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ORIGINAL ARTICLE

Genetic diversity and population structure of pear (*Pyrus* spp.) collections revealed by a set of core genome-wide SSR markers

Qingwen Liu[1] · Yue Song[1] · Lun Liu[1] · Mingyue Zhang[1] · Jiangmei Sun[1] · Shaoling Zhang[1] · Jun Wu[1]

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Abstract Pear is one of the most important temperate fruits, with high genetic diversity, but controversial classification for some genotypes or species. Our study evaluates the polymorphism of 385 pear resources belonging to five cultivated species or interspecies of *Pyrus*, based on a set of 134 core simple sequence repeat (SSR) markers. A total of 690 variant alleles were detected, from 2 to 12 per locus, with an average of 5.45, as well as 30 rare alleles. The clustering relationship divided the pear genotypes into three groups, with the primary division between occidental and oriental pears, revealing separate evolution processes, followed by division of *Pyrus ussuriensis*, *Pyrus pyrifolia*, and *Pyrus bretschneideri*. Population structure analysis with *K* values of 2 to 8 reflected a clear genetic composition within different genotypes, supporting *Pyrus sinkiangensis* as a hybrid of oriental and occidental pears and *P. pyrifolia* and *P. bretschneideri* sharing a common ancestor. However, the division of genetic components also revealed separate evolution at the different geographic and environmental conditions of South China and North China. The varieties "Pingguoli" and "Chaoxianyangli," which currently have controversial classification, were classified into *P. bretschneideri* and *Pyrus communis*, respectively. A core collection of 88 accessions was chosen, covering all of the rare alleles and 95.54 % of all alleles. The high-quality and comprehensive evaluation of a wide range of pear cultivars by core SSR markers covering the whole genome demonstrated their excellent application for the study of genetic diversity, genetic relationships, and building a core collection for pear.

Keywords Pear (*Pyrus* spp.) · Core SSR markers · Genetic diversity · Genetic structure · Core collection

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✉ Jun Wu
wujun@njau.edu.cn

[1] Center of Pear Engineering Technology Research, State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China

Introduction

Pear (*Pyrus* spp.) is one of the most important temperate fruit trees, with the third largest growing area worldwide, after apple and grape, and has a long cultivation history (Oliveira et al. 1999). Pear belongs to the genus *Pyrus*, in the family Rosaceae, and originates from China, with three known secondary centers of origin: the Chinese center, the Central Asian center, and the Near Eastern center (Vavilov 1951). According to its origination and distribution, pear germplasm has mainly been divided into two categories, known as occidental pears (European pear) and oriental pears (Asian pear) (Bailey 1919). Occidental pears include more than 20 populations, mainly distributed in Europe, North Africa, Asia Minor, Iran, Central Asia, and Afghanistan. Oriental pears contain 13 populations with about a thousand cultivars (Rubtsov 1944). The division of pear into populations has been studied over years by many researchers but remains ambiguous, with at least 22 widely recognized primary species (Challice and Westwood 1973). However, the main cultivated species of pear are commonly divided into 5 populations: *Pyrus ussuriensis*, *Pyrus pyrifolia*, *Pyrus bretschneideri*, *Pyrus communis*, and *Pyrus sinkiangensis* (Pu 1988). With long-term natural selection and genetic flow, modern cross-breeding between different species, and self-incompatibility, many cultivars show highly complex genetic backgrounds, undoubtedly making it very difficult to classify pear resources.

Pear classification has been studied since the 1920s, when the methods mostly relied on were geographic distribution and pear morphological taxonomy (Bailey 1919; Rehder 1940). Results showed great differences between occidental and oriental pears. More accurate methods arose such as palynology and chemical components in the 1970s (Westwood and Challice 1978; Challice and Westwood 1973) and enzymatic analysis from the 1980s to the 1990s (Lin and Shen 1983), but still, all these methods are easily affected by the environment, especially when using morphology characteristics to study the diversity of pear. Additionally, with the increase in new cultivars and quantities of pears, those methods could not meet the requirement of identifying the diversity of cultivars with similar morphological traits. With the rapid development of science and technology, molecular markers were soon being used for many of species, including pear. The development of molecular techniques has allowed ready access to the information provided by DNA, which is stable regardless of environment, tissue, and growth stage. There are many types of molecular markers, such as AFLP (Teng et al. 2001), RFLP (Kim et al. 2002; Castillo et al. 2001; Takasaki et al. 2004), RAPD (Chen et al. 2005; Ahmed et al. 2012), ISSR (Monte-Corvo et al. 2000), IRAP (Sun et al. 2015), SRAP (Xu et al. 2011), and simple sequence repeat (SSR) (Fan et al. 2013; Song et al. 2014) which provide a good choice for genetic diversity analysis and resource classification. With the excellent characteristics of polymorphism, reproducibility, and codominant nature, the diversity of SSR markers are better for genetic diversity analysis, molecular marker development, marker-assisted selection (MAS), fingerprinting, map construction, and comparative studies in several plant families, including Poaceae (Huang et al. 2012), Moraceae (Saleh 2013), Rosaceae (Pluess and Stöcklin 2004), Brassicaceae (Chen et al. 2005), and pear. For example, six SSR loci were used to evaluate the diversity of 98 pear cultivars native to East Asia and occidental pears generally had low affinities to Asian pears (Bao et al. 2007). One hundred thirty-four SSR markers with high polymorphism were developed to study the genetic variability and relationships of 99 *P. pyrifolia* cultivars, and it has been shown that pears from Yangtze River Basin and Japan had a close relationship (Song et al. 2014). A high-density linkage map was firstly constructed by 98 SSR markers and other markers with a total of 32 potential QTLs for 11 traits identified and positioned on the genetic map (Wu et al. 2014). A high-density genetic linkage map consisting of 734 loci distributed along all 17 linkage groups was constructed, with a total length of 1661.4 cM and an average marker interval of 2.26 cM with 894 SSRs covering the whole genome of pear (Chen et al. 2014). Sixty-seven SSR markers selected from the pear genome with good transferability were applied to construct comparative mapping between seven other Rosaceae species (Fan et al. 2013). Recently, the genome data for many species has been released and the markers selected from genome-wide data can provide favorable access for evaluating genetic diversity; for example, after the release of apple genome, researchers such as Khan et al. (2012) and Costa et al. (2010) developed a number of SNP markers. The genome data for pear "Dangshansuli" (*P. bretschneideri*) was released in 2012 by our lab, providing an invaluable new resource for developing new SSR markers covering the whole genome. The SSR markers developed based on genome-wide SSR loci would be a favorable method to evaluate the diversity of pear germplasm and identify the relationships of pear.

Recently, genetic structure has been increasingly concerned on germplasm collection, protection, and utilization in some species, such as wheat (Hao et al. 2011), rice (Zhang et al. 2011), grape (Emanuelli et al. 2013), banana (de Jesus et al. 2013), rye (Bolibok-Brągoszewska et al. 2014), soybean (Dong et al. 2014), etc. Estimating population structure is important for avoiding false genetic trends and identifying good alleles and for identifying cultivars with specific or minor alleles that will be important for molecular breeding programs. However, as far as we know, not enough information on the population structure of pear collections is available or has been assessed with a large and comprehensive set of SSR markers, besides the smaller populations used by Song et al. (2014) with 99 *P. pyrifolia* and by Rana et al. (2015) with 48 pear accessions belonging to six species.

Therefore, despite the abundant research on pear germplasm by SSR markers, most relied on a small number of markers with few cultivars and were non-representative for all species, the genetic structure of many pears remained unknown. In this study, we used a set of 134 pairs of genome-wide core SSR markers covering all 17 chromosomes of pear to study 385 genotypes covering 5 populations and spread over all production regions, which contained 127 *P. pyrifolia*, 90 *P. bretschneideri*, 57 *P. ussuriensis*, 8 *P. sinkiangensis*, 61 *P. communis*, and 42 interspecific hybridization cultivars, in order to evaluate the diversity of pear germplasm, reveal the population structure, and construct a preliminary core collection. The results will be useful for making rational and scientific conservation and management strategies for pear germplasm.

Materials and methods

Plant materials

A total of 385 accessions, collected from Chinese National Pear Germplasm Repository in Wuhan, Jiangpu, a pear resource depository in Nanjing Agricultural University, were used in this study, including 127 *P. pyrifolia* (sand pear), 90 *P. bretschneideri* (white pear), 57 *P. ussuriensis*, 8 *P. sinkiangensis*, 61 *P. communis*, and 42 interspecific

hybridization cultivars (Table 1). These cultivars were chosen based on their economic value and geographical distribution. Young leaves of different accessions were collected in spring 2013.

DNA extraction and quality determination

DNA extraction from the young leaves was conducted using the improved CTAB method (Yan et al. 2008). The quality of DNA was tested on 0.8 % agarose gels (Invitrogen, China) and then stored in a freezer at −80 °C until use in subsequent experiments. NanoDrop 2000 (Thermo Scientific, USA) was used to detect the concentration, and then all DNA was diluted to 30–50 ng/μL for PCR amplification.

SSR markers and PCR amplification

Of the 134 pairs of SSR markers, 81 are from pear genome (http://peargenome.njau.edu.cn; Fan et al. 2013; Song et al. 2014; Chen et al. 2014), 46 are from apple genome, and 7 are from pear derived from expressed sequence tag (EST) (Yamamoto et al. 2002; Song et al. 2014). The marker selection steps were according to the article of Chen et al. (2014) and Song et al. (2014).

PCR reactions were carried out in a 10 μL volume containing 30 ng genomic DNA template, 1 μL of 10× PCR buffer (Mg^{2+} contained), 0.1 μL each of forward and reverse primer (10 pmol/L), 0.25 μL of 10 Mm dNTP, and 0.1 μL of 5 U/μL Taq polymerase (Takara Biotechnology Company, Dalian, China) with the protocol described by Zhang et al. (2014). PCR products (10 μL) were mixed with 2.0 μL formamide loading buffer (98 % formamide, 10 mM EDTA, 0.25 % bromophenol blue, 0.25 % xylene cyanol, pH 8.0) and 1.3 μL of each mixture (each PCR product (10 μL) mixed with formamide loading buffer (2.0 μL)), and two molecular size markers of 100 and 20 bp DNA ladders were loaded onto an 8 % non-denaturing polyacrylamide gel in 1× TBE buffer (Tris-borate, EDTA, pH 8.0) and then run at 200 V for 2–2.5 h and visualized using the silver staining protocol described by Bassam et al. (1991).

