,248,249,253,256,261,266,271,272,273	31

Q15	106,115,126,200,218	5
Q16	9,51,79,93,103,105,110,166,167,169,170,171,174,184,201,204,207,211,214,220,240	21

251

252 **Figure 4** Cross-validation error rate of each K value of Admixture.(A)
253 Cross-validation error rate for each K value, Group structure analysis of 273 cotton
254 materials, calculated CV error. at K of 1~20 . (B) Sample clustering results for each K
255 value, Group structure at K =1-16 in which each individual is represented by lines of
256 different colours, inferred which subgroup the breed belongs to from the proportion of
257 color.

258 Kinship calculations were performed by TASSEL 3.0 software, and a heat map
259 was generated in R (Figure 5), revealing that the 273 cotton varieties were closely
260 related to most varieties (blue) and a few other materials (red).

261

262 **Figure 5** Heat map distribution map of genetic relationship

263 2.3 Analysis of cotton genetic diversity

264 The experimental cotton group was divided into 6 subgroups according to source, and
 265 1,313,331 high-quality SNPs were evaluated to determine the genetic diversity of the
 266 experimental cotton group by calculating the diversity index, flavor index, and PIC.
 267 Through VCFtools calculations, the diversity index of the cotton population was
 268 determined to be 0.306, ranging between 0.314 for groups 1~6 and ~0.390, where
 269 group 2 had the lowest genetic diversity index (0.314) and group 4 had the highest
 270 (0.390) of the 6 groups. The aroma index (0.551) and PIC (0.296) were also
 271 determined. Group 4 had a relatively high genetic diversity level, but the overall
 272 genetic diversity level was still relatively low, which is consistent with the group
 273 structure analysis results(Table 2).

274 The population differentiation index (F_{st}) was used to evaluate the degree of
275 differentiation between cotton groups and revealed that the groups were all
276 moderately or weakly genetically differentiated, with genetic differentiation indexes
277 between groups ranging between 0.02368 and 0.10664 (Table 3). Among the groups,
278 there was moderate differentiation between group 1 and groups 3, 4, and 5; there was
279 moderate differentiation between group 2 and groups 3 and 4; there was moderate
280 differentiation between groups 3, 4, 5 and 6; and the remaining groups were weakly
281 equally divided. These results showed a moderate or weak degree of genetic
282 differentiation among the groups; that is, the genetic relationship between the groups
283 was relatively close. The genetic difference between group 3 and group 6 was the
284 largest, and the difference between group 3 and group 5 was the smallest. This is
285 related to the more effective promotion of selfing cotton varieties in the cotton
286 planting area of the Yangtze River Basin. In addition, the genetic distance analysis of
287 the cotton population used in this study showed that the genetic distance between
288 these cotton germplasms ranged from 0.000332651~0.562664014. The average
289 genetic distance was 0.25240429, and the genetic distances were quite different,
290 indicating that there were large differences in gene exchange between subgroups. The
291 genetic distance between Wanmian 8407 and Keyuan 1 was the closest (0.000332651),
292 while that between Xinluzhong 4 and Ekangmian 33 was the longest (0.562664014).

293 **Table 2** Statistics of genetic diversity

Grouping	Diversity index (H)	Shannon Index(I)	PIC
1	0.340962993	0.513301254	0.273711106
2	0.313688456	0.478088588	0.253233419
3	0.336734808	0.503163381	0.268215802
4	0.390081647	0.551293397	0.295768827
5	0.347723988	0.513940149	0.274302057

6	0.355438627	0.518915162	0.277185405
overall	0.306138549	0.470906956	0.248870833

294 **Table 3** Statistics of population differentiation index

	1	2	3	4	5	6
1	0.00000					
2	0.02655	0.00000				
3	0.09008	0.06711	0.00000			
4	0.06625	0.06038	0.02638	0.00000		
5	0.06801	0.03543	0.02368	0.03440	0.00000	
6	0.04768	0.04231	0.10664	0.09887	0.07326	0.00000

295

296 3 Discussion

297 3.1 Analysis of cotton population structure

298 Studies have shown that there will be false associations between genotypes and traits,
 299 which may be caused by population structure and an uneven distribution of alleles
 300 (Wu *et al.* 2019). To eliminate false positives in association analysis, we need to
 301 analyze the group structure of a test group first and control for that group structure.
 302 This study used ADMIXTURE software to analyze the population structure of 273
 303 natural populations of cotton varieties at home and abroad. The results showed that
 304 when K=16, the CV error value was the smallest. Therefore, the 273 cotton
 305 germplasms were divided into 16 subgroups. According to the grouping results, in the
 306 same subgroup, most varieties were genetically related. The analysis results showed
 307 that some varieties with different origins were of one type, which may have been
 308 caused by crossover between varieties and environmental factors, but most varieties
 309 with the same origin and with similar genetic background information could be better
 310 classified. For subgroups, the results of population structure analysis were more in

