



## Estimates of selection in breeding of wheat based on phenotypes and microsatellite markers

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**Abstract:** Intense selection for desirable phenotypes within a breeding pool gives rise to the difference of genotypes in parental stocks. The objective of this study was to reveal the frequency and magnitude of selection in breeding of wheat (*Triticum aestivum* L.). Twenty-five cultivars, representing historically important cultivars and deriving from six representative crosses, were evaluated using 7 morphological traits in five environments and 379 SSR markers across the genome. These cultivars employed included four founder parents used widely in China. The result indicated that phenotypic characters of the progeny from a single cross were very various, and most of them approached their parents or had intermediate quantitative variables between their both parents. Nevertheless, transgressive inheritance was recorded for certain characters. Microsatellite data revealed that the remains of two parents among a set of sister cultivars were very diverse in all parts of the genome and of different chromosomes, which might derive from rigorous selection pressure due to different purposes. Among 25 cultivars derived from single crosses, 14 was significantly different from the expected parental contribution (0.5) based on SSR data. The comprehensive data indicated that genealogical relationships alone were not predictive of progeny performance for yield components and parental contribution. Utilizing molecular markers is necessary for diversity analysis.

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**Keywords:** Phenotype; microsatellite marker; parental inheritance; selection

### 1. Introduction

The monitoring of genetic variation in breeding programs is recommended to reveal changes in variability caused by genetic drift, inbreeding, or selection (Allendorf and Ryman 1987). Such information is also extremely useful for germplasm management, variety protection, as well as to using them as source of useful genes for other cultivated wheats (*Triticum aestivum* L.) (Engles et al. 2002).

Randomly derived lines from a single cross would be expected to receive half of their nuclear genetic information from each parent in the absence of selection according to Mendelian inheritance generally. Fähr et al. (1993) investigated the origin of chromosome segments of famous maize inbred (*Zea mays* L.) B 86 from its parental inbred B 52 and Oh 43 using RFLP and found that the proportion of its genome from two parents was almost equal as a whole, but varied significantly among different chromosomes. Bernardo et al. (1997), determining the extent of deviations from the expected parental contribution among F<sub>2</sub>- and BC<sub>1</sub>- derived maize inbreds by RFLP, found that more than 20% of all derived inbreds investigated deviated significantly from the expected values and the selection during backcrossing generally favored the recurrent parent

over the donor parent. Bernardo et al. (2000) also investigated the genetic composition of 10 public maize inbreds derived from three founder maize inbreds (B 37, B 73 and GE 440) based on pedigree, RFLP and SSR data. They found that the founder parent inbreds have presented various proportion of their genome to the progeny generally and there was a deviation between observed and expected parental contributions among maize inbreds. Terzi et al. (2007), evaluating six advanced breeding lines and three cultivars which were all obtained from an emmer wheat (*Triticum dicoccon* Schrank) × durum wheat (*Triticum durum* Desf.) cross by using pedigree selection, found that the genetic contribution of the two parents to all progenies showed diverse among different chromosomes on the basis of AFLP and SSR markers. Similar results were also observed in barley (*Hordeum vulgare* L.) (Sjakste et al. 2003) and soybean (*Glycine max* L. Merr.) (Lorenzen et al. 1995). However, the frequency and magnitude of genetic selection have not been thoroughly studied in bread wheat, especially the difference of selection existing among improved genotypes derived from elite parents.

In the present study, the genetic diversity and inheritance of microsatellite alleles in a set of

important varieties derived from six representative crosses in China were evaluated with their immediate parents using morphological traits and SSR markers across the genome. The main objective of this study was to give useful insight on the effects of selection in breeding programs.

## 2. Material and Methods

### 2.1 Materials

Twenty-five accessions representing historically important cultivars and developed from six representative crosses in breeding of China were assayed in this study. Of these cultivars, Bima 4, Orofen, Beijing 8 and Lovrin 10 designed as founder parents have played a particularly key role in the improvement of wheat. At the same time, Bima 4, Orofen and Beijing 8 have been widely grown commercially in main agro-ecological zones for many years in China, whose annual maximum acreage reached 667,000 hm<sup>2</sup>. Seeds of these genotypes were obtained from the National Crop Gene Bank, Chinese Academy of Agricultural Sciences (CAAS), Beijing.