Data collection

The 100 and 20 bp DNA ladders (Dye Plus, DNA MW Standard Marker; Takara Biotechnology Co., Ltd., Dalian) were used as standard sizes to measure allele sizes, as SSR allelic composition was determined as codominant markers, and only clear and distinct bands were used for further analysis. Different putative alleles

Table 1 Three hundred eighty-five pear cultivars studied in this research

No.	Cultivars	Species	Origin
1	3000lundali	Pb	Jilin
2	Baipisu	Pb	Anhui
3	Baiqiaoli	Pb	Liaoning
4	Bayuexue	Pb	Jiangxi
5	Beifeng	Pb	Neimenggu
6	Bingxianlaoyisheng	Pb	Henan
7	Boli	Pb	Hebei
8	Chili	Pb	Shandong
9	Chonghuadali	Pb	Sichuan
10	Chongyanghong	Pb	Shaanxi
11	Da'aoao	Pb	Shandong
12	Dacili	Pb	Jilin
13	Da'enli	Pb	Shandong
14	Daguochuanli	Pb	Sichuan
15	Dahuanghua	Pb	Yunnan
16	Dalijitui	Pb	Unknown
17	Dangshansuli	Pb	Anhui
18	Datouli	Pb	Liaoning
19	Dianli No. 1	Pb	Yunnan
20	Dongguoli	Pb	Gansu
21	Dongmali	Pb	Shandong
22	Eli	Pb	Hebei
23	Enli	Pb	Shandong
24	Fengxianjitui	Pb	Shaanxi
25	Fojianxi	Pb	Hebei
26	Ganzi	Pb	Unknown
27	Guihuali	Pb	Guangxi
28	Gunzili	Pb	Jilin
29	Haichengci	Pb	Liaoning
30	Hanlu	Pb	Liaoning
31	Hansu	Pb	Liaoning
32	Hebeili	Pb	Hebei
33	Hongguochuanli	Pb	Sichuan
34	Hongxiangmi	Pb	Henan
35	Hongxiao	Pb	Henan
36	Hongzhimuyang	Pb	Hebei
37	Huangcuili	Pb	Shandong
38	Huangxianchangba	Pb	Shandong
39	Huangxiang	Pb	Anhui
40	Jinchuanxueli	Pb	Sichuan
41	Jingbaili	Pb	Beijing
42	Jinhua No. 1	Pb	Sichuan
43	Jinhua4hao	Pb	Sichuan
44	Jinli	Pb	Shanxi
45	Jinqiuli	Pb	Hunan
46	Jinzhui	Pb	Hebei
47	Lijiangbaili	Pb	Yunnan
48	Lijiangxiangli	Pb	Yunnan

Table 1 (continued)

No.	Cultivars	Species	Origin
49	Lixiandongguoli	Pb	Unknown
50	Mali	Pb	Hebei
51	Maogongli	Pb	Sichuan
52	Maotouli	Pb	Jilin
53	Mianbaoli	Pb	Gansu
54	Mianli	Pb	Unknown
55	Mili	Pb	Hebei
56	Mingzhu	Pb	Liaoning
57	Pingguoli	Pb	Jilin
58	Qiubaili	Pb	Liaoning
59	Qiupingguo	Pb	Liaoning
60	Qixiadaxiangshui	Pb	Shandong
61	Qixiaxiaoxiangshuei	Pb	Shandong
62	Qiyuehongxiangsu	Pb	Unknown
63	Qiyuesu	Pb	Henan
64	Ruanzhiqing	Pb	Jiangsu
65	Shageda	Pb	Gansu
66	Shuidonggua	Pb	Anhui
67	Sumei	Pb	Qinghai
68	Wowoli	Pb	Gansu
69	Xiali	Pb	Shanxi
70	Xiaobaili	Pb	Liaoning
71	Xiaobaixiao	Pb	Liaoning
72	Xiaoguochuanli	Pb	Sichuan
73	Xiaoyifu	Pb	Henan
74	Xinpingli	Pb	Liaoning
75	Xixiantianli	Pb	Unknown
76	Xuefang	Pb	Zhejiang
77	Xuehuali	Pb	Hebei
78	Yali	Pb	Hebei
79	Youli	Pb	Shanxi
80	Youyanci	Pb	Jilin
81	Yuluxiang	Pb	Shanxi
82	Yunnanhongxiangsu	Pb	Yunnan
83	Yunnanmali	Pb	Yunnan
84	Zaobaili	Pb	Liaoning
85	Zaoli No. 18	Pb	Jilin
86	Zaomeisu	Pb	Henan
87	Zaosumi	Pb	Shaanxi
88	Zaoxiangcui	Pb	Shaanxi
89	Zhengzili	Pb	Henan
90	Zisu	Pb	Anhui
91	Anngurem	Pc	America
92	Avocado	Pc	Italy
93	Bartlett	Pc	England
94	Becurre Giffard	Pc	France
95	Bo10	Pc	Poland
96	Bo12	Pc	Unknown
97	Bo19	Pc	Unknown

Table 1 (continued)

No.	Cultivars	Species	Origin
98	Bosc	Pc	America
99	Bunte Julibirne	Pc	England
100	Butirra Rosata Morettini	Pc	Italy
101	Cascade	Pc	America
102	Chaoxianyangli	Pc	North Korea
103	Charles Ernest	Pc	France
104	Clapp's Favorite	Pc	America
105	Clapp's Liebling	Pc	German
106	Docteur Jules Guyot	Pc	France
107	Condo	Pc	Holland
108	Conference	Pc	England
109	Coscia	Pc	Italy
110	Cure	Pc	France
111	Cure15	Pc	France
112	D'Anjou	Pc	France
113	Dekora	Pc	France
114	Dell avzzana	Pc	France
115	Dilibairui	Pc	Unknown
116	Doyenne du Comice	Pc	America
117	Dr. Jules Guyot	Pc	France
118	Etrusca	Pc	Italy
119	Etruska	Pc	Italy
120	Fanshan	Pc	Sichuan
121	Feilaiyin	Pc	Unknown
122	Flemish Beauty	Pc	Belgium
123	Hardy	Pc	France
124	Kujieli	Pc	Unknown
125	La France	Pc	France
126	Le Conte	Pc	Italy
127	Le Lectier	Pc	France
128	Louise	Pc	France
129	Mishirazi	Pc	Japan
130	Olia	Pc	Russia
131	P. salicifolia pall	Pc	Unknown
132	Packham's Triumph	Pc	England
133	PALL	Pc	Unknown
134	Radana	Pc	Poland
135	Red Bartlett	Pc	America
136	Red Conference	Pc	France
137	Red D'Anjou	Pc	England
138	Early red Doyenne du Comice	Pc	America
139	Red Hardy	Pc	France
140	Rocha	Pc	Portugal
141	Shengma	Pc	Unknown
142	Stakrimson	Pc	America
143	Summer Bloodbirn	Pc	German
144	Taxiamute	Pc	Unknown
145	Tema	Pc	Russia
146	Toska	Pc	Italy

Table 1 (continued)

No.	Cultivars	Species	Origin
147	Wuyuexian	Pc	Unknown
148	Yourika	Pc	France
149	Yubilen Dar	Pc	Bulgaria
150	Abate Fetel	Pc	France
151	Alexand Douillard	Pc	France
152	Aikansui	Pp	Japan
153	Akaho	Pp	Japan
154	Akibae	Pp	Japan
155	Annong1hao	Pp	Hubei
156	Annong2hao	Pp	Hubei
157	Aokusankichi	Pp	Japan
158	Atago	Pp	Japan
159	Baozhuli	Pp	Yunnan
160	Cangwudashali	Pp	Guangxi
161	Cangxiliuyuexue	Pp	Sichuan
162	Cangxixueli	Pp	Sichuan
163	Cangxiyou	Pp	Sichuan
164	Chikusui	Pp	Japan
165	Choju	Pp	Japan
166	Chubixiang	Pp	Hubei
167	Chuwhangbae	Pp	Korea
168	Chuxialv	Pp	Zhejiang
169	Cuifeng	Pp	Unknown
170	Cuiguan	Pp	Zhejiang
171	Cuiyu	Pp	Zhejiang
172	Daguohuanghua	Pp	Jiangsu
173	Daqing	Pp	Sichuan
174	Deshengxiang	Pp	Sichuan
175	Ejima	Pp	Japan
176	Fengyue	Pp	Japan
177	Ganquan	Pp	Unknown
178	Gaoyaodanshuili	Pp	Yunnan
179	Gion	Pp	Japan
180	Gold-Nijisseik	Pp	Japan
181	Guali	Pp	Zhejiang
182	Guiguan	Pp	Zhejiang
183	Haidongli	Pp	Yunnan
184	Hakko	Pp	Japan
185	Hanfeng	Pp	Unknown
186	Hangqing	Pp	Zhejiang
187	Hengxianlingshanli	Pp	Guangxi
188	Hokushin	Pp	Japan
189	Hongfenli	Pp	Guizhou
190	Housui	Pp	Japan
191	Huahongli	Pp	Unknown
192	Huali No. 1	Pp	Hubei
193	Huangpieli	Pp	Unknown
194	Huiyangsuanli	Pp	Sichuan
195	Huobali	Pp	Yunnan

Table 1 (continued)

No.	Cultivars	Species	Origin
196	Imamttraaki	Pp	Japan
197	Ishiiwase	Pp	Japan
198	Jindiaozi	Pp	Sichuan
199	Jinshui No. 1	Pp	Hubei
200	Jinshui No. 3	Pp	Hubei
201	Jinshuiqiu	Pp	Hunan
202	Jinshuisu	Pp	Hubei
203	Josengwhangkeum	Pp	Korea
204	Juli	Pp	Unknown
205	Kefali	Pp	Hubei
206	Kikusui	Pp	Japan
207	Kisui	Pp	Japan
208	Kotobukishinsui	Pp	Japan
209	Kousui	Pp	Japan
210	Kouzou	Pp	Japan
211	Lijiangmianli	Pp	Yunnan
212	Lijiangzhima	Pp	Hubei
213	Linxiaxiangshuili	Pp	Neimenggu
214	Lvbaoshi	Pp	Unknown
215	Lvxin	Pp	Zhejiang
216	Lvyun	Pp	Zhejiang
217	Mandingxue	Pp	Fujian
218	Manpungbae	Pp	Korea
219	Meigetsu	Pp	Japan
220	Miduxiaohongli	Pp	Yunnan
221	Miduxiaomianli	Pp	Yunnan
222	Mixue	Pp	Unknown
223	Mugua	Pp	Sichuan
224	Nanpingli	Pp	Liaoning
225	Nasui	Pp	Japan
226	Niitaka	Pp	Japan
227	Nijisseiki	Pp	Japan
228	Okan	Pp	Japan
229	Okusankichi	Pp	Japan
230	Osa-Nijisseiki	Pp	Japan
231	Puchengxue	Pp	Fujian
232	Pugua	Pp	Zhejiang
233	Qinghuali	Pp	Unknown
234	Qingkui	Pp	Zhejiang
235	Qingyun	Pp	Zhejiang
236	Sanmenjiangshali	Pp	Hubei
237	Shanzhali	Pp	Unknown
238	Shinkou	Pp	Japan
239	Shinsetsu	Pp	Japan
240	Shinseiki	Pp	Japan
241	Shounan	Pp	Japan
242	Shuijing	Pp	Korea
243	Sichuanhanyuan	Pp	Sichuan
244	Suanhuangli	Pp	Sichuan

Table 1 (continued)

No.	Cultivars	Species	Origin
245	Sunhwang	Pp	Korea
246	Taihaku	Pp	Japan
247	Wakahikar	Pp	Japan
248	Wanmi	Pp	Yunnan
249	Waseaka	Pp	Japan
250	Weiningsuanhuangli	Pp	Guizhou
251	Weiningxiangmianli	Pp	Guizhou
252	Whangkeumbae	Pp	Korea
253	Whasan	Pp	Korea
254	Wonhwang	Pp	Korea
255	Xiangmali	Pp	Unknown
256	Xinfeng	Pp	Jiangsu
257	Xinhang	Pp	Zhejiang
258	Xinjiuli	Pp	Guangxi
259	Xinpuxueli	Pp	Yunnan
260	Xinxiangli	Pp	Unknown
261	Xinxue	Pp	Taiwan
262	Xiuyu	Pp	Japan
263	Xuefeng	Pp	Unknown
264	Xueshan No. 1	Pp	Yunnan
265	Xuexin	Pp	Zhejiang
266	Xueying	Pp	Zhejiang
267	Yangchengli	Pp	Hubei
268	Yanshanhuangpixiao	Pp	Yunnan
269	Yaqing	Pp	Zhejiang
270	Yunhong No. 1	Pp	Unknown
271	Yushui	Pp	Hubei
272	Zaocui	Pp	Zhejiang
273	Zaohuangjin	Pp	Korea
274	Zaosuxiang	Pp	Hubei
275	Zaoxiang No. 2	Pp	Liaoning
276	Zaoxiangsu	Pp	Unknown
277	Zhaojiaoli	Pp	Sichuan
278	Zhaotongxiaohuangli	Pp	Yunnan
279	Changbazi	Ps	Jilin
280	Guidechangba	Ps	Qinghai
281	Huachangba	Ps	Lanzhou
282	Kuerlexiangli	Ps	Xinjiang
283	Lanzhouchangba	Ps	Gansu
284	Linxiagadiaodan	Ps	Gansu
285	Zhangyechangba	Ps	Gansu
286	Shayidongheisuanli	Ps	Unknown
287	Aishanli	Pu	Heilongjiang
288	Anli	Pu	Liaoning
289	Balixiang	Pu	Liaoning
290	Chaoyangchuan	Pu	Jilin
291	Dadaxiangshuili	Pu	Liaoning
292	Dali	Pu	Jilin
293	Dananguo	Pu	Liaoning