line with the evolutionary trends of the genetic background during breeding. According to the different origins of the experimental cotton materials, they were divided into 6 groups. The population differentiation indexes ranged between 0.02368 and 0.10664, and the population differentiation indexes between most groups were less than 0.07, indicating that the cotton population has moderately weak genetic differentiation. In the later period, the genetic relationship of varieties cultivated through continuous introduction and germplasm innovation gradually increased. China is not the origin of upland cotton. Previous studies have shown that since the introduction of tetraploid cotton to China, Daizimian 15 has been the most widely planted variety in China. In 1958 alone, more than 3.5 million hectares were planted, accounting for approximately 50% of the country's cotton planting area that year. More than 100 varieties have been directly selected from Daizimian 15. In addition, there are quite a few varieties that are crossed with Daizimian 15. Principal component analysis and population structure analysis have also shown that the genetic range of cotton is relatively narrow, and most cotton varieties are derived from the same ancestor, Stormproof. The group differentiation of soybeans is driven by differences in geographical locations. Conversely, the formation of populations during the process of cotton breeding was mainly due to different ancestors, which is similar to the situation in wheat (Ye.2011;Mei.2012). There were some differences among the 33 local upland cotton varieties bred in southern Xinjiang collected by the Economic Crop Research Institute of the Xinjiang Academy of Agricultural Sciences, but the overall differences were not large; in cultivated cotton from other regions, the population differentiation indexes were between 0.01 and 0.05 (Ai *et al.*2010). This shows that cotton collected in China has a low degree of genetic differentiation. In comparison, the population differentiation index of cotton germplasm in this study is similar to that of cotton germplasm collected in China, and varieties from different ecological regions show obvious mixed characteristics.

3.2 Analysis of cotton genetic diversity

339 The sum of genetic information carried by all organisms is defined as genetic diversity,
 340 which can also be called species diversity. Genetic diversity is usually regarded as the
 341 sum of genetic variation among individuals within a species. In this study, the
 342 diversity index, Shannon index and PIC were calculated to evaluate the genetic
 343 diversity of the cotton population. Previous studies such as that of Ai (2017)
 344 genotyped 288 upland cotton germplasms; the genetic diversity was 0.31, and the PIC
 345 was 0.25. Fang *et al.* (2013) detected an average PIC of 0.29 in 193 upland cotton
 346 varieties collected from 26 countries. These results indicate that the diversity of cotton
 347 collected in China is relatively low and that the degree of genetic differentiation is low.
 348 Moreover, the level of genetic differentiation between landraces and modern cultivars
 349 is low (Tyagi *et al.* 2014). According to the cotton SNP molecular marker
 350 development and genetic diversity comparison, the genetic diversity index of each
 351 subgroup in the cotton population in this study was between 0.314 and 0.390, and the
 352 overall genetic diversity index was 0.306, with a PIC of 0.249. Thus, the genetic
 353 diversity index of the cotton germplasm is consistent with that of the cotton
 354 germplasm collected in China. The largest genetic diversity was observed between
 355 varieties from the the US and extra-early cotton regions, and the largest differentiation
 356 index was between varieties from the the US and the Yangtze River Basin.
 357 High-quality cotton varieties can be selected and introduced to China to enrich the
 358 existing cotton germplasm resources. In addition, the genetic relationship between the
 359 samples will also have a certain impact on the results of the association analysis. In
 360 this study, the genetic distances between 273 cotton germplasms were analyzed, and
 361 the kinship value was used to infer the genetic relationships between different
 362 materials. The results showed that the average genetic distance of these cotton
 363 germplasms was 0.252. Part of the results indicated that some varieties with different
 364 origins were clustered into one category. Varieties with similar genetic backgrounds
 365 can be better clustered into one category, and the clustering results are more in line
 366 with the evolutionary trends of the genetic background of the varieties.

367 4 Conclusion

Chinese cotton planting area is very large, but upland cotton was not domesticated in China. Upland cotton was first domesticated in the United States and was then introduced to China in the 1940s and 1950s. Among upland cotton varieties, Daizimian 15 and Sizimian 2B are the two most widely planted in China. On the basis of these two varieties, many modern cotton cultivars were bred by Chinese cotton breeders through genealogy and crossbreeding. After domestication and improvement, the yield of cotton was higher, the fiber quality was better, and the planting range was wider. Thus, these improved upland cotton cultivars can replace Asian cotton grown in China. The results of domestic and foreign studies have consistently shown that the genetic range of upland cotton varieties is narrow. The intraspecific genetic diversity was far lower than the interspecific differences (Liu *et al.* 2003; Curt *et al.* 1994). Using multiple methods to expand the genetic range of upland cotton varieties will be an important aspect of cotton germplasm resource innovation and breeding in the future. In this study, 1,313,331 SNP loci from the analyzed population were used to determine the population genetic structure of 273 domestic and foreign cotton germplasm resources. The results of the genetic diversity analysis revealed that the genetic diversity of the experimental cotton population was average; the results of the population genetic structure analysis showed that the population was divided into 16 subgroups (K=16). In addition, this cotton population was less differentiated than and closely related to the domestically collected cotton germplasm. Genetic distance analysis revealed that Wanmian 8407 is the closest genetically to Keyuan 1 and that Xinluzhong 4 is the most genetically distant from Ekangmian 33. The results of the genetic diversity analysis showed that the greatest genetic diversity occurred between cultivars from the US and early cotton regions, and the largest differentiation index was observed between cultivars from the US and the Yangtze River Valley. This study will provide a basis for genome-wide association analysis for mining elite genes and obtaining elite cotton germplasms.