### 2.2 Methods

#### 2.2.1 Morphological evaluation

All accessions were planted in a randomized block design with two or three replications in 2006 in each of Shaanxi, Shandong, Hebei, Sichuan and Jiangsu provinces in China. They represent four main agro-ecological zones of China: Northern Winter Wheat Region, Yellow and Huai River Facultative Winter Wheat Region, Middle and Low Yangtze Valley Winter Wheat Region, and Southwestern Winter Wheat Region. Each plot consisted of 90 plants that were grown in three rows 2 m long and spaced 30 cm apart. Crop management was carried out following the local practices. Seven morphological characters were scored on 10 plants from each plot. They were plant height, spike length, spikelet number, grains per spike, thousand-grain weight, spike number per plant and days to heading (days from sowing to the half heading). Means of each character were compared using Duncan's multiple range procedure ( $p < 0.05$ ) generated by SAS general linear model (GLM) procedure (SAS Institute Inc., Cary, NC).

#### 2.2.2 Microsatellite analysis

Bulk genomic DNA was extracted according to the previously described procedure (Saghai Maroof et al. 1984) using leaves of 5-10 plants for each accession. Twenty microlitres of each reaction mixture contained 10 mM of Tris-HCl (pH 8.3), 50 mM of KCl, 3.0 mM of MgCl<sub>2</sub>, 5.0 mM of dNTP each, 5.0 mM of primers each, 60 ng of genomic DNA, and 1 unit of Taq polymerase. DNA amplification was performed in a PTC-200

thermocycler (MJ Research, Watertown, MA), which was programmed for 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 50-60°C, and 1 min at 72°C, and 10 min at 72°C for a final extension. PCR products were separated on 6% polyacrylamide denaturing gels and were visualized following silver-staining. Each DNA fragment amplified was scored as present (1) or absent (0).

The thirty-three accessions were screened with 379 previously published SSR primer pairs. All markers and their sequences were obtained from database GrainGenes. They distribute 21 wheat chromosomes, ranging from 8 (chromosome 4D) to 37 (chromosome 2A) with an average of 18 per chromosome. The average map distance between adjacent markers was 6.78 cM according to the consensus map of wheat (Somers et al. 2004).

#### 2.2.3 Cluster analysis

The data matrixes of SSR and means for each morphological phenotype were used to compute Nei's genetic distance coefficients (Nei 1972) by the NTSYS-pc version 2.1 software, respectively. Distance coefficients were used to construct a dendrogram using the UPGMA (Unweighted pair group method with arithmetic average) and the SHAN (Sequential agglomerative hierarchical non-overlapping clustering) routines in the NTSYS program (Rohlf 2000).

## 3. Results and Discussion

### 3.1 Morphological comparison

The results of ANOVA indicated that there existed significant difference among progeny and their parents for certain agronomic traits in each cross. The comparison among the progeny and their parents based on mean values for each morphological trait measured were displayed in Figure 1. In general, the progeny from a single cross approached their parents or had intermediate quantitative variables between both parents for most traits. Nevertheless, transgressive inheritance was recorded for certain morphological traits. For example, Shannong 17 derived from 'Bima 4 × Jubileina II' showed lower values of plant height and shorter days to heading than its parents. All the evaluated progenies from 'Youmangbai 4 × Lovrin 10' were characterized by lower stature compared to their parents. Similarly, transgressive inheritance has been reported in recent studies such as Terzi et al. (2007) and Li et al. (2007). As a result, genealogical relationships alone were not predictive of progeny performance for yield components to some extent. On the other hand, the phenotypic characters among different sister cultivars from a single cross were very various, which could be observed from the extent of decentralization of phenotypic data (Figure 1). Otherwise, based on

morphological characters, it is difficult to classify an over expression of only one of the two parents in each cross as a whole. Among four cultivars obtained from 'Beijing 8 × Orofen', Kedong 81 have spike length and thousand-grain weight significantly different from Orofen and closer to Beijing 8, which may indicate the latter contributed more for the two morphological traits.