Table 1 (continued)

No.	Cultivars	Species	Origin
294	Daxiangshui	Pu	Liaoning
295	Dongmili	Pu	Heilongjiang
296	Dongxiangli	Pu	Liaoning
297	Dongxiangshui	Pu	Liaoning
298	Fuanjianba	Pu	Gansu
299	Fuxiangli	Pu	Liaoning
300	Ganguhongxia	Pu	Gansu
301	Guanhongxiao	Pu	Liaoning
302	Hei520	Pu	Heilongjiang
303	Heiheshanli	Pu	Jilin
304	Honghuagai	Pu	Liaoning
305	Hongqibaitangxingli	Pu	Jilin
306	Houguoyuan No. 11	Pu	Jilin
307	Hufu	Pu	Jilin
308	Humengxiushuixiang	Pu	Jilin
309	Jianbali	Pu	Liaoning
310	Kaiyuanwuqibai	Pu	Liaoning
311	Liaoyangdaxiangshui	Pu	Liaoning
312	Lintaobairuan'er	Pu	Gansu
313	Longxiangli	Pu	Liaoning
314	Maidili	Pu	Unknown
315	Maili	Pu	Jilin
316	Manyuanxiang	Pu	Liaoning
317	Maqiuzi	Pu	Jilin
318	Matihuang	Pu	Jilin
319	Naiyetian	Pu	Liaoning
320	Nanguoli	Pu	Liaoning
321	Pingdingxiang	Pu	Jilin
322	Qiuzili	Pu	Liaoning
323	Reli	Pu	Jilin
324	Sha01	Pu	Liaoning
325	Shanli No. 1	Pu	Jilin
326	Shanshiji	Pu	Jilin
327	Suanchengtuo	Pu	Liaoning
328	Suanguoli	Pu	Liaoning
329	Suanli	Pu	Liaoning
330	Suanliguozi	Pu	Liaoning
331	Tangli	Pu	Jilin
332	Tiantanggengzi	Pu	Liaoning
333	Tianxiadiyixing	Pu	Liaoning
334	Wuxiangli	Pu	Liaoning
335	Xiangshui	Pu	Liaoning
336	Xiaohebai	Pu	Jilin
337	Xiaoshanli	Pu	Jilin
338	Xiaoxiangshui	Pu	Liaoning
339	Xiehuatian	Pu	Liaoning
340	Yanbiandaxiangshui	Pu	Jilin
341	Yaoli	Pu	Jilin
342	Zaohuangli	Pu	Neimenggu

Table 1 (continued)

No.	Cultivars	Species	Origin
343	Zhenhongxiao	Pu	Jilin
344	Hanxiang	Pi	Jilin
345	Jinxiang	Pi	Liaoning
346	Cuilv	Pi	Zhejiang
347	Eli No. 2	Pi	Hubei
348	Eli No. 1	Pi	Hubei
349	Ganlizao8	Pi	Gansu
350	Ningmenghuang	Pi	Liaoning
351	Hanhong	Pi	Jilin
352	Jinxingli	Pi	Henan
353	Hongxiangsu	Pi	Henan
354	Hongjinqiu	Pi	Heilongjiang
355	Huajin	Pi	Liaoning
356	Huangguan	Pi	Hebei
357	Huanghua	Pi	Zhejiang
358	Huasu	Pi	Liaoning
359	Jumi	Pi	Liaoning
360	Jingfeng	Pi	Liaoning
361	Jinmili	Pi	Shanxi
362	Wujiuxiang	Pi	Liaoning
363	Hongtaiyang	Pi	Henan
364	Hanyu	Pi	Shanxi
365	Hongxiu No. 3	Pi	Xinjiang
366	Mantianhong	Pi	Henan
367	Meirensu	Pi	Henan
368	Xiangyanghong	Pi	Henan
369	Pingboxiang	Pi	Jilin
370	Pingxiangli	Pi	Jilin
371	Qinfeng	Pi	Shaanxi
372	Qingxiang	Pi	Zhejiang
373	Qinsu	Pi	Shaanxi
374	Shuofeng	Pi	Shanxi
375	Bayuehong	Pi	Shaanxi
376	Xingyeli	Pi	Jilin
377	Xizilv	Pi	Zhejiang
378	Zaoguan	Pi	Hebei
379	Zaojinsu	Pi	Liaoning
380	Zaokui	Pi	Hebei
381	Zheli	Pi	Jilin
382	Xinli No. 4	Pi	Xinjiang
383	Zhong'ai No. 2	Pi	Liaoning
384	Zaosu	Pi	Liaoning
385	Zhongli No. 1	Pi	Henan

Pb P. bretschneideri, *Pc P. communis*, *Pp P. pyrifolia*, *Pu P. ussuriensis*, *Ps P. sinkiangensis*, *Pi Pyrus* of interspecies

were represented by different letters in alphabetical order according to size.

Polymorphism and population structure analysis

To evaluate the genetic diversity of the cultivars, observed number of alleles (N_a), effective number (N_e), expected heterozygosity (H_e), observed heterozygosity (H_o), Shannon information index (I), and Wright's fixation index (F_{is}) were calculated with PopGene software (version 2.0) (Yeh et al. 1997) and pairwise differentiation among subpopulations (F_{ST}) was calculated with Weir and Cockerham estimator (Antao et al. 2008) (Table 2).

The MEGA4.0 (Tamura et al. 2007) program was used to evaluate genetic relationships based on information from all polymorphic markers. The model-based Bayesian clustering method STRUCTURE 2.3 software (Pritchard et al. 2000) was used to identify the number of populations, capturing the major structure in data and for construction of a population structure. The parameters burn-in period and Markov chain Monte Carlo (MCMC) were set as 10,000 and 100,000, respectively. The average value of ln likelihood when K changed from 2 to 8 was calculated according to their genetic similarity, with each K value run at least ten times (Song et al. 2014), while genetic relationships between genotypes were analyzed by principal coordinate analysis (PCA) with NTSYSpc2.11 software (Nirgude et al. 2014) to determine the optimal number of genotypes in our study.

Construction of core collection

A core collection is based on the least number of accessions representing the most genetic diversity within the population. PowerCore v. 1.0 (Kim et al. 2007) was used to construct a core collection. In this study, two methods of random and nonrandom modes were chosen to analyze the genetic polymorphism of 385 genotypes. Both modes were used to construct the core collection, and then the better one was selected by comparing the preservation of alleles, particularly the rare alleles (Table 3). Jaccard's coefficient value was used to visualize the relationships between core collection and original germplasm with the NTSYSpc program (version 2.10s) (Rohlf 1998), and the results calculated using the variance-covariance matrix of allele frequencies. The software SPSS 18.0 was used to calculate the parameters with the t value.

Results

High polymorphism of 385 pear accessions revealed by SSR markers

A set of 134 core SSR markers evenly distributed in 17 chromosomes was used to identify 385 pear accessions. All of the SSR markers produced clear bands and effectively analyzed the polymorphism of pear germplasm. In this study, 690

Table 2 Diversity statistics for 134 SSR loci studied in 385 pear cultivars

Locus	LG	Allele size range (bp)	*Na*	*I*	*He*	*Ho*	*Fis*	F_{ST}
17000000	LG15	100–220	6	1.6475	0.8065	0.7798	0.3892	0.0987
1D7	LG13	290–300	3	0.9276	0.8508	0.5449	0.8507	0.2431
AF057134	LG10	200–240	5	1.7181	0.8416	0.8064	0.4695	0.1233
AU223486	LG13	240–290	5	1.0204	0.5979	0.5978	0.0377	0.1058
au301431	LG16	160–240	5	1.7773	0.6915	0.6914	0.0377	0.1195
CH01D09	LG12	130–190	8	1.7297	0.6528	0.6525	0.0775	0.0519
CH01H01	LG17	100–140	4	1.3317	0.6599	0.652	0.2694	0.1791
CH02H11A	LG4	110–170	12	1.8594	0.8013	0.8008	0.0988	0.0144
CH02D12	LG11	170–200	6	1.6102	0.6845	0.6806	0.2176	0.106
CH02E12	LG15	190–210	7	1.6959	0.6974	0.686	0.3157	0.107
CH03D08	LG14	130–160	4	1.58	0.7891	0.789	0.0447	0.1672
CH03E03	LG3	100–200	5	1.5562	0.6875	0.6787	0.2789	0.1351
CH03g12	LG3	160–210	5	1.3607	0.7355	0.7243	0.3118	0.1297
CH04H02	LG11	160–250	5	1.2876	0.6494	0.6432	0.242	0.1224
CH05A04	LG16	150–200	6	1.6334	0.7646	0.7645	0.0397	0.1091
CH05F04	LG13	160–180	7	1.7133	0.7752	0.7738	0.1586	0.0827
CN544851	LG4	130–180	4	1.4855	0.6462	0.6425	0.216	0.2074
CN862645	LG11	135–170	6	1.4761	0.7465	0.7441	0.1949	0.119
CN863717	LG2	180–260	6	1.7544	0.8229	0.8207	0.1922	0.1176
CN869104	LG1	120–300	5	1.1941	0.6444	0.6442	0.0732	0.1486
CN875141	LG2	190–270	6	1.3832	0.6979	0.6901	0.2657	0.0834
CN899844	LG6	140–200	6	1.5646	0.6488	0.6415	0.254	0.0987
CN904191	LG12	220–250	5	1.4398	0.7178	0.7177	0.0379	0.107
CN919375	LG12	140–200	6	1.7316	0.7129	0.7117	0.1506	0.0905
ctg1059711	LG6	180–250	5	1.5602	0.78	0.7793	0.1187	0.12
ctg1060101	LG2	100–190	5	1.5478	0.7806	0.7751	0.2382	0.1209
CTG1060251	LG17	140–260	8	1.7989	0.7688	0.7064	0.4889	0.0966
CTG1060382	LG13	200–240	4	1.1692	0.5699	0.5687	0.1516	0.175
CTG1062183	LG10	170–230	5	1.457	0.7558	0.727	0.4134	0.1486
CTG1062302	LG9	250–270	4	1.1958	0.5805	0.5435	0.4758	0.2027
CTG1062559	LG5	250–320	5	1.5701	0.7857	0.7842	0.1661	0.1649
CTG1063619	LG5	190–280	5	1.5614	0.7873	0.7803	0.2464	0.1228
CTG1064300	LG10	160–260	8	1.8517	0.8291	0.8265	0.1989	0.0477
CTG1064376	LG4	250–270	4	1.2219	0.6571	0.6554	0.1681	0.1573
CTG1064670	LG11	250–320	5	1.5397	0.7778	0.7701	0.2635	0.1408
CTG1064726	LG1	250–300	6	1.5025	0.7545	0.7506	0.217	0.073
CTG1065053	LG12	240–270	3	1.2495	0.694	0.6856	0.2758	0.154
CTG1065662	LG8	220–240	5	1.2849	0.6987	0.6887	0.2957	0.1672
CTG1069672	LG8	150–230	5	1.3844	0.6955	0.6904	0.2321	0.1209
CTG1070530	LG7	230–280	3	1.0692	0.6714	0.6492	0.38	0.2412
CTG1074717	LG17	240–260	6	1.4006	0.7299	0.7007	0.4356	0.173
EMPC11	LG11	130–190	5	1.5128	0.774	0.7605	0.3495	0.1039
HI04A05	LG9	190–230	6	1.5231	0.7218	0.7161	0.2407	0.1031
IPPN12	LG14	200–270	5	1.5395	0.7848	0.7704	0.3604	0.1167
MES136	LG14	140–170	5	1.321	0.6599	0.6504	0.2873	0.1605
MES138	LG3	190–220	5	1.557	0.7892	0.7785	0.3064	0.1587
NAUpy02E	LG2	200–260	7	1.6563	0.7905	0.7861	0.2286	0.1186
NAUpy03d	LG2	200–250	4	1.4889	0.7117	0.7095	0.1932	0.1722
NAUpy04h	LG2	130–180	7	1.7398	0.8016	0.7917	0.2953	0.0844

