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North American study on essential derivation in maize: inbreds developed without and with selection from F_2 populations

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Abstract An essentially derived variety largely retains the characteristics of a parental or ancestral variety. A consensus has not been reached regarding the threshold for declaring essential derivation in maize (*Zea mays* L.), partly because benchmark data are lacking. Our objective in this study, commissioned by the American Seed Trade Association, was to determine the range of parental contribution among maize inbreds developed without and with selection. Seed companies in North America contributed existing proprietary data on the molecular marker similarity of 100 or more families, developed without selection from F_2 populations, with each of their parents. The companies also sent us seed samples of elite inbreds, developed with selection from F_2 populations, for analysis using 60 RFLP marker loci and 20 SSR marker loci. Among the families developed without selection, the average parental contribution was close to the expected value of 0.50 for F_2 populations. Specific families received up to 79% of their alleles from one parent. Although selection tended to increase the frequency of such transgressive segregants, it did not necessarily increase the maximum parental contribution in an F_2 population. Parental contributions were consistent between the elite inbreds and their early-generation families. We conclude that inbreds with 70% to nearly 80% of their genome derived from one parent can be obtained from an F_2 population. Further empirical data would be valuable particularly for backcross populations, which were not available in this study.

Keywords Essential derivation · Parental contribution · *Zea mays* L. · F_2 populations

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Introduction

Essential derivation has become an important concept in plant variety protection (Smith et al. 1995). In 1991, the Union Internationale pour la Protection des Obtentions Végetales defined an essentially derived variety as being ''predominantly derived from (an) initial variety, or from a variety that is itself predominantly derived from the initial variety, while retaining the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety'' (UPOV 1991). The concept of essential derivation implies that a threshold will be established, beyond which a variety will be declared as essentially derived from a parental or ancestral variety.

Molecular markers have been generally accepted as a means for determining essential derivation (Dillmann et al. 1995). Specifically, parental contribution–the proportion of the genome contributed by a parent to its inbred progeny–can be estimated with molecular markers (Lorenzen et al. 1995; Bernardo et al. 1997). An inbred is declared essentially derived from a parental inbred if the parental contribution exceeds the threshold. The appropriate thresholds for different crop species are yet undetermined, although a