### 3.2 Microsatellite analysis

Of three hundred and seventy-nine SSR markers used in this study, 306 (80.7%) displayed polymorphism among all examined genotypes, and the remaining 73 produced a monomorphic pattern, respectively. All monomorphic loci were not included below the analysis. The number of polymorphic markers on each chromosome ranged from 5 on chromosome 4D to 31 on chromosome 2A. A total of 1413 alleles were recorded with an average of 4.61 polymorphic bands per locus. The average number of alleles detected per SSR locus varied from 3.25 on chromosome 5D to 5.86 on chromosome 1B.

Table 1 showed the transmittance of microsatellite alleles for 25 cultivars from their immediate parents. A high level of genetic variability was revealed among the accessions. Although significant difference was not observed in morphological traits among progeny from the same cross, for example, the four cultivars from 'Youmangbai 4 × Lovrin 10' and the genotypes Fengkang 8, Fengkang 9 and Fengkang 15 from 'Youmanghong 7 × Lovrin 10', the genetic differentiations were clearly shown by SSR data. The parental contribution showed two situations as a whole: some parents were over-represented on their progeny. Some representative cases were: Bima 4 in Qingchun 2, Shannong 17, Jinan 8 and Dexuan 1, Beijing 8 in Kedong 81 and Xingxuan 7, Lovrin 10 in Jingshuang 16. Additionally, a balanced contribution in the parental stocks was observed in some cultivars such as 54405, Fengkang 10 and Fengkang 15. Keim et al. (1990) found that only two of 60 F2 progeny differed significantly from the expected value. Lorenzen (1995) demonstrated that four of 26 soybean cultivars deviated from the expected. In the present study, fourteen of 25 wheat cultivars was significantly different their expected parental contribution based on a Chi-square analysis ( $\alpha=0.05$ ). Furthermore, each of four cultivars (Qingchun 2, 54405, Fengkang 7 and Fengkang 10) received equal or over 70% genomic information from the first parent. Such transmittance of microsatellite alleles observed in this study produced a contradiction with previous prediction according to St. Martin (1982), who underlined that selection of an inbred line with a 70% or greater genetic contribution from one parent would be very unlikely. This might arise from the

influence of linkage drag among adjacent molecular markers (Bernardo et al. 1997). In addition, founder parents with the exception of Lovrin 10 presented more genetic contribution to their progeny compared to another parent generally in each cross, indicating that more desirable phenotypes were selected from them in breeding.

The cross 'Beijing 8 × Orofen' has been undertaken as an example to evaluate the character of proportion of parental inheritance in each of 21 wheat chromosomes. Of 306 SSR markers investigated, 234 were found to produce polymorphisms between Beijing 8 and Orofen. This set of molecular markers were used to evaluate the genetic contribution of the two parents to the four genotypes obtained from their cross. Figure 2 shows graphically the percentages of nuclear genomes from both parents to their progeny. The progeny seemed to have been subjected to intense selection during breeding. For example, when compared with remaining two sister cultivars, Beijing 8 contributed more genome information to Kedong 81 and Xingxuan 7. Nevertheless, genetic differences could be observed between these two cultivars: Kedong 81 inherited more genome information from Beijing 8 on chromosomes 1A, 3A, 6A, 2B, 1D, 4D, 5D and 7D, and yet Xingxuan 7 obtained a little proportion in the same chromosomes from Beijing 8. On the contrary, Beijing 8 contributed much to Xingxuan 7 on chromosome 4B, but smallness to Kedong 81 on the same chromosome. In a like manner, differences were observed between Kedong 83 and Kechun 14, which inherited nearly equal genome information from both parents.

Inconsistent alleles, which were detected in progeny and could not be tracked to either of both parents, were observed in this study with a frequency of 19.7% of all cases. The maximum of non-parental bands were recorded between the cultivar Fengkang 9 and its parents (148 from 306 markers studied). Approximate 52% of all monitored cases could be determined for the transmittance of microsatellite alleles from parents to their progeny. Similarly, non-parental bands were previously described by RFLP markers (4%) and SSR markers (2%) in maize by Smith et al. (1997) and in barley (13.9%) by Sjakste et al. (2003). It is complicated in practice to illustrate the reasons that inconsistent alleles were produced. Several problems might be associated with it according to Smith et al. (1997): the differentia exists among parental genotype used for original crossing and samples for molecular analysis afterward, and ideally, DNA samples should be applied from the actual plants used to make the original cross. On the other hand, mutation, contamination or physical mixing during increasing of seeds may have occurred.