Table 2 (continued)

Locus	LG	Allele size range (bp)	*Na*	*I*	*He*	*Ho*	*Fis*	F_{ST}
NAUpy07h	LG17	120–160	6	1.6083	0.7904	0.7868	0.2143	0.1025
NAUpy07m	LG17	100–180	8	1.6943	0.7862	0.7837	0.1967	0.0626
NAUpy07s	LG17	120–160	3	1.1448	0.6395	0.6304	0.2838	0.2125
NAUpy08T	LG4	170–200	5	1.5327	0.7678	0.7676	0.0739	0.0986
NAUpy09T	LG11	160–220	6	1.4414	0.7004	0.6972	0.2099	0.0795
NAUpy10U	LG9	90–150	10	1.7031	0.7914	0.7813	0.3015	0.0467
NAUpy14R	LG6	100–150	7	1.5827	0.7699	0.7671	0.2035	0.0762
NAUpy16m	LG15	100–170	9	1.9683	0.8188	0.8177	0.1478	0.0621
NAUpy17h	LG16	130–160	5	1.4685	0.7422	0.7324	0.2876	0.1596
NAUpy17M	LG10	70–190	6	1.8273	0.7956	0.7936	0.1776	0.1047
NAUpy20b	LG2	190–200	2	0.9311	0.5571	0.555	0.1778	0.2589
NAUpy22D	LG6	140–170	5	1.3786	0.6506	0.6342	0.3792	0.1612
NAUpy22F	LG2	130–190	6	1.6698	0.7854	0.7847	0.1597	0.0945
NAUpy23f	LG15	130–230	4	0.8311	0.5532	0.5598	−0.2047	0.1785
NAUpy23M	LG2	130–180	7	1.7674	0.8454	0.8119	0.4614	0.0812
NAUpy24f	LG12	140–170	5	1.3895	0.7035	0.6918	0.3289	0.1432
NAUpy25n	LG17	120–190	5	1.6721	0.7912	0.7859	0.2361	0.1503
NAUpy26a	LG3	140–180	4	1.1728	0.6666	0.6665	0.0397	0.1812
NAUpy26K	LG3	100–130	4	1.3404	0.6726	0.6721	0.0953	0.1715
NAUpy26r	LG10	110–160	4	1.3808	0.7035	0.6979	0.2392	0.1734
NAUpy26s	LG5	180–200	5	1.4541	0.6832	0.671	0.3327	0.1615
NAUpy27D	LG14	230–280	5	1.2848	0.6771	0.67	0.2532	0.1539
NAUpy28i	LG5	120–150	5	1.4734	0.6843	0.6814	0.2075	0.2598
NAUpy28R	LG7	100–120	5	1.4642	0.7322	0.7312	0.1449	0.1649
NAUpy29X	LG12	130–170	6	1.7505	0.8194	0.8192	0.0832	0.1316
NAUpy30F	LG6	100–200	5	1.5403	0.7764	0.7708	0.2682	0.1499
NAUpy30H	LG15	130–180	6	1.2834	0.6506	0.6463	0.2255	0.1359
NAUpy34k	LG15	90–160	7	1.6458	0.743	0.7387	0.2455	0.0845
NAUpy34m	LG13	110–140	6	1.6535	0.78	0.7564	0.3869	0.1159
NAUpy35C	LG10	200–240	6	1.6687	0.792	0.7846	0.2601	0.1241
NAUpy36E	LG4	120–160	6	1.8681	0.861	0.832	0.4322	0.1195
NAUpy38K	LG3	100–130	5	1.5671	0.7919	0.7824	0.2973	0.1689
NAUpy45b	LG1	160–240	5	1.6404	0.7731	0.7724	0.1887	0.1574
NAUpy45d	LG1	105–150	7	1.8071	0.8139	0.8126	0.1517	0.0569
NAUpy46k	LG6	120–180	7	1.6568	0.7658	0.7604	0.2376	0.0713
NAUpy46N	LG11	70–180	5	1.3861	0.7361	0.7326	0.2128	0.1538
NAUpy47k	LG15	130–170	6	1.6158	0.7839	0.7803	0.2144	0.085
NAUpy47N	LG2	110–150	6	1.7769	0.8302	0.8292	0.1539	0.1095
NAUpy50h	LG1	100–260	7	1.6067	0.7401	0.7396	0.0963	0.0729
NAUpy51B	LG15	100–160	5	1.5678	0.7882	0.7822	0.2414	0.1641
NAUpy52a	LG8	130–160	5	1.2306	0.7059	0.6031	0.5102	0.1653
NAUpy52s	LG4	120–190	7	1.7428	0.7885	0.7865	0.1786	0.0802
NAUpy53F	LG11	100–180	6	1.5863	0.761	0.7494	0.3266	0.1162
NAUpy53k	LG11	100–190	5	1.4007	0.7091	0.7023	0.2451	0.1591
NAUpy54a	LG17	150–230	5	1.6172	0.7732	0.7714	0.1729	0.1619
NAUpy54b	LG13	190–220	5	1.5332	0.783	0.7673	0.3721	0.1589
NAUpy54f	LG15	150–270	7	1.5924	0.7451	0.7436	0.1673	0.0832
NAUpy55D	LG8	120–170	5	1.3585	0.7008	0.7006	0.0833	0.1543
NAUpy55E	LG3	180–230	4	1.5876	0.7925	0.7917	0.1216	0.2035

Table 2 (continued)

Locus	LG	Allele size range (bp)	*Na*	*I*	*He*	*Ho*	*Fis*	F_{ST}
NAUpy58b	LG7	230–260	4	1.278	0.7002	0.6986	0.1666	0.1895
NAUpy60e	LG11	90–140	4	1.2	0.748	0.643	0.5503	0.1875
NAUpy61N	LG4	210–260	4	1.0829	0.6425	0.6385	0.2224	0.1739
NAUpy63n	LG3	110–170	5	1.3276	0.7589	0.6588	0.4969	0.1612
NAUpy64M	LG2	100–130	5	1.5144	0.7568	0.7564	0.0798	0.1543
NAUpy65d	LG8	120–160	3	1.5403	0.7712	0.7706	0.1055	0.2812
NAUpy65U	LG12	90–150	4	1.0817	0.6122	0.6111	0.1498	0.1912
NAUpy68F	LG3	70–110	5	1.5123	0.7581	0.7562	0.1744	0.1614
NAUpy70f	LG7	130–260	7	1.7404	0.7821	0.7818	0.0757	0.1621
NAUpy71e	LG15	120–160	7	1.4408	0.7371	0.7363	0.1316	0.0895
NAUpy77s	LG13	100–170	5	1.693	0.7999	0.7995	0.0998	0.1691
NAUpy80c	LG15	160–240	6	1.7103	0.8073	0.8064	0.1331	0.1132
NAUpy80F	LG10	100–170	8	1.8045	0.8172	0.8165	0.1412	0.0599
NAUpy81c	LG9	170–290	9	1.8366	0.7705	0.77	0.0979	0.0758
NAUpy82d	LG2	210–260	5	1.5556	0.7785	0.7774	0.1478	0.1543
NAUpy82E	LG12	100–270	4	1.2344	0.6686	0.6637	0.2314	0.1993
NAUpy82r	LG6	130–290	5	1.4064	0.8307	0.5294	0.6618	0.1624
NAUpy83F	LG4	130–170	6	1.8201	0.8251	0.8236	0.1861	0.1013
NAUpy86b	LG5	260–280	4	1.4501	0.7269	0.7251	0.1825	0.1798
NAUpy87s	LG16	130–150	4	1.1317	0.4116	0.4103	0.1585	0.1883
NAUpy90d	LG17	100–170	4	1.4008	0.6982	0.6976	0.1173	0.1912
NAUpy90E	LG5	100–210	6	1.591	0.7545	0.7526	0.1735	0.1093
NAUpy91D	LG2	130–160	4	1.5411	0.7781	0.7707	0.2593	0.1887
NAUpy91H	LG8	240–280	5	1.5407	0.7718	0.7709	0.1533	0.1584
NAUpy91m	LG16	120–180	7	1.628	0.7881	0.7815	0.2436	0.1735
NAUpy91n	LG10	210–240	5	1.4203	0.6095	0.6081	0.1613	0.1491
NAUpy97a	LG16	260–300	5	1.6323	0.7683	0.7674	0.1622	0.1616
NAUpy97X	LG7	130–180	6	1.727	0.8139	0.8105	0.2112	0.1143
NAUpy98f	LG7	100–120	5	1.398	0.6959	0.6949	0.1551	0.1602
nb103A	LG10	80–280	4	1.4791	0.7098	0.7093	0.1973	0.1965
nb106A	LG9	150–180	4	1.4412	0.7196	0.7189	0.1887	0.1874
NH20A	LG5	130–190	7	1.7386	0.812	0.8098	0.1938	0.0792
NH26A	LG16	110–270	4	1.6836	0.7925	0.7903	0.1924	0.1795
NH35A	LG14	160–190	7	1.6803	0.8084	0.7771	0.4552	0.0806
NH36B	LG8	170–200	5	1.484	0.7429	0.7424	0.0989	0.1549
NH39A	LG10	120–150	6	1.6784	0.7961	0.7934	0.202	0.1011
Average			5.45	1.52	0.74	0.73	0.23	0.1368

Locus is the name of the primer. Size is the accession number. Allele size range (bp) was the allele size amplified by the markers, Na was the observed number of alleles, Ne was the effective number of alleles, I was the Shannon's Information index, Ho was the observed heterozygosity, and He was the expected heterozygosity, Fis was Wright's fixation index, FST was pair-wise differentiation among subpopulations

LG linkage group

alleles, ranged from 2 (NAUpy07s) to 12 (NAUpy81c), were produced by the core SSR markers, with an average of 5.45 alleles per marker. The allele frequency was different from each SSR locus, strongly supporting the evidence explaining the effective number of alleles from different loci. A total of 30 (4.7 % of all 629 alleles) rare alleles with a frequency of less than 5 % were identified, indicating that many rare alleles were present in these pear collections and that the accessions in our research represented high pear germplasm biodiversity. The Shannon information index (*I*) varied from 0.83 of NAUpy23f to 1.96 of NAUpy16m, with an average of 1.52; the observed heterozygosity (H_o) varied from 0.41 of

Table 3 Comparison of the statistics obtained from the original germplasm and random and nonrandom polymorphism analysis based on 134 core SSR markers

	Allele retention ratio (%)	Rare allele retention ratio (%)	*Na*	*He*	*Ho*
Original germplasm			5.15[a]	0.7[a]	0.73[a]
Random	95.58	100	5.23[a]	0.72[a]	0.74[a]
Nonrandom	86.23	80.46	4.76[b]	0.60[b]	0.62[b]

Through the *t* value test, the parameters of N_a, H_e, and H_o values of both original germplasm and random core collection show no significant difference but a significant difference exists between original germplasm and nonrandom core collection (significant at 0.05 probability)

[a, b] The significance value at 0.05 probability of each index between Original germplasm and Nonrandom core collection

NAUpy87s to 0.86 of NAUpy36E, with an average of 0.74; the expected heterozygosity (H_e) varied from 0.41 of NAUpy87s to 0.83 of NAUpy36E, with an average of 0.73; Wright's fixation index (F_{is}) varied from −0.20 of NAUpy23F to 0.85 of NAUpy60E, with an average of 0.23, implying that inbreeding increased among populations (shown in Table 2). A higher value of H_o than H_e observed in one locus (NAUpy23f) resulted in negative Wright's fixation index (F_{is}) values, indicating a slight excess of heterozygosity for this locus. The pairwise differentiation among subpopulations (F_{ST}) was estimated at each locus for all individuals, and the values varied from 0.0144 of CH02H11A to 0.2812 of NAUpy65d, with an average of 0.1368. The mean F_{ST} values of the four populations (*P. pyrifolia*, *P. bretschneideri*, *P. ussuriensis*, and *P. communis*) were 0.1440, 0.1438, 0.1363, and 0.1229, respectively, indicating a slight genetic variation between populations.