Dota availability statements

396 The raw sequence data reported in this paper have been deposited in the Genome
397 Sequence Archive (Genomics, Proteomics & Bioinformatics 2017) in National
398 Genomics Data Center (Nucleic Acids Res 2021), China National Center for
399 Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences,
400 project number PRJCA 005438, under accession number CRAxxxxxx that are
401 publicly accessible at <https://ngdc.cncb.ac.cn/gsa>.

402

403

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413 **Literature cited**

414 Alkes L Price, Nick J Patterson, et al. 2006. Principal components analysis corrects
415 for stratification in genome-wide association studies. Nature Genetics. 38(8),
416 904-909. doi:10.1038/ng1847.

417 Alexander D H, Novembre J, Lange K. 2009. Fast model-based estimation of ancestry
418 in unrelated individuals. Genome research. 19(9): 1655-1664. doi:10.1101/gr.094
419 052.109.

420 Ai X-T, Li X-Y, et al. 2010. Genetic Diversities of Upland Cotton Varieties in South
421 Xinjiang. *Cotton Science*. 6:603-610.

422 Ai X-T. 2017. Genome-wide Association Analysis on Yield and Fiber Quality Traits
423 in Upland Cotton. Xinjiang Agricultural University.

424 Beasley, J. O. 1940. The production of polyploids in gossypium. 31(1): 39-48.

425 Chen G, Du X-M. 2006. Genetic Diversity of Source Germplasm of Upland Cotton in
426 China as Determined by SSR Marker Analysis. *Journal of Genetics and*
427 *Genomics*. 33: 733-745.

428 Cingolani P, Platts A, Wang le L, et al. 2012. A program for annotating and predicting
429 the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of
430 *Drosophila melanogaster* strain w1118; iso-2; iso-3., *Fly (Austin)*.
431 *Apr-Jun;6(2):80-92*. doi:10.4161/fly.19695.

432 Curt.L. , Brubaker , Jonathan , F.Wendel , F Jonathan. 1994. Reevaluating the Origin
433 of Domesticated Cotton (*Gossypium hirsutum*; Malvaceae) Using Nuclear
434 Restriction Fragment Length Polymorphisms (RFLPs). *American Journal of*
435 *Botany*. doi:10.2307/2445407.

436 Dong W. 2007. Genetic Diversity and SSR Abundance Analysis of Cotton Germplasm
437 Resources. Master's Thesis of Chinese Academy of Agricultural Sciences.

438 Fryxell PA. 1979. The Natural History of the Cotton Tribe (Malvaceae, Tribe
439 *gossypieae*). Texas A&M University Press. p 4-7.

440 Fryxell PA. 1992. A Revised Taxonomic Interpretation of *Gossypium* L. (Malvaceae).
441 *Rheede*. 2:108-165.

442 Fang DD, Hinze LL, Percy RG, et al.2013.A microsatellite-based genome-wide analy
443 sis of genetic diversity and linkage disequilibrium in Upland cotton (*Gossypium*

444 hirsutum L.) cultivars from major cotton- growing countries. Euphytica.
445 191: 391~401. doi:10.1007/s10681-013-0886-2.

446 Grover CE, Gallagher JP, Jareczek JJ, Page JT, Udall JA, Gore MA, Wendel JF. 2015.
447 Re-evaluating the Phylogeny of Allopolyploid Gossypium L. Molecular
448 Phylogenetics and Evolution. 92:45-52. doi:10.1016/j.ympev.2015.05.023.

449 Gallagher JP, Grover CE, Rex K, Moran M, Wendel JF. 2017. A New Species of
450 Cotton from Wake Atoll. Gossypium stephensii (Malvaceae). Systematic Botany.
451 42:115-123. doi :10.1600/036364417x694593.

452 Gao W, Liu F, Li S-H, Wang K-B, et al. 2010. Genetic Diversity of Allotetraploid
453 Cotton Based on SSR Markers. ACTA AGRONOMICA SINICA. 36(11):
454 1902-1909.

455 Hardy O J, Vekemans X. 2002. SPAGeDi: a versatile computer program to analyse
456 spatial genetic structure at the individual or population levels. Molecular ecology
457 notes. 2(4): 618-620. doi:10.1046/j.1471-8286.2002.00305.x.

458 Kuang M, Yang W-H, Xu H-X. 2011. Construction of DNA Fingerprinting and
459 Analysis of Genetic Diversity with SSR Markers for Cotton Major Cultivars in
460 China. China Agricultural Sciences.44(1):20-27.doi:10.3864/j.issn.
461 0578-1752.2011.01.003.

462 Kozich J J, Westcott S L, Baxter N T, et al. 2013. Development of a dual-index
463 sequencing strategy and curation pipeline for analyzing amplicon sequence data
464 on the MiSeqIllumina sequencing platform. Applied and environmental
465 microbiology. 79(17): 5112-5120. doi:10.1128/aem.01043-13.

466 Liu Q. 2015. Identification of red star grass cotton-Australian cotton diploid and
467 MSAP detection of genomic DNA methylation. Nanjing Agricultural University.

468 Liu W-X, Kong F-L, Guo Z-L, et al. 2003. Molecular Marker Analysis of Cotton Seed
469 Inheritance in China since the Founding of the People's Republic of China.
470 Journal of Genetics and Genomics. 30(6):560-570.