In addition, examples of heterozygous phenotypes were detected with a frequency of 1.4% of 7650 monitored cases in this study, indicating that the genotypes have not been fixed in these SSR loci. The highest level of heterozygosity was recorded in the cultivar Dai 179 (10 from 306 markers studied).

### 3.3 Cluster analysis from phenotypes and SSRs

Two dendrograms were generated and compared based on the genetic distance coefficients from agronomic and SSR data. The morphology clusters reflected the pedigree relationship of the accessions to some extent, however, the dendrogram based on SSR data accorded highly with their pedigrees (Figure 3). Such indicated that molecular markers were more effective for the analysis of genetic diversity. Similar result was obtained as previously reported (Fufa et al. 2005; Sorkheh et al. 2007). In the SSR clusters, each of three founder parents (Beijing 8, Bima 4 and Lovrin 10) with its progeny was clustered within a small group. Three parents, Orofen, Ckopocnelka JI-1 and Jubileina II

which were introduced from other countries, were located far from their progeny, indicating they contributed relative small genome and probably played special roles in the genetic construction of its progeny.

In conclusion, twenty-five cultivars representing historically important accessions and developed from six representative crosses in the breeding of China were assayed using morphological traits and SSR markers in this study. The comprehensive data indicated that a same cross could produce quite different varieties in different breeding programs, emphasizing that strong selection existed during breeding. Therefore, genealogical relationships alone were not predictive of progeny performance for yield components and parental contribution. Based on the analyses of morphological traits, pedigree and SSR marker, SSR marker seems to be more accurate to reflect genetic relationships among wheat cultivars.

Table 1. Inheritance of 306 SSR loci in 25 wheat cultivars from their immediate parents and Chi-square tests of deviation from the expected ratio (0.5)

Cultivar	Pedigree	Parent 1	Parent 2	Same	Incon-sistent	Hetero-geneous	P value
Qingchun 2	Bima 4×Jubileina II	110	43	76	73	4	*
Shannong 17	Bima 4×Jubileina II	99	55	80	71	1	*
54405	Bima 4×Ckopocnelka JI-1	59	58	57	130	2	NS
Jinan 8	Bima 4×Ckopocnelka JI-1	117	45	63	79	2	*
Dexuan 1	Bima 4×Ckopocnelka JI-1	130	40	68	66	2	*
Nongda 198	Beijing 8×Nongda 45	78	64	121	37	6	NS
Dai 179	Beijing 8×Nongda 45	96	46	132	22	10	*
Youmangbai 4	Beijing 8×Nongda 45	88	58	132	26	2	*
Youmangbai 15	Beijing 8×Nongda 45	74	58	119	48	7	NS
Youmanghong 8	Beijing 8×Nongda 45	82	66	129	25	4	NS
Kedong 81	Beijing 8×Orofen	138	49	58	59	2	*
Kedong 83	Beijing 8×Orofen	112	84	67	42	1	*
Kechun 14	Beijing 8×Orofen	110	84	67	44	1	NS
Xingxuan 7	Beijing 8×Orofen	140	60	69	29	8	*
Fengkang 1	Youmangbai 4×Lovrin 10	98	72	90	40	6	*
Fengkang 2	Youmangbai 4×Lovrin 10	98	80	93	33	2	NS
Fengkang 4	Youmangbai 4×Lovrin 10	100	72	87	42	5	*
Fengkang 5	Youmangbai 4×Lovrin 10	97	73	90	38	8	NS
Jingshuang 16	Youmanghong 7×Lovrin 10	56	122	72	53	3	*
Fengkang 7	Youmanghong 7×Lovrin 10	61	107	66	70	2	*
Fengkang 8	Youmanghong 7×Lovrin 10	69	85	64	82	6	NS
Fengkang 9	Youmanghong 7×Lovrin 10	68	43	45	148	2	*
Fengkang 10	Youmanghong 7×Lovrin 10	73	76	65	84	8	NS
Fengkang 11	Youmanghong 7×Lovrin 10	72	98	63	71	2	*
Fengkang 15	Youmanghong 7×Lovrin 10	71	79	68	81	7	NS
Total		2296	1717	2041	1493	103	
Percentage		30.0%	22.4%	26.7%	19.5%	1.4%	