Cluster analysis revealed three distinct groups of 385 accessions

The dendrogram of 385 pear cultivars was constructed with the software MEGA4.0. In Fig. 2, all the cultivars were divided into two clusters, with occidental pear separated out from oriental pear. The oriental pears were further divided into two subclusters, with *P. ussuriensis* separated out from *P. pyrifolia* and *P. bretschneideri*.

Cluster I contained 69 cultivars including all the 61 *P. communis*, 2 *P. sinkiangensis* ("Shayidongheisuanli" and "Linxiagadiaodan"), and 6 other cultivars ("Hongtaiyang," "Zaosu," "Jinxiangli," "Wujiuxiang," "Bayuehong," and "Zaojinsu") which are hybrid cultivars with one parent or grandparent belonging to *P. communis*. In this cluster, the cultivars of *P. communis* from America and Europe showed a mixed genetic background with no distinct genetic distance and having a better gene flow. "Bartlett" and its bud mutation "Red Bartlett" were clustered together, but "Doyenne du Comice" and the deduced bud mutation variety named "Early red Doyenne du Comice" were distinct from each other. The Early red Doyenne du Comice was clustered in the group of "Clapp's Favorite," indicating a close relationship of the two cultivars. The ambiguous cultivar "Chaoxianyangli" was clustered in this cluster, confirming that the cultivar has a high genetic component from occidental pear.

Cluster II included 127 *P. pyrifolia*, 90 *P. bretschneideri*, 57 *P. ussuriensis*, 6 *P. sinkiangensis*, and 36 interspecific hybridization cultivars. This cluster was divided into two subclusters, I and II. Subcluster I contained 57 *P. ussuriensis* and 11 interspecific hybridization cultivars that were hybrids of *P. ussuriensis*. At the same time, *P. ussuriensis* from Northwest China was clustered together with cultivars from North China, indicating that genetic communication occurred between cultivars originating from areas near to each other. Subcluster II contained 90 *P. bretschneideri*, 127 *P. pyrifolia*, 6 *P. sinkiangensis*, and 25 interspecific hybridization cultivars, confirming the previous hypothesis that *P. pyrifolia* and *P. bretschneideri* originated from the same ancestor and gene flow has occurred. Six *P. sinkiangensis* varieties, "Zhangyechangba," "Guidechangba," "Changbazi," "Lanzhouchangba," "Huachangba," and "Kuerlexiangli," were clustered with some varieties of *P. bretschneideri* and *P. pyrifolia*. In subcluster II, "Huanghua" and its bud mutation "Daguohuanghua" were clustered together but "Nanguoli" and "Dananguo" were separated. "Mili" and "Qiubaili" were clustered with "Pingguoli," while "Huangguan" and "Zaokui" were clustered with "Xuehuali," indicating that the filial generation has a close relationship with their parents. The controversial cultivar Pingguoli, which has been considered as either *P. pyrifolia* or *P. bretschneideri* in previous studies, was clustered together with most cultivars of *P. bretschneideri*, indicating a closer genetic relationship. At the same time, varieties from Korea such as "Whangkeumbae" and "Wonhwang" and varieties from Japan such as "Kousui" and "Choju" were all clustered together with Chinese *P. pyrifolia*, which is distributed in the Yangtze River Basin, indicating that *P. pyrifolia* originating from Japan and Korea might share an ancestor with the cultivars from the Yangtze River Basin.

The population structure declared the clear relationship of accessions and species of pear

The codominant nature of the core SSR markers was used to analyze the structure of the populations using the model-based Bayesian clustering method. In order to analyze the population genetic structure of the pear germplasm, STRUCTURE (v. 2.3.4) was used with different *K* values from 2 to 8 to reveal the genetic components of the population (Fig. 1).

When *K*=2, all accessions were divided into two populations, Pop1 and Pop2, with Pop1 containing all occidental pears and a few interspecific hybridization cultivars, all sharing the genetic background of occidental pear, and with Pop2 containing all cultivars of oriental pear. This structure indicated a great difference in genetic components between occidental and oriental pears. It was interesting to find out that occidental pears were all clustered together in Pop1 with no distinctive variation with increasing *K* values; however, the oriental pear was divided into different subpopulations. With the increase of *K* to 3, the accessions were divided into three populations, with oriental pear divided into two populations (Pop2 and Pop3). *P. ussuriensis* and closely related species were separated out from *P. pyrifolia* and *P. bretschneideri*, while Pop2 contained most of *P. pyrifolia* and *P. bretschneideri*, along with the cultivars of *P. sinkiangensis* and some interspecific hybrid cultivars. Pop3 contained all *P. ussuriensis* cultivars and some cultivars of *P. pyrifolia*, *P. bretschneideri*, and some interspecific hybrids that have a close relationship with *P. ussuriensis*. In this grouping, gene introgression in the three populations was clearly revealed and showed a great amount of gene flow within and among populations, especially the oriental pear species. When *K*=4, all accessions were divided into four parts, which largely clustered with accessions within species range, except for *P. sinkiangensis*. In this population structure, *P. pyrifolia* was separated out from *P. bretschneideri*. Pop2 contained most of *P. pyrifolia* and several varieties of *P. bretschneideri*, such as "Daguochuanli," "Yunnanhongxiangsu," and "Jinchuanxueli," originating from South China, and the varieties from Japan and Korea. Pop3 contained most *P. bretschneideri* and several *P. pyrifolia* such as "Nanpingli" and "Linxiaxiangshuili," which mostly originated from North China. Numerous varieties in these two populations share similar genetic components, with some cultivars intersecting each other because of their distribution areas. The information indicated that over evolution, great gene flow occurred between *P. pyrifolia* and *P. bretschneideri*. Particularly, varieties belonging to *P. pyrifolia* but cultivated in North China communicated with *P. bretschneideri* and varieties belonging to *P. bretschneideri* but cultivated in South China communicated a lot with *P. pyrifolia*. These results confirmed that varieties distributed in the same area, with the same climate and soil nutrient status, might gradually evolve into having similar gene components, either due to natural hybridization or human intervention. When *K*=5, *P. pyrifolia* was divided into two populations (Pop2 and Pop3), mainly reflecting their geographic distribution. Pop2

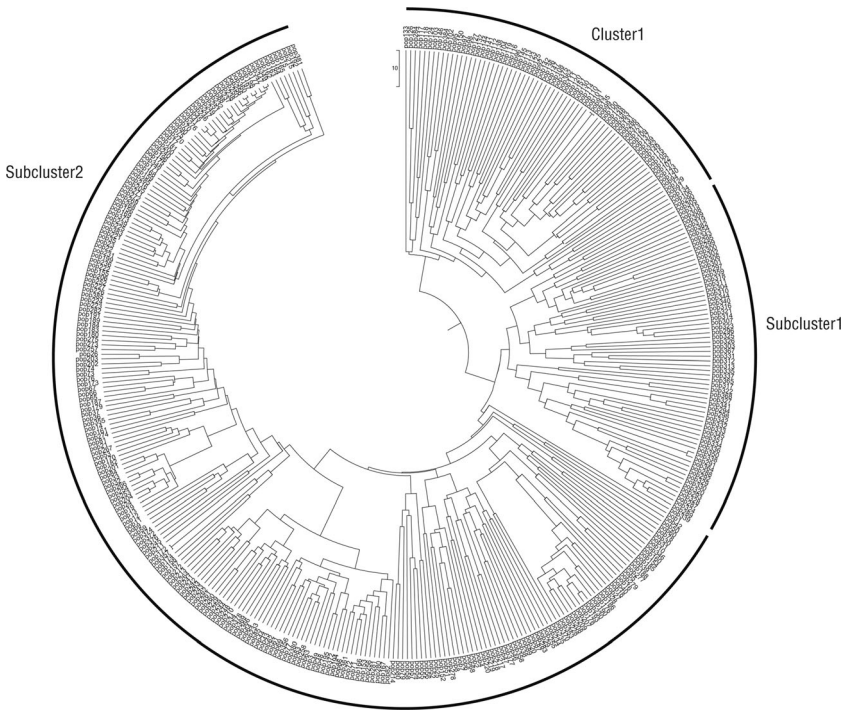

Fig. 1 All the 61 cultivars in *P. communis*, 2 cultivars of "Shayidongheisuanli" and "Linxiagadiaodan" in *P. sinkiangensis*, and 6 hybrid cultivars with 1 parent or grandparent cultivar belong to *P. communis*. Subcluster 1 refers to 57 cultivars in *P. ussuriensis* and 11 interspecific hybridization cultivars. Subcluster 2 refers to 90 cultivars in *P. bretschneideri*, 127 cultivars in *P. pyrifolia*, 6 cultivars in *P. sinkiangensis*, and 25 interspecific hybridization cultivars

contained the cultivars of *P. pyrifolia* mostly originating from South China, while Pop3 contained the other cultivars of *P. pyrifolia*, mostly originating from the middle and lower reaches of the Changjiang River, Japan, and Korea, and their hybrid cultivars. The result indicated that geographic barriers might restrict gene flow of different cultivars, even if the cultivars came from the same species. When K=6, *P. bretschneideri* was divided into two populations (Pop5 and Pop6) based on different areas of origination. Pop5 contained *P. bretschneideri* mainly originating from Northeast China, such as "Yali," "Beifeng," and Mili, while Pop6 contained *P. bretschneideri* mostly originating from the Yellow River Basin and the west, such as "Mianbaoli," Zaosu, "Chili," and "Jinli." Most cultivars in these two populations had a complex genetic relationship with the other species, indicating an extensive gene exchange between different cultivars. When K=7, the cultivars from North China were clustered together in a single population including some *P. bretschneideri*, *P. pyrifolia*, and interspecies hybrids, indicating great gene flow between different cultivars from the same area, and the environment and geographic traits are important factors in structure components. When K=8, the cultivars originating from Japan and Korea were separated out from other *P. pyrifolia* from China, the differences among them also gave us a better understanding that geographical location would be an important factor affecting gene flow between different cultivars.

We saw that, at K=2, two cultivars of *P. sinkiangensis* (Shayidongheisuanli and Linxiagadiaodan) were clustered with occidental pears and the other six (Lanzhouchangba, Zhangyechangba, Guidechangba, Changbazi, Huachangba, and Kuerlexiangli) with oriental pears and that the genetic background of all eight cultivars contained gene structures of both occidental and oriental pears, suggesting a close relationship of *P. sinkiangensis* with occidental as well as oriental pears. When K was 4, the cultivars Zhangyechangba and Kuerlexiangli were clustered together with most of *P. bretschneideri*; the cultivars Lanzhouchangba, Guidechangba, and Huachangba were clustered with *P. pyrifolia*; the cultivar Changbazi was clustered with *P. ussuriensis*; and the genetic components of each variety shared all four genetic backgrounds. Then, with the increase of K to 5, the cultivars Lanzhouchangba, Guidechangba, and Huachangba were all clustered together with the *P. pyrifolia*, originating from South China, and with the increase of K to 6, Zhangyechangba and Kuerlexiangli were clustered together with most cultivars of *P. bretschneideri* from North China. Genetic components of the eight varieties of *P. sinkiangensis* displayed in our study showed complicated genetic components including *P. communis*, *P. bretschneideri*, *P. ussuriensis*, and also *P. pyrifolia*, revealing that it had experienced much gene flow with occidental and oriental pears.