471 Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler
472 transform. Bioinformatics. 25(14):1754-1760.doi:10.1093/bioinformatics/btp324.

473 Li H, Handsaker B, Wysoker A, et al. 2009. The Sequence Alignment/Map Format
474 andSAMtools.Bioinformatic.25(16):2078-2079.doi:10.1093/bioinformatics
475 /btp352.

476 Multanid S,Lyon B R.1995. Genetie Finger Printing of Australian Cotton Culivars w
477 ith RAPD Markers. Genome. 38: 1005 - 1008. doi:10.1139/g95-132.

478 McKenna A, Hanna M, Banks E, et al. 2010. The Genome Analysis Toolkit: a
479 MapReduce framework for analyzing next-generation DNA sequencing data.
480 Genome research. 20(9): 1297-1303. doi:10.1101/gr.107524.110.

481 Mei H-X. 2012. Genetic Diversity and Association Analysis of Main Breeding Target
482 Traits in Uplang Cotton Cultivars of China. Nanjing Agricultural University.

483 Tyagi P, Gore M A, Bowman D T, et al. 2014. Genetic diversity and population struc
484 ture in America Upland cotton (*Gossypium hirsutum* L.). Theoretical and Applie
485 d Genetics. 127: 283~ 295. doi:10.1007/s00122-013-2217-3.

486 Wu Y-T, Zhang T-Z, Yin J-M. 2001. An Analysis about Genetic Basis of Cotton
487 Cultivars in China since 1949 with Molecular Markers. Journal of Genetics and
488 Genomics. 28 (11): 1040-1050.

489 Wu L-Y. 2012. Genetic Diversity Analysis of Sea-Island Cotton Germplasm
490 Resources and Studies on Heterosis Utilisation between Upland Cotton and
491 Island Cotton in the South China cotton region. Guangxi University.

492 Wu M, Wang N, Lin Z-X, et al. 2019. Development and evaluation of InDel markers
493 in cotton based on whole-genome re-sequencing data. ACTA AGRONOMICA
494 SINICA. 45(2).

495 Ye G-X. 2011. Genetic Analysis of cotton Cultivars Evolution in China. Nanjing
496 Agricultural University.

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522 **Appendix 1 273 cotton materials**

Num berin g	Variety name	Origin	Num berin g	Variety name	Origin	Num berin g	Variety name	Origin
1	Xinlu early 1	Inland Northwest	92	Jin Cotton 6	Yellow River Basin	183	Su Mian 12	Yangtze River Basin
2	Xinlu Morning 2	Inland Northwest	93	Shaanxi Cotton No. 9	Yellow River Basin	184	Su Mian 15	Yangtze River Basin
3	Xinlu early 3	Inland Northwest	94	Shaanxi Cotton No. 6	Yellow River Basin	185	Xuzhou 514	Yangtze River Basin
4	Xinlu Morning 4	Inland Northwest	95	Shaan 63-1	Yellow River Basin	186	Ganmian 10	Yangtze River Basin
5	Xinlu Morning 5	Inland Northwest	96	Shaan 5245	Yellow River	187	Ganmian 17	Yangtze River Basin

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6	Xinlu early 7	Inland Northwest	97	Shaan 401	Yellow River Basin	188	Chuan 169-6	Yangtze River Basin
7	Xinlu Morning 8	Inland Northwest	98	Shaan 2812	Yellow River Basin	189	Chuan 73-27	Yangtze River Basin
8	Xinlu Morning 9	Inland Northwest	99	Shaanxi 2754	Yellow River Basin	190	Sichuan cotton 65	Yangtze River Basin
9	New Land 10 a.m	Inland Northwest	100	Sprinkle cotton No.2	Inland Northwest	191	Yu cotton 1	Yangtze River Basin
10	New Land 11 a.m	Inland Northwest	101	Cotton No.1	Yellow River Basin	192	Liao cotton No.1	Special precocious cotton area
11	New Land 12 early	Inland Northwest	102	Dun cotton 2	Inland Northwest	193	Liao cotton No.9	Special precocious cotton area
12	New Land 13 early	Inland Northwest	103	Dunhuang 77-126-8	Inland Northwest	194	Liao cotton 16	Special precocious cotton area
13	New Land 14	Inland Northwest	104	Dunhuang 77-166	Inland Northwest	195	Liao no 1201	Special precocious cotton area
14	New Land 16	Inland Northwest	105	Tashkent 2	Central Asia	196	Liao 632-124	Special precocious cotton area
15	Xinluzao 17	Inland Northwest	106	108 husbands	Central Asia	197	Liao 7334-7728	Special precocious cotton area
16	Xinluzao 18	Inland Northwest	107	KK-351	Central Asia	198	Nylon 1	Special precocious