\* Significant difference (at 5%). NS= Non-significant difference

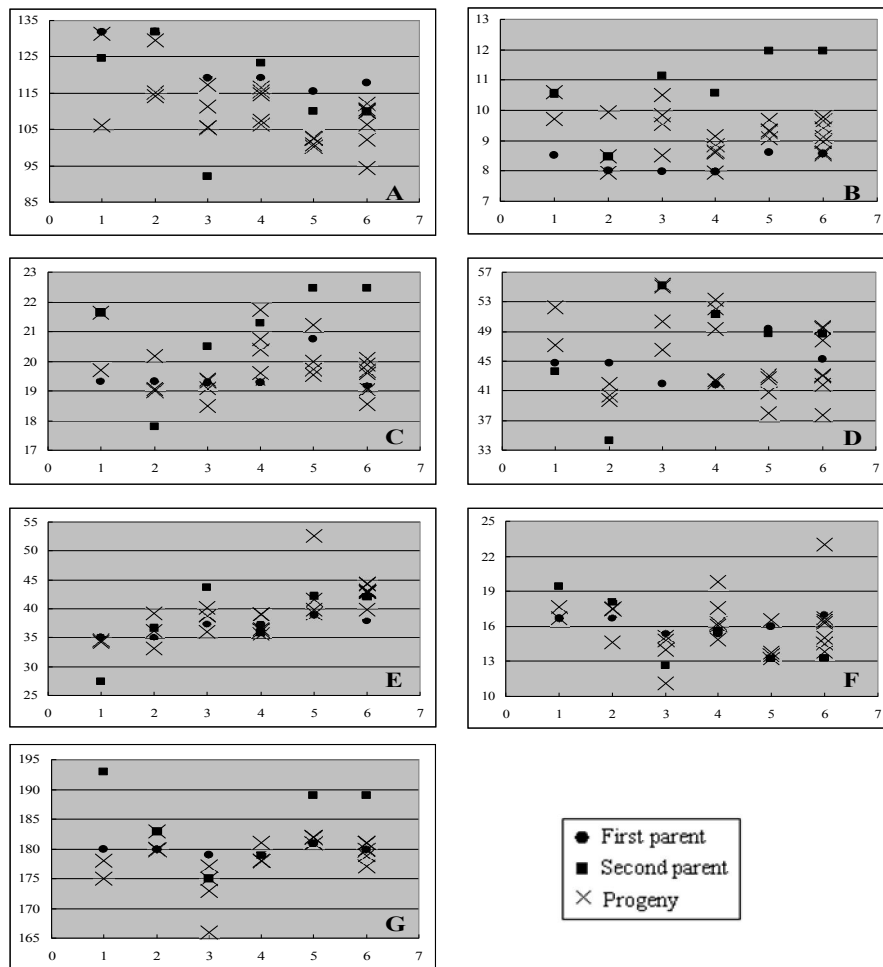


Figure 1. Comparison of morphological traits among parents and their progeny based on mean values of samples from five geographical regions. X axes were the crosses: 1 ‘Bima 4 × Jubileina II’; 2 ‘Bima 4 × Скороспелка JI-1’; 3 ‘Beijing 8 × Orofen’; 4 ‘Beijing 8 × Nongda 45’; 5 ‘Youmangbai 4 × Lovrin 10’; 6 ‘Youmanghong 7 × Lovrin 10’. A plant height; B spike length; C spikelet number; D grain number per spike; E thousand-grain weight; F spike number per plant; G days half to heading