In genetic population structure, Pingguoli was clustered together with oriental pear in the first group of K=2 and then, with the increase of K values, it was clustered with *P. bretschneideri*. The genetic components were similar with most *P. bretschneideri* and also contained a genetic background from *P. pyrifolia*, but little from *P. ussuriensis*, suggesting a close relationship of Pingguoli and *P. bretschneideri*, and also, a lot of gene communication has occurred in it. Chaoxianyangli was clustered together with most occidental pears and contained a genetic background from *P. pyrifolia* and *P. bretschneideri*, indicating a closer relationship between Chaoxianyangli and *P. communis*.

According to the results of our structure analysis, with the increase of K value, the pear accessions clustered with stratification and each increased population gave a better interpretation of the evolution of pear. However, with higher K values, these accessions were clustered into distinct clusters, better reflecting the genetic exchange between pears from different varieties. As with the increase of K value, the cultivars were divided into smaller populations based on geographical distribution, which was strong evidence for identifying the relationships between different pear cultivars and better analyzing the gene flow and evolution of pear germplasm.

Principal component analysis revealed a similar pattern with population structure and cluster analysis

Principal component analysis (PCA) was also performed to analyze the genetic relationship and population structure. Most accessions were divided into three groups along the circles (Fig. 3), although there were some redundant or overlapping cultivars. The most aggregates among the three populations were *P. pyrifolia*, *P. bretschneideri*, *P. ussuriensis*, and *P. communis*, in accordance with STRUCTURE and cluster analysis. P1 (the yellow circle) contained most *P. bretschneideri* and *P. pyrifolia*, P2 (the red circle) contained most *P. ussuriensis*, and P3 (the blue circle) contained most *P. communis*. There were also some varieties of genotypes scattering out of the circles, giving us a better understanding of the higher diversity of the pear genome and the close genetic relationship between each population.

Finally, we found that the phylogenetic tree based on MEGA, population structure analysis, and PCA all strongly supported that pear varieties have high diversity but genes have been communicated between different clusters. All the results from the above support that pear has three well-differentiated genetic populations.

Eighty-eight genotypes selected as the core collection of 385 pear accessions

The main purpose of constructing a core collection is to have a small quantity of accessions to represent the maximum genetic diversity and avoid repetitiveness. The random method

selected 88 accessions to represent all 385 pear collections and contained 10 *P. communis*, 30 *P. bretschneideri*, 30 *P. pyrifolia*, 1 *P. sinkiangensis*, 11 *P. ussuriensis*, and 6 interspecies. The alleles in those 88 accessions covered all rare alleles and 95.54 % of all alleles (Table 4). The nonrandom method was used to select 52 accessions, only including 6 *P. communis*, 14 *P. bretschneideri*, 20 *P. pyrifolia*, 6 *P. ussuriensis*, and 4 interspecies, covering 80.46 % of rare alleles and 86.23 % of all alleles; however, these cultivars did not comprehensively represent the alleles and rare alleles and did not give us an optimum result. A core collection of 88 cultivars representing all 385 pear collections covering all rare alleles was developed, and it has been indicated that the core collection can be used to identify the pear diversity (Table 5). Abundant genetic diversity was detected by 88 pear genotypes based on 134 SSR markers, with a total of 601 alleles detected ranging from 2 to 11 per locus. The average value of the Shannon information index (I) was 1.49, the average value of the observed heterozygosity (H_o) was 0.73, the average value of the expected heterozygosity (H_e) was 0.70, and the average value of Wright's fixation index (F_{is}) was 0.18. Then, the SPSS 18.0 software was used and the parameters of I, H_o, H_e, and F_{is} were detected by t value, showing that no significant difference occurred between the 385 pear germplasm and 88 core collections. The results also indicated that the core collection of 88 can represent the 95.54 % of the total diversity of all pear germplasm in our study.

Identification of the molecular fingerprinting among different accessions

Besides the polymorphism of different accessions revealed by SSR markers, the specific or rare alleles identified could be used as markers to distinguish the different genotypes. The fingerprinting of 385 pear accessions was constructed based on the fragment diversity developed from 134 SSR markers. The result from 30 randomly selected pear accessions by eight SSR markers (Table 2) revealed that a combination of at least two markers could be used to identify the genotypes. For example, the marker 'NH039a" could distinguish the genotypes "Baipisu, " "Hanfu," Chili, "Daaoao," "Youli," "Fanshan," Hongtaiyang, "Hongxiao," "Jinhua No. 4," Jinli, "Jinxing," "Jinzhui," "Qixiadaxiangshui," and "Xiaobaixiao"; combined with the marker "NAUpy20b," all other cultivars could be separated. Using the same method, a total of 23 selected markers (NAUpy97a, CH01D09, CH03E03, CH05F04, NAUpy81c, NAUpy45b, NAUpy58b, NAUpy27D, NAUpy45d, CH05A04, CN863717, CN875141, NAUpy53k, NAUpy16m, NAUpy17M, HI04A05, NAUpy25n, NAUpy47N, NAUpy63n, NAUpy52s, NAUpy26s, NAUpy10U, and NH39A) could clearly distinguish all 385 pear accessions. The fingerprinting of 385 pear cultivars with different SSR markers can act as

Table 4 The varieties obtained from random and nonrandom core collection

No.	Name	Species
Random		
1	Chili	Pb
2	Beifeng	Pb
3	Binxianlaoyisheng	Pb
4	Daenli	Pb
5	Dalijitui	Pb
6	Dangshansuli	Pb
7	Dianli1hao	Pb
8	Dongmianli	Pb
9	Eli	Pb
10	Ganzi	Pb
11	Guihuali	Pb
12	Hanlu	Pb
13	Hongguochuanli	Pb
14	Hongxiangmi	Pb
15	Jinmili	Pb
16	Lijiangxiangli	Pb
17	Lixiandongguoli	Pb
18	Mali	Pb
19	Mantianhong	Pb
20	Mili	Pb
21	Qiyuehomgxiangli	Pb
22	Qiyuesu	Pb
23	Ruanzhiqing	Pb
24	Sumei	Pb
25	Xuehuali	Pb
26	Yunnanhongxiangsu	Pb
27	Zaobaili	Pb
28	Zaomeisu	Pb
29	Zaosumi	Pb
30	Zheli	Pb
31	Saint Maria	Pc
32	Anguliemu	Pc
33	Colomerina tardiva	Pc
34	Packham's	Pc
35	Red Bartlett	Pc
36	Early red Doyenne du Comice	Pc
37	Red Hardy	Pc
38	SummerBloadBir	Pc
39	Toska	Pc
40	Yourika	Pc
41	Chongyanghong	Pi
42	Cuifeng	Pi
43	Hongsucui	Pi
44	Jinxiang	Pi
45	Meirensu	Pi
46	Ningmenghuang	Pi
47	Akibae	Pp

Table 4 (continued)

No.	Name	Species
48	Baozhuli	Pp
49	Cangxixueli	Pp
50	Chuwhangbae	Pp
51	Cuiguan	Pp
52	Daguohuanghua	Pp
53	Deshengxiang	Pp
54	Ejima	Pp
55	Guiguan	Pp
56	Hangqing	Pp
57	Hongfenli	Pp
58	Hongxiu No. 3	Pp
59	Huanghua	Pp
60	Huiyangsuanli	Pp
61	Kefali	Pp
62	Kousui	Pp
63	Miduxiaomianli	Pp
64	Mugua	Pp
65	Nijisseiki	Pp
66	Okusankichi	Pp
67	Pingguoli	Pp
68	Qingkui	Pp
69	Suanhuangli	Pp
70	Wakahikar	Pp
71	Whangkeumbae	Pp
72	Xuexin	Pp
73	Xueying	Pp
74	Yaqing	Pp
75	Yushui	Pp
76	Zaocui	Pp
77	Guidechangba	Ps
78	Donghuagai	Pu
79	Dongmili	Pu
80	Fuanjianba	Pu
81	Longxiangli	Pu
82	Maidili	Pu
83	Matihuang	Pu
84	Suanguoli	Pu
85	Suanliguozi	Pu
86	Tangli	Pu
87	Zaohuangli	Pu
88	Zhenhongxiao	Pu
Nonrandom		
1	Baipisu	Pb
2	Dongli	Pb
3	Guihuali	Pb
4	Hanlu	Pb
5	Hansu	Pb
6	Mantianhong	Pb
7	Qiyuesu	Pb
8	Sumei	Pb
9	Zheli	Pb
10	Daenli	Pb
11	Lixiandongguoli	Pb
12	Qiyuehomgxiangli	Pb
13	Jinmili	Pb
14	Ganzi	Pb
15	SummerBloadBire	Pc
16	Toska	Pc
17	Feilaiyin	Pc
18	Cascade	Pc
19	Red Hardy	Pc
20	Early red Doyenne du Comice	Pc
21	Cuifeng	Pi
22	Jinxiang	Pi
23	Ningmenghuang	Pi
24	Hongsucui	Pi
25	Kefali	Pp
26	Hakko	Pp
27	Pingboxiang	Pp
28	Kouzou	Pp
29	Xueshan No. 1	Pp
30	Zaoxiang No. 2	Pp
31	Xinhang	Pp
32	Yaqing	Pp
33	Okusankichi	Pp
34	Pingguoli	Pp
35	Suanhuangli	Pp
36	Zaocui	Pp
37	Baozhuli	Pp
38	Suanhuangli	Pp
39	Deshengxiang	Pp
40	Whangkeumbae	Pp
41	Chuwhangbae	Pp
42	Miduxiaomianli	Pp
43	Huanghua	Pp
44	Ejima	Pp
45	Hongqibaitangxingli	Pu
46	Matihuang	Pu
47	Zhenhongxiao	Pu
48	Maidili	Pu
49	Suanguoli	Pu
50	Donghuagai	Pu

Pb P. bretschneideri, *Pc P. communis*, *Pp P. pyrifolia*, *Pu P. ussuriensis*, *Ps P. sinkiangensis*

individual allele patterns and has potential application for further cultivar identification.