								cotton area
17	Xinlu early 19	Inland Northwest	108	KK-1543	Central Asia	199	Nylon 6	Special precocious cotton area
18	Xinluzao 21	Inland Northwest	109	KK-1047	Central Asia	200	Big boll cotton	Yellow River Basin
19	Xinluzao 22	Inland Northwest	110	Coker310	Central Asia	201	Dai 4554	United States
20	Xinluzao 23	Inland Northwest	111	C6524	Central Asia	202	Dai 45A	United States
21	Xinlu morning 24	Inland Northwest	112	C-4744	Central Asia	203	Dai-80	United States
22	Xinlu Zao 25	Inland Northwest	113	C464	Central Asia	204	Dai word cotton 15	United States
23	New Land 27	Inland Northwest	114	C460	Central Asia	205	Guan Nong 1	Special precocious cotton area
24	New Land 29 early	Inland Northwest	115	C-405-555	Central Asia	206	Montenegrin Cotton 1	Special precocious cotton area
25	New Land 30 morning	Inland Northwest	116	C-3174	Central Asia	207	Tess cotton	Yellow River Basin
26	New Land 31	Inland Northwest	117	Bazhou 6501	Inland Northwest	208	McNair 210	United States
27	New Land 32 early	Inland Northwest	118	Library T94-4	Inland Northwest	209	Coyuan 1	Yellow River Basin
28	New Land 33	Inland Northwest	119	8024 anti-	Inland Northwest	210	Cloth 3363	United States
29	New Land 34	Inland Northwest	120	65-201	Inland Northwest	211	Chad 3	Africa

30	New Land 35	Inland Northwest	121	Car 61-72	Inland Northwest	212	Turkmen land cotton	Central Asia
31	New Land 36	Inland Northwest	122	Sacar cotton	Inland Northwest	213	U.S. B-35	United States
32	New Land 37	Inland Northwest	123	Farming 5	Inland Northwest	214	African cotton E-40	Africa
33	New Land 38	Inland Northwest	124	Moyu 11	Inland Northwest	215	Australia V21-757	Australia
34	New Land is 39 early	Inland Northwest	125	New Land 202	Inland Northwest	216	Miscot7803-52	United States
35	New Land 40 morning	Inland Northwest	126	New Land 201	Inland Northwest	217	T-word cotton 16	United States
36	New Land 41	Inland Northwest	127	New Land 71	Inland Northwest	218	Aussie Siv2	Australia
37	New Land 42	Inland Northwest	128	Xinluzhong 70	Inland Northwest	219	Division 6524	Central Asia
38	Xinluzao 45	Inland Northwest	129	Xinluzhong 69	Inland Northwest	220	Thin floc H10	United States
39	Xinluzao 47	Inland Northwest	130	Xinluzhong 65	Inland Northwest	221	Yinmian 1	Yellow River Basin
40	Xinluzao 48	Inland Northwest	131	Xinluzhong 64	Inland Northwest	222	Us 28114-313	United States
41	Xinluzao 49	Inland Northwest	132	Xinluzhong 63	Inland Northwest	223	Filgan 175	Central Asia
42	Xinluzao 51	Inland Northwest	133	Xinluzhong 62	Inland Northwest	224	Xinluzao 44	Inland Northwest
43	Xinluzao 52	Inland Northwest	134	Xinluzhong 61	Inland Northwest	225	Jizhong cotton 315	Yellow River Basin
44	Xinluzao 53	Inland Northwest	135	Xinluzhong 60	Inland Northwest	226	Xinluzao 43	Inland Northwest

45	Xinluzao 57	Inland Northwest	136	Xinluzhong 59	Inland Northwest	227	J206-5	Inland Northwest
46	Xinluzao 58	Inland Northwest	137	Xinluzhong 58	Inland Northwest	228	Xinluzhong 82	Inland Northwest
47	Xinlu early 60	Inland Northwest	138	Xinluzhong 54	Inland Northwest	229	Xinluzao 82	Inland Northwest
48	Xinluzao 61	Inland Northwest	139	Xinluzhong 52	Inland Northwest	230	Xinlu early 80	Inland Northwest
49	Xinluzao 62	Inland Northwest	140	Xinluzhong 50	Inland Northwest	231	Xinluzao 77	Inland Northwest
50	Xinluzao 63	Inland Northwest	141	Xinluzhong 48	Inland Northwest	232	Xinluzao 73	Inland Northwest
51	Xinluzhong 2	Inland Northwest	142	Xinluzhong 47	Inland Northwest	233	Xinluzao 65	Inland Northwest
52	Xinluzhong 4	Inland Northwest	143	Xinluzhong 46	Inland Northwest	234	Xinluzao 55	Inland Northwest
53	Xinluzhong 5	Inland Northwest	144	Xinluzhong 45	Inland Northwest	235	Luyan cotton 27	Inland Northwest
54	Xinluzhong 6	Inland Northwest	145	Xinluzhong 42	Inland Northwest	236	17N11	Inland Northwest
55	Xinluzhong 8	Inland Northwest	146	Xinluzhong 40	Inland Northwest	237	17N10	Inland Northwest
56	Xinluzhong 9	Inland Northwest	147	Xinluzhong 39	Inland Northwest	238	17N9	Inland Northwest
57	Xinluzhong 10	Inland Northwest	148	Xinluzhong 38	Inland Northwest	239	17N8	Inland Northwest
58	Xinluzhong 14	Inland Northwest	149	Xinluzhong 36	Inland Northwest	240	17N7	Inland Northwest
59	Xinluzhong 15	Inland Northwest	150	Lu 34	Yellow River	241	17N6	Inland Northwest