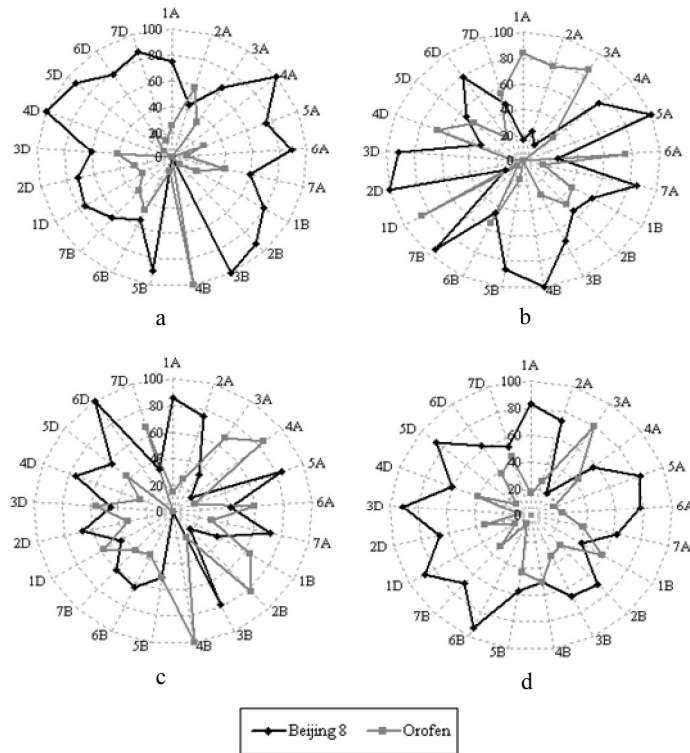


Figure 2. Percentages of the parental inheritance to progenies from the cross ‘Beijing 8 × Orofen’ in each of twenty-one wheat chromosomes based on SSR mapped markers (A: Kedong 81; B: Kedong 83; C: Kechun 14; D: Xingxuan 7)

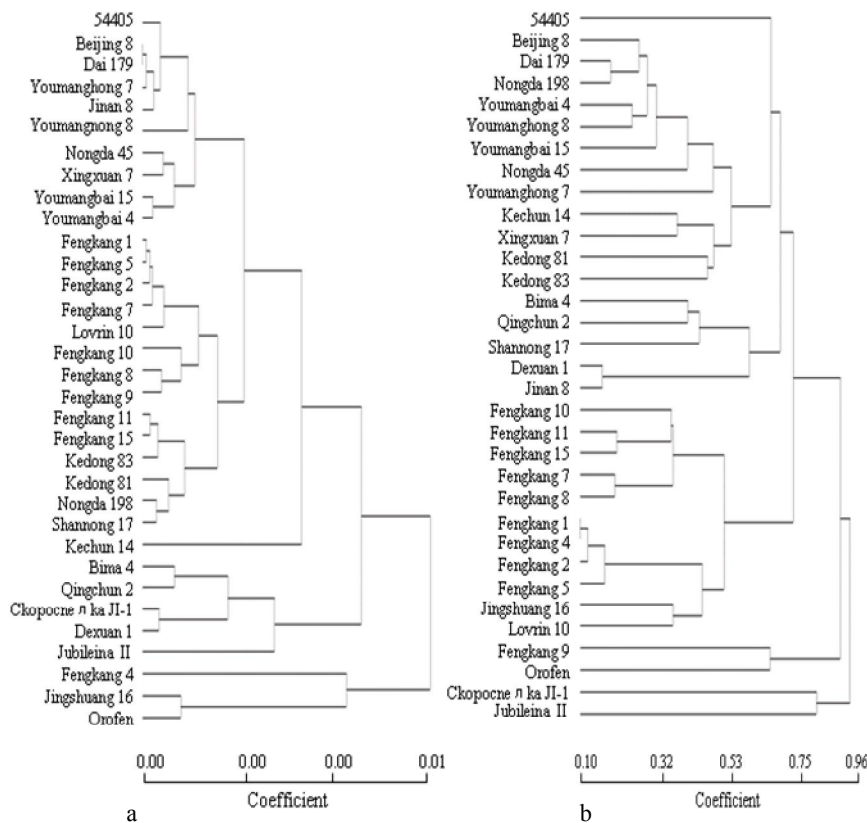


Figure 3. UPGMA dendrogram of thirty-three cultivars based on agronomic (a) and SSR data (b)

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