Table 5 The fingerprints of 30 varieties identified by 8 SSR markers

Code	Cultivars	NH039A					NAUpy45d						CH03g12			NAUpy87s			NAUpy28i				NAUpy08t				NAUpy46k				HI04A05				
		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	z	ab	ac	ad	ae	af	ag	ah	ai
1	Baipisu	0	1	1	1	1	0	1	0	1	1	1	0	1	0	0	1	1	1	1	0	0	1	1	1	1	0	1	0	0	1	0	0	1	0
2	Baiqiaoli	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
3	Beifeng	1	1	1	0	0	0	1	0	1	1	1	0	1	0	0	0	1	0	1	0	0	0	0	1	0	0	1	1	1	0	1	0	0	1
4	Hanlu	0	1	1	0	1	1	0	0	0	0	0	1	0	0	0	0	1	1	1	1	0	0	0	0	1	0	1	1	0	0	0	1	1	1
5	Chili	0	0	1	0	1	0	0	0	1	1	1	1	0	1	0	1	1	0	1	1	0	1	0	1	0	1	1	0	0	1	1	1	0	0
6	Daaoao	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	1	0	1	0	0	1	0	0
7	Dahuangli	1	1	0	0	0	1	1	1	0	1	0	0	0	0	1	1	1	0	1	0	1	0	0	1	0	0	1	0	1	1	0	1	0	1
8	Dangshansuli	1	1	1	0	0	0	0	1	0	1	1	0	1	0	0	1	0	0	1	0	0	0	1	0	1	0	0	0	0	0	1	0	1	0
9	Youli	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0
10	Eli	1	0	1	1	1	0	0	0	1	1	1	1	1	1	0	1	1	1	1	0	1	0	1	0	1	0	1	1	0	0	1	1	1	0
11	Enli	1	1	1	0	0	0	1	0	1	1	1	0	1	0	0	0	1	1	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0
12	Fanshan	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0
13	Hongtaiyang	1	0	0	1	1	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	1	1	0	1	0	1	0	0	0
14	Hongxiangmi	1	1	0	0	0	1	1	1	0	0	0	0	0	0	1	1	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
15	Hongxiao	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1	1	0
16	Huachangba	1	0	1	1	1	1	1	1	0	1	0	0	0	0	0	0	1	1	0	1	0	1	0	1	1	1	1	0	0	0	1	1	0	0
17	Huangguan	1	0	1	0	0	0	0	0	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	0	1	0	1	1	0	0	1	1	1	0
18	Jinhua No. 4	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1
19	Jinli	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0	0
20	Jinxing	0	1	0	1	0	1	1	1	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	1	1	0	1	0
21	Jinzhui	0	1	1	0	0	0	1	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	1	1	0	0	1	1	0	1	0
22	Jingfeng	0	0	1	1	1	0	0	0	1	1	0	1	1	1	0	1	0	1	0	1	0	1	1	1	1	1	0	0	0	0	0	1	1	1

Letters represent different band sizes, which represent the different fingerprints: a=150 bp, b=160 bp, c=165 bp, d=170 bp, e=180 bp, f=80 bp, g=100 bp, h=120 bp, i=130 bp, j=140 bp, k=160 bp, l=160 bp, m=190 bp, n=200 bp, o=130 bp, p=160 bp, q=180 bp, r=150 bp, s=155 bp, t=160 bp, u=170 bp, v=160 bp, w=180 bp, x=200 bp, y=210 bp, z=90 bp, ab=110 bp, ac=150 bp, ad=170 bp, ae=110 bp, af=130 bp, ag=150 bp, ah=170 bp, and ai=190 bp; 1=the presence of a band and 0=the absence of a band

Discussion

Polymorphism and heterozygosity of pear resources evaluated by a set of SSR markers

In previous studies, SSR markers used to evaluate pear resources were mainly developed from the apple genome and the available markers developed from pear were still few and usually only scattered in a few linkage groups (Bao et al. 2007; Fernandez-Fernandez et al. 2006; Terakami et al. 2009). However, with the increasing amount of new cultivars and some controversial pear varieties, these limited SSR markers did not meet the requirement of evaluating the diversity of pear resources. In this study, 134 high polymorphic core SSR markers distributed over the whole pear genome were chosen to analyze the diversity of 385 pear varieties and evaluate the polymorphism of SSR markers.

Marker polymorphism is an important factor for evaluating germplasm diversity, and high marker polymorphism is important. In previous studies of pear, 277 alleles were detected on 7 pear genotypes by 67 SSR primer pairs with an average of 4.13 alleles per locus (Fan et al. 2013) and 311 alleles were detected by 108 EST-SSR primers with dinucleotide and trinucleotide motifs, with an average of 2.88 and 2.86 alleles per locus, respectively (Zhang et al. 2014). Other studies found 65 putative alleles generated by 7 primer pairs with a mean of 9.2 (Yamamoto et al. 2002); 173 different alleles were detected based on 14 loci with an average of 12.4 alleles per locus (Zong et al. 2014), and 133 putative alleles were detected by 9 SSR markers with a mean of 14.8 per locus (Kimura et al. 2002). In our study, all 134 SSR markers amplified 629 alleles, with a mean of 5.45 per locus, and also detected 30 rare alleles.

There was a higher average value of the detected alleles than previous studies of pear, possibly because our core SSR markers could comprehensively detect the distinctive bands distributed in each variety and thus find out a lot of alleles. Of many markers used in our study, some only detected a small number of alleles, bringing the average down, so some other studies have detected more alleles. All the results indicate that a core set of SSR markers distributed genome-wide is reasonable and necessary in comprehensive evaluation of germplasm genetic diversity. In addition, pear self-incompatibility, prior long

cross-pollination history, and the different genotypes of pollination widespread among the pear cultivars caused high heterozygosity and the high heterozygosity was always measured by the observed heterozygosity (H_o) in the previous studies; for example, 142 apple accessions were studied with the value of H_o at 0.62 (Hokanson et al. 2001), 28 peach accessions were studied with the value of H_o at 0.28 (Sosinski et al. 2000), 106 pear accessions were evaluated by 9 SSR markers with the value of H_o at 0.6 (Cao et al. 2011), and 92 *P. bretschneideri* accessions were evaluated by 10 SSR markers derived from apple and pear with the average value of H_o at 0.61 (Tian et al. 2012), and in our study, there were 385 pear accessions with H_o of 0.72. The low value in peach might be because of self-pollination, while in apple, it might be because of the restricted materials or markers. Otherwise, the SSR markers derived from the pear genome could detect more polymorphic loci than the pear derived from apple and the amount of SSR markers would also be an important factor.

The inbreeding coefficient F_{is} ranged from −0.23 to 0.85, with an average of 0.23. The high F_{is} values greater than 0.5 were only detected in three loci, and the average F_{is} value was also quite low, which were similar with the results reported by Liu et al. (2012), suggesting no loss of heterozygosity and also proving the validity of our results. In addition, the same phenomenon was also detected in Tibetan poplar (Shen et al. 2014). However, in this study, the positive values of F_{is} for different alleles imply the increased inbreeding among populations, which was very surprising in pear, because it is self-incompatible and thus always experiences heterologous hybridization. Iketani et al. (2010) considered the increased inbreeding arisen from human activities, such as selection, transportation, and propagation. Liu et al. (2012) attributed the positive F_{is} values to the Wahlund effect resulting from the subpopulation structure or the existence of null alleles, which could increase the inbreeding index.

Generally, insect-pollinated and outcrossing species would have relatively low genetic differentiation, which supports the view that genetic diversity mainly exists within populations of outcrossed and widespread species (Hamrick et al. 1992; Liu et al. 2012). In our study, the similar genetic differentiation of *P. pyrifolia* and *P. bretschneideri* was in accordance with cluster analysis and PCA, and they were clustered together. However, the relatively low level of genetic differentiation of *P. communis* indicates that wide gene flow existed in the population, which forced it to become less differentiated than *P. pyrifolia* and *P. bretschneideri*. From PCA, we found distant relationships between *P. communis* and *P. pyrifolia* and *P. bretschneideri* populations, in accordance with the F_{ST} values.

Genetic relationships of 385 pears

Overall, pear is a complex population with no obvious genetic differentiation and great amount of gene flow between different species. Gene flow among accessions influences the capability of STRUCTURE to correctly evaluate the genetic diversity and components of germplasm (Hubisz et al. 2009). Also, pear varieties with uncertain relationships exist, as has been reported by a number of studies (Yamamoto et al. 2001; Teng et al. 2002; Bao et al. 2008; Erfani et al. 2012; Song et al. 2014). Understanding the population structure is essential for efficient germplasm classification, protection, and utilization. The software STRUCTURE might be the most popular method to detect the diversity of germplasm; furthermore, combined with cluster analysis and PCA, it is an effective method to analyze genetic relationships, population structure, and structure components. Previous studies have used these methods on germplasm of different species, such as poplar (Shen et al. 2014), grape (Emanuelli et al. 2013), rice (Zhang et al. 2011), soybean (Dong et al. 2014), apple (Hokanson et al. 2001), and smaller sets of pear (Song et al. 2014; Bao et al. 2008; Zhou et al. 2013).

Occidental pears displayed lower levels of diversity compared to Asian species and have been inferred to derive from oriental species based on geographical spread (Zheng et al. 2014). In our research based on MEGA, the first cluster divided all 385 pear accessions into two populations: oriental pear and occidental pear, indicating that occidental and oriental pears have evolved independently for a long time and a great difference exists between them. This result was consistent with Bailey (1919), Bao et al. (2008), Erfani et al. (2012), and Yue et al. (2014), which considered that the two categories of occidental and oriental pears have a little similarity in the pear germplasm, supporting their separate evolution. Meanwhile, occidental pears were all clustered together regardless of geographical distribution, consistent with the previous research of Liu et al. (2015), who evaluated 45 *P. communis* based on 134 SSR markers and found that pears from America and Europe were all clustered together. The possible reason might be that the occidental pear pollinated without restriction of geographic distribution through self-incompatibility, encouraging gene exchange. Meanwhile, with the increase of K values, the material of occidental pears preserved a smooth and steady variation and showed a relatively clear gene background, all of which demonstrate the limited gene background of occidental pears and great gene communication and recombination among them. Meanwhile, according to the simplex material components of occidental and oriental pears, we could conclude that with the evolution of pear germplasm, the two branches were geographically and ecologically isolated. The same conclusion was also reached for other species such as soybean. The genetic structure of 100 cultivars was constructed by 53 SSR markers based on the STRUCTURE method, revealing that soybean germplasm in each classified group showed great consistency with their origins, seed coat colors, and pedigrees (Dong et al. 2014).

In oriental populations, *P. ussuriensis* was separated out from *P. pyrifolia* and *P. bretschneideri*, indicating many natural barriers limiting gene flow between *P. ussuriensis* and other oriental pears. Then, when $K=4$, *P. pyrifolia* was separated out from *P. bretschneideri*. Previous studies found that *P. pyrifolia* and *P. bretschneideri* clustered intercross, supporting a common ancestor of these two species (Teng et al. 2002; Bao et al. 2008), and Teng et al. (2002) considered *P. bretschneideri* as a cultivated group or an ecotype of *P. pyrifolia*. However, the genetic division found in our study revealed that the two species separately evolved specific genetic components at different geographic and environmental conditions in South China and North China. At $K=8$, *P. pyrifolia* formed three populations with cultivars from Japan and Korea, cultivars originating from South China, and cultivars originating from North China. *P. bretschneideri* also formed three populations with cultivars from the Yellow River Basin and the western region, cultivars from North China, and cultivars originating from East China. All these indicate that geographical and ecological divisions affect gene communication between different varieties.

P. sinkiangensis was a controversial species, previous studies have evaluated that some cultivars have a close relationship with *P. communis* and some have a close relationship with oriental pear (Teng et al. 2001; Pan et al. 2001; Lu et al. 2011). In our research, two cultivars of *P. sinkiangensis* clustered with *P. communis* and the other six clustered with oriental pear (Fig. 2), suggesting a complex genetic background of this species. Alternatively, combined with population structure (Fig. 3), the steady variation of genetic materials identified that *P. sinkiangensis* might be a hybrid offspring of occidental and oriental pears.