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60	Xinluzhong 17	Inland Northwest	151	Lu Mianyan 36	Yellow River Basin	242	17N5	Inland Northwest
61	Xinluzhong 18	Inland Northwest	152	Lu Mianyan 37	Yellow River Basin	243	17N3	Inland Northwest
62	Xinluzhong 20	Inland Northwest	153	Yumian 11	Yellow River Basin	244	17N2	Inland Northwest
63	Xinluzhong 22	Inland Northwest	154	Yumian 15	Yellow River Basin	245	17N1	Inland Northwest
64	Xinluzhong 23	Inland Northwest	155	Yumian 17	Yellow River Basin	246	Xuzhou 142	Yellow River Basin
65	Xinluzhong 25	Inland Northwest	156	Yumian 19	Yellow River Basin	247	Soviet 8911	Central Asia
66	Xinluzhong 28	Inland Northwest	157	Zhongzhi Cotton 372	Yellow River Basin	248	Kexin 001	Yellow River Basin
67	Xinluzhong 29	Inland Northwest	158	China Cotton Institute 12	Yellow River Basin	249	150030	Central Asia
68	Xinluzhong 32	Inland Northwest	159	Middle cotton 16	Yellow River Basin	250	150028	Central Asia
69	Xinluzhong 33	Inland Northwest	160	China Cotton Institute 17	Yellow River Basin	251	150022	Central Asia
70	Xinluzhong	Inland	161	Cotton 19	Yellow	252	150021	Central Asia

	34	Northwest			River Basin			
71	Xinluzhong 35	Inland Northwest	162	Medium cotton 35	Yellow River Basin	253	150019	Central Asia
72	Lu 25	Yellow River Basin	163	Cotton 41	Yellow River Basin	254	17N13	Inland Northwest
73	Lu 24	Yellow River Basin	164	China Cotton Institute 43	Yellow River Basin	255	17N12	Inland Northwest
74	Lu Mianyan 21	Yellow River Basin	165	China Cotton Institute 60	Yellow River Basin	256	Miscott 8711	United States
75	Lu 9	Yellow River Basin	166	Hunan Cotton 11	Yangtze River Basin	257	coker139	United States
76	Lu 28	Yellow River Basin	167	Ekan cotton 8	Yangtze River Basin	258	Darmian 20	Yangtze River Basin
77	Lumian 17	Yellow River Basin	168	Ekan cotton 10	Yangtze River Basin	259	19(Taihu)	Yangtze River Basin
78	Lumian 11	Yellow River Basin	169	Ekan cotton 9	Yangtze River Basin	260	Silver cotton 2	Yellow River Basin
79	Shi Yuan 321	Yellow River Basin	170	Ekan cotton 33	Yangtze River Basin	261	Liaomian 17	Special precocious cotton area
80	Ji Mian 12	Yellow River Basin	171	Emian 6	Yangtze River Basin	262	Hunan Cotton 10	Yangtze River Basin

81	Ji Mian 11	Yellow River Basin	172	Emian 10	Yangtze River Basin	263	Zhong93001	Yellow River Basin
82	Ji Mian 10	Yellow River Basin	173	Emian 14	Yangtze River Basin	264	Tashkent 6	Central Asia
83	Ji Mian 8	Yellow River Basin	174	Emian 21	Yangtze River Basin	265	Jinmian 29	Yellow River Basin
84	Ji 168	Yellow River Basin	175	Dongting 1	Yangtze River Basin	266	China Cotton Institute 41	Yellow River Basin
85	Ji 169	Yellow River Basin	176	Wanmian 8407	Yangtze River Basin	267	Medium 1132	Yellow River Basin
86	Yun 93 Anti 354	Yellow River Basin	177	Simian 2	Yangtze River Basin	268	Huamian No. 1	Inland Northwest
87	Taiyuan 4	Yellow River Basin	178	Simian 3	Yangtze River Basin	269	Lumian 28	Yellow River Basin
88	Jin Cotton 31	Yellow River Basin	179	Su Mian No. 1	Yangtze River Basin	270	Lu Mianyan 27	Yellow River Basin
89	Jin Cotton 19	Yellow River Basin	180	Su Cotton 5(12)	Yangtze River Basin	271	Ji Mian 938	Inland Northwest
90	Jin Cotton 12	Yellow River Basin	181	Su Mian 8	Yangtze River Basin	272	18N3	Inland Northwest
91	Jin 11	Yellow River	182	Su Mian 9	Yangtze River	273	18N4	Inland Northwest

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541 **Appendix 2 Statistical Table of Sample SNP Information**

Sample ID	SNP num	Integrity	Heter ratio	Sample ID	SNP num	Integrity	Heter ratio	Sample ID	SNP num	Integrity	Heter ratio
1	434,940	33.12%	1.98%	92	398,290	30.33%	6.63%	183	372,651	28.37%	4.04%
2	404,396	30.79%	1.75%	93	355,575	27.07%	9.44%	184	366,593	27.91%	1.53%
3	346,050	26.35%	1.69%	94	360,834	27.47%	2.69%	185	429,669	32.72%	1.81%
4	356,469	27.14%	1.88%	95	346,255	26.36%	7.03%	186	421,258	32.08%	4.02%

5	387,519	29.51%	1.82%	96	402,924	30.68%	4.91%	187	384,530	29.28%	2.90%
6	395,712	30.13%	3.31%	97	400,893	30.52%	6.37%	188	382,419	29.12%	2%
7	419,970	31.98%	7.02%	98	337,635	25.71%	2.25%	189	425,245	32.38%	5.01%
8	344,481	26.23%	1.77%	99	371,512	28.29%	6.45%	190	438,126	33.36%	1.95%
9	368,142	28.03%	5.43%	100	380,645	28.98%	6.25%	191	419,716	31.96%	1.85%
10	359,658	27.39%	5.67%	101	368,512	28.06%	4.76%	192	371,816	28.31%	4.25%
11	406,512	30.95%	1.80%	102	398,505	30.34%	1.97%	193	381,356	29.04%	1.78%
12	428,670	32.64%	4.89%	103	405,013	30.84%	6.31%	194	422,487	32.17%	4.14%
13	407,643	31.04%	2.20%	104	393,738	29.98%	3.17%	195	395,536	30.12%	1.65%
14	351,700	26.78%	1.71%	105	400,240	30.48%	3.45%	196	401,132	30.54%	3.99%
15	362,723	27.62%	6.88%	106	362,316	27.59%	1.59%	197	416,455	31.71%	1.80%
16	346,777	26.40%	1.78%	107	413,228	31.46%	5%	198	342,310	26.06%	8.13%
17	425,605	32.41%	1.78%	108	405,613	30.88%	4.54%	199	371,032	28.25%	4.48%
18	406,179	30.93%	1.76%	109	359,280	27.36%	3.98%	200	399,414	30.41%	2.07%
19	358,576	27.30%	1.62%	110	371,168	28.26%	2.74%	201	440,223	33.52%	3.33%
20	389,335	29.64%	5.48%	111	398,243	30.32%	1.82%	202	444,033	33.81%	3.98%
21	417,327	31.78%	1.72%	112	398,822	30.37%	2.45%	203	424,247	32.30%	1.76%
22	433,431	33.00%	1.83%	113	420,994	32.06%	7.32%	204	404,954	30.83%	1.66%
23	417,988	31.83%	5.37%	114	398,432	30.34%	3.36%	205	430,315	32.77%	2.27%
24	366,549	27.91%	1.74%	115	364,089	27.72%	1.80%	206	412,801	31.43%	1.82%
25	366,817	27.93%	1.68%	116	345,660	26.32%	2.18%	207	389,324	29.64%	7.63%
26	411,664	31.35%	1.65%	117	371,624	28.30%	1.68%	208	384,411	29.27%	1.65%
27	402,222	30.63%	3.76%	118	386,846	29.46%	6.56%	209	413,280	31.47%	1.52%
28	397,127	30.24%	1.75%	119	330,553	25.17%	3.22%	210	378,617	28.83%	1.67%

29	417,399	31.78%	3.95%	120	378,615	28.83%	8.11%	211	423,858	32.27%	1.98%
30	348,578	26.54%	5.54%	121	380,015	28.94%	3.46%	212	418,915	31.90%	3.91%
31	368,995	28.10%	1.72%	122	406,505	30.95%	3.67%	213	393,101	29.93%	1.53%
32	394,821	30.06%	1.71%	123	408,282	31.09%	5.86%	214	385,355	29.34%	2.35%
33	425,061	32.37%	1.92%	124	366,610	27.91%	11.53%	215	399,485	30.42%	1.63%
34	432,987	32.97%	1.83%	125	384,027	29.24%	4.89%	216	443,888	33.80%	2.26%
35	429,669	32.72%	1.78%	126	400,793	30.52%	5.55%	217	419,395	31.93%	1.78%
36	403,553	30.73%	1.54%	127	392,742	29.90%	2.27%	218	384,402	29.27%	1.59%
37	425,604	32.41%	2.05%	128	408,697	31.12%	2.09%	219	387,147	29.48%	1.60%
38	427,422	32.54%	2.36%	129	412,864	31.44%	4.08%	220	402,916	30.68%	1.52%
39	403,257	30.70%	1.85%	130	362,779	27.62%	1.52%	221	393,220	29.94%	1.68%
40	353,197	26.89%	1.58%	131	354,145	26.97%	1.61%	222	424,769	32.34%	3.63%
41	421,349	32.08%	4.34%	132	381,547	29.05%	1.56%	223	375,430	28.59%	1.48%
42	354,833	27.02%	2.77%	133	348,341	26.52%	1.40%	224	431,499	32.86%	2.49%
43	413,331	31.47%	1.71%	134	384,133	29.25%	2.05%	225	374,640	28.53%	1.61%
44	421,040	32.06%	1.69%	135	377,131	28.72%	1.86%	226	434,324	33.07%	1.86%
45	393,095	29.93%	1.65%	136	359,475	27.37%	2.20%	227	420,923	32.05%	1.77%
46	371,182	28.26%	1.81%	137	414,540	31.56%	1.74%	228	423,923	32.28%	1.72%
47	404,044	30.76%	1.66%	138	375,535	28.59%	1.65%	229	390,694	29.75%	1.82%
48	409,550	31.18%	3.06%	139	377,312	28.73%	7.24%	230	387,537	29.51%	4.50%
49	427,854	32.58%	1.88%	140	379,476	28.89%	6.35%	231	357,978	27.26%	1.61%
50	375,473	28.59%	4.94%	141	350,884	26.72%	1.80%	232	434,914	33.12%	3.50%
51	380,337	28.96%	1.77%	142	382,052	29.09%	1.65%	233	432,206	32.91%	5.68%
52	421,356	32.08%	1.78%	143	408,789	31.13%	2.12%	234	382,565	29.13%	1.74%