Chaoxianyangli has been a controversial variety, but clustered with *P. communis* in our study, agreeing with Cao and Qu (2006). Combined with the structural component showing that the cultivar also carries the genetic material of oriental pears, we conclude that Chaoxianyangli may be a hybrid of occidental and oriental pears. Pingguoli is a unique and desirable variety, as it looks like an apple, with morphological features similar to *P. bretschneideri*, such as the color of fruit and fresh branches, while other features such as the shape of fruit and leaves are just like *P. pyrifolia*, making its classification contentious. Previous studies on the taxonomic status of Pingguoli classified it to *P. pyrifolia* (Pu and Wang 1963), but the reverse conclusion also exists, classifying Pingguoli with *P. bretschneideri* (Song et al. 2014; Qu et al. 2001). In our study, Pingguoli clustered closely together with "Xinpingli" and other *P. bretschneideri*, and combined with the structure component, we found that most genome components of Pingguoli were in accordance with white pear. From this, we inferred that Pingguoli might be a variety of *P. bretschneideri*. Most of the cultivars share complicated genetic components, and classification is difficult; these results may solve the mystery of attribution of some controversial varieties but may not finally classify them into different species. Further studies including sequencing technology would be an effective method for analyzing controversial varieties. Some bud mutation cultivars did not cluster with their original cultivars, such as Huanghua and Daguohuanghua as well as Bartlett and Red Bartlett, consistent with a previous study (Lu et al. 2011), which indicates the limitation of simplex SSR markers, as they cannot detect any loci with a somatic mutation. Lu et al. (2011) also concluded that the SSR markers have little capacity to detect bud mutations, and the further studies with SRPA (Sun et al. 2015), SNP, or other markers would be better for identifying bud mutations. The cultivar Early red Doyenne du Comice, with an appearance like "Starkrimson," a bud mutation of Clapp's Favorite, was originally recognized as the bud mutation of Doyenne du Comice in China but did not cluster with Doyenne du Comice but with Clapp's Favorite in our study, providing further evidence that the Early red Doyenne du Comice was not the bud mutation of Doyenne du Comice. The close relationship of Early red Doyenne du Comice and Clapp's Favorite supported that the Early red Doyenne du Comice is a false name for Starkrimson or another bud mutant of Clapp's Favorite.

A preliminary core collection of 385 pears

The essence of constructing a preliminary core collection is to use the minimum quantity of germplasm samples to represent the maximum genetic diversity of the species (Frankel 1984). Previous studies have confirmed that the number of alleles is a key factor for evaluating the genetic diversity, especially the rare alleles (Marshall and Brown 1975). A higher diversity of pear accessions would be more likely to capture the total genetic diversity with a small number of individuals, as was found for grape (Emanuelli et al. 2013). The 30 rare alleles retained in the core collection in our study indicated that our core collection is representative of most of the genetic polymorphism, especially as our accessions include all pear species, originating from China, Korea, Japan, Europe, and America. The core collection can help us efficiently select varieties with good properties, utilize and breed for further production, and then use an optimum method to select the representative varieties needed. In this study, two methods, random and nonrandom, were used for constructing a core collection, which were then compared for the ratio of covering rare alleles, covering all alleles, and polymorphism parameters. Finally, the random method was chosen to construct a core collection, although a previous study by Song et al. (2014) considered that the two methods were both appropriate for constructing a core collection and constructed two core collections of sand pear based on 99 cultivars of *P. pyrifolia*. Our larger core collection from the random method than nonrandom was in accordance with Song et al. (2014). The inconsistency between the two different methods might be ascribed to the different kinds and amounts of materials; with more

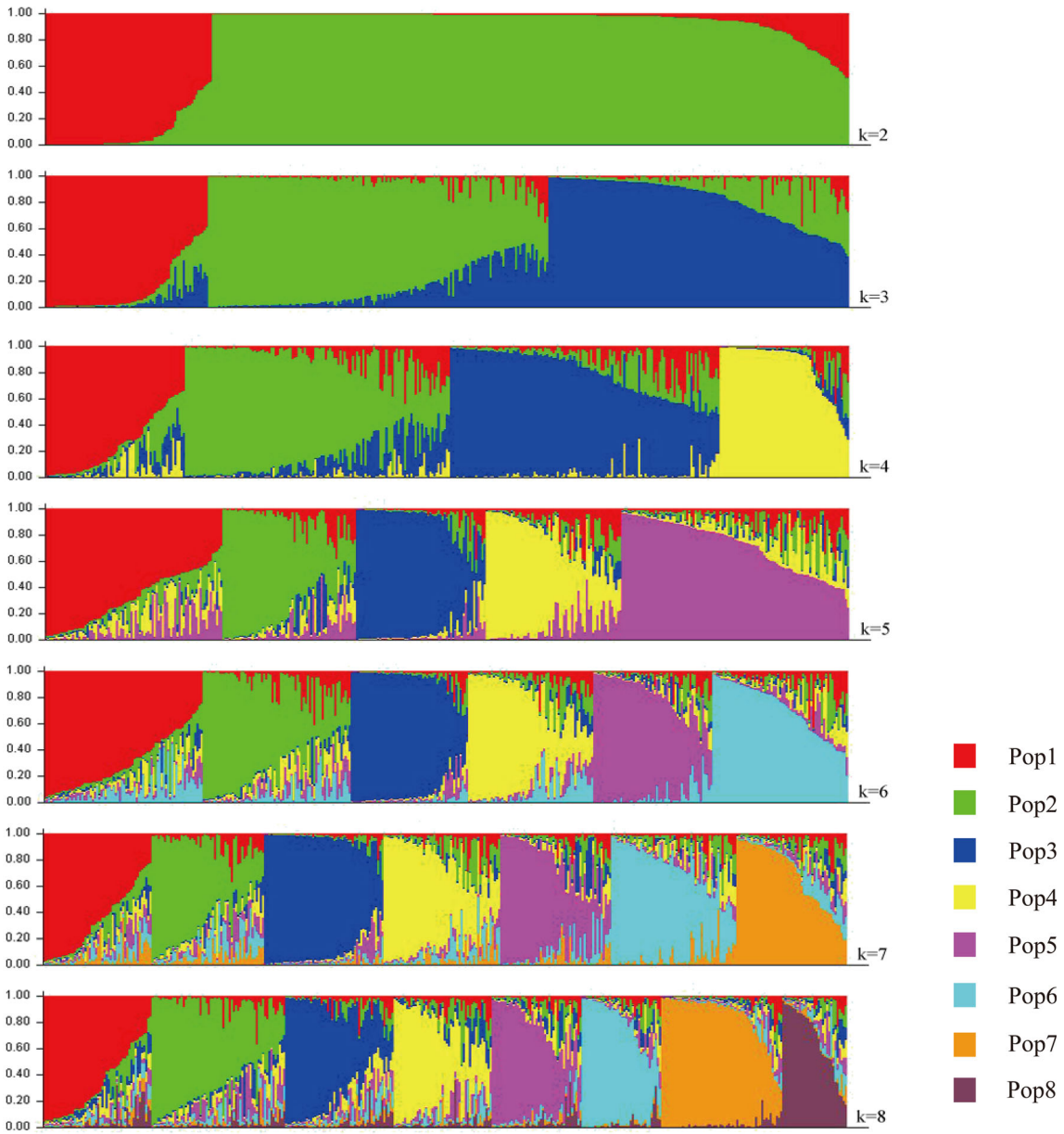

Fig. 2 Population structure of 385 accessions with *K* of 2 to 8. Each individual was shown as a *vertical line* divided into segments representing the estimated membership proportion. *Y-axis* refers to the proportion of genetic background, and *the height of each line with different colors* represents the probability of varieties belonging to different genetic backgrounds

materials and diversity, the comprehensive core collection should be constructed using the random method. The core collection of *P. pyrifolia* obtained in our study included 30 varieties, of which 6 originated from Japan, 2 originated from Korea, and 22 originated from China covering all of the production areas of sand pear, indicating the great genetic diversity of our collection. Comparing with the previous study of Song et al. (2014) who constructed a core collection of *P. pyrifolia* with 24 varieties using the nonrandom method and 32 varieties using the random method while the numbers of common varieties with our 30 varieties of core collection is 11 and 14, the reason might be that some different species studied in our study and their genetic diversity could be represented by other varieties. In the core collection, about 33 % (30 out of 90) of *P. bretschneideri* accessions and 24 % (30 out of 127) of *P. pyrifolia* accessions were in the core collection, larger than the *P. communis* of 16 % (10 out of 61), which revealed that the wide distribution of *P. bretschneideri* and *P. pyrifolia* determined their higher diversity than *P. communis*, which is distributed in a narrow area and had extensive genetic communication. Previous diversity studies of *P. bretschneideri* and *P. pyrifolia* have found that N_a mainly varied from 9 to 20 (Zhang et al. 2007; Iketani

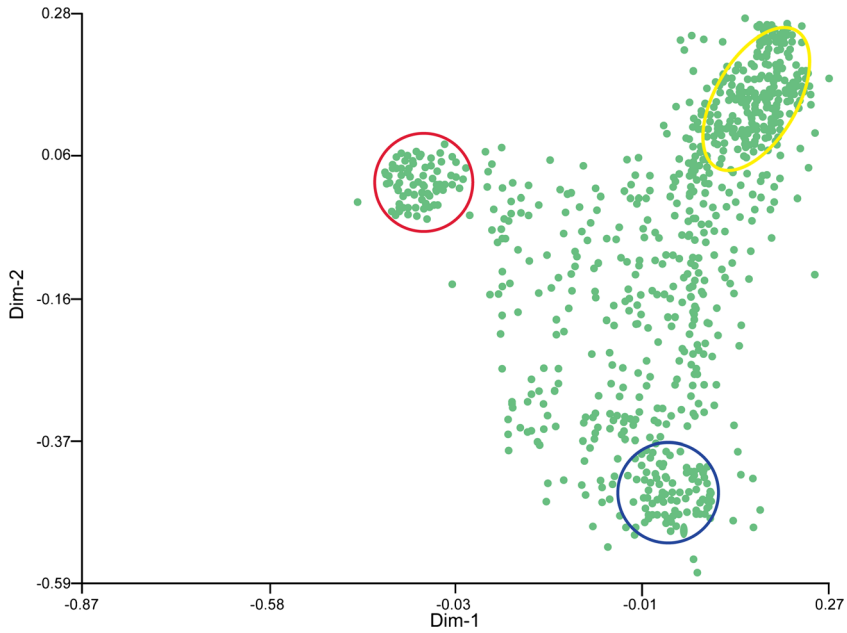

Fig. 3 Principal component plots for 385 pear accessions based on 134 core SSR markers. *Each integer point* represents one cultivar, and dense integer points are *circled*. The *yellow circle* refers to most cultivars from *P. pyrifolia* and *P. bretschneideri*, the *red circle* refers to most cultivars from *P. ussuriensis*, and the *blue circle* refers to most cultivars from *P. communis*. *X-axis* and *Y-axis* refer to the first and second principal components, respectively

et al. 2012) and the N_a values of *P. communis* mostly varied from 5 to 7 (Erfani et al. 2012; Liu et al. 2015), clearly confirming the higher diversity of *P. bretschneideri* and *P. pyrifolia*.

Conclusion

This study constructs both a phylogenetic tree and builds a population structure to analyze the population diversity and phylogenetic relationships among the extensive collections of *Pyrus* species, based on a genome-wide core set of SSR markers. The results revealed that occidental and oriental pears are clearly distinguishable and cultivars from Japan and Korea might share an ancestor with *P. pyrifolia*, originating from China. Meanwhile, *P. sinkiangensis* had a genetic background of oriental and occidental pears and was determined to be a hybrid of both. Genetic structures of *P. pyrifolia* and *P. bretschneideri* supported a common ancestor for these two species; however, the division based on the increasing *K* value also revealed a separate evolution at different geographic and environmental conditions in South China and North China. The bud mutation Dananguo, from Nanguoli, was identified, and Early red Doyenne du Comice was not the bud mutant of Doyenne du Comice. The population structure gave us a better understanding of the genetic relationships and composition within different pear genotypes; meanwhile, a core collection was chosen to represent the composite diversity of pear germplasm. These conclusions give us evidence for further study of controversial pear cultivars. In addition, our genome-wide core SSR markers displayed high polymorphism, which is valuable for molecular breeding, investigation of population genetic diversity, and evolutionary studies among pears. The genotypes used and information obtained in this study can provide a guide for further exploration of pear genetic diversity and population structure as well as genome organization and evolution.

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Compliance with ethical standards

Data archiving statement The authors declare that all the work described in this manuscript followed the standard Tree Genetics and Genomes policy. All the primers used were in accordance with the article of Song et al. (2014).

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