53	403,303	30.71%	3.28%	144	382,242	29.10%	1.88%	235	383,969	29.24%	1.55%
54	418,254	31.85%	5.45%	145	380,524	28.97%	6.02%	236	403,449	30.72%	1.61%
55	385,327	29.34%	1.68%	146	417,955	31.82%	4.24%	237	433,385	33.00%	5.49%
56	424,481	32.32%	1.95%	147	350,340	26.68%	1.52%	238	360,771	27.47%	3.74%
57	377,631	28.75%	4.39%	148	407,595	31.04%	2.38%	239	419,835	31.97%	1.98%
58	415,037	31.60%	3.20%	149	382,006	29.09%	1.53%	240	383,080	29.17%	1.50%
59	418,553	31.87%	1.75%	150	376,016	28.63%	4.67%	241	364,755	27.77%	1.62%
60	405,432	30.87%	1.57%	151	371,336	28.27%	4.18%	242	307,038	23.38%	1.49%
61	377,245	28.72%	2.08%	152	373,996	28.48%	1.50%	243	397,387	30.26%	1.53%
62	378,597	28.83%	8.20%	153	340,280	25.91%	1.53%	244	425,251	32.38%	1.74%
63	370,030	28.17%	4.12%	154	386,129	29.40%	6.10%	245	386,212	29.41%	1.62%
64	400,675	30.51%	2.18%	155	365,701	27.85%	1.31%	246	360,541	27.45%	1.49%
65	426,418	32.47%	5.97%	156	381,831	29.07%	1.59%	247	411,842	31.36%	1.59%
66	375,172	28.57%	3.84%	157	375,794	28.61%	1.51%	248	405,759	30.90%	1.67%
67	373,745	28.46%	3.84%	158	359,096	27.34%	7.09%	249	407,020	30.99%	1.71%
68	383,177	29.18%	1.58%	159	386,452	29.43%	6.24%	250	370,818	28.23%	2.64%
69	419,576	31.95%	3.94%	160	433,567	33.01%	1.71%	251	341,905	26.03%	1.34%
70	343,639	26.17%	7.17%	161	412,427	31.40%	1.48%	252	392,718	29.90%	1.67%
71	405,923	30.91%	5.93%	162	367,058	27.95%	1.57%	253	406,359	30.94%	4.76%
72	366,423	27.90%	3.67%	163	366,890	27.94%	5.07%	254	412,915	31.44%	2.24%
73	364,232	27.73%	5.05%	164	392,907	29.92%	1.55%	255	372,600	28.37%	2.51%
74	432,997	32.97%	7.68%	165	357,611	27.23%	1.49%	256	389,704	29.67%	2.90%
75	391,367	29.80%	3.10%	166	401,102	30.54%	5.18%	257	376,592	28.67%	1.49%
76	405,920	30.91%	1.80%	167	406,770	30.97%	5.97%	258	409,829	31.21%	1.64%

77	359,165	27.35%	1.65%	168	416,563	31.72%	3.03%	259	386,984	29.47%	1.49%
78	440,316	33.53%	3.35%	169	431,051	32.82%	4.15%	260	416,587	31.72%	3.17%
79	399,286	30.40%	1.68%	170	416,577	31.72%	1.90%	261	360,637	27.46%	7.74%
80	395,603	30.12%	4.81%	171	355,087	27.04%	5.14%	262	354,067	26.96%	1.47%
81	408,905	31.13%	1.89%	172	349,741	26.63%	6%	263	336,657	25.63%	1.50%
82	344,019	26.19%	4.78%	173	397,827	30.29%	2.33%	264	401,962	30.61%	2.87%
83	357,174	27.20%	1.32%	174	520,267	39.61%	3.57%	265	386,537	29.43%	3.33%
84	397,512	30.27%	3.32%	175	441,371	33.61%	2.28%	266	344,766	26.25%	1.93%
85	401,092	30.54%	1.61%	176	357,995	27.26%	1.58%	267	322,448	24.55%	1.63%
86	402,273	30.63%	7.84%	177	397,769	30.29%	6.66%	268	343,393	26.15%	1.57%
87	404,460	30.80%	1.59%	178	376,710	28.68%	4.97%	269	385,205	29.33%	2.72%
88	391,250	29.79%	2.30%	179	417,191	31.77%	2.31%	270	412,979	31.45%	1.58%
89	364,635	27.76%	4.41%	180	426,798	32.50%	1.86%	271	396,283	30.17%	2.29%
90	389,678	29.67%	1.97%	181	405,897	30.91%	3.46%	272	360,236	27.43%	1.67%
91	399,869	30.45%	8.46%	182	352,575	26.85%	5.81%	273	404,182	30.78%	1.87%

542 Note: Sample ID: sample number; SNP num: the number of SNP detected in the corresponding sample; Integrity:
543 the integrity of the SNP detected in the sample; Heter ratio: the heterozygosity rate of the SNP in the sample.

Population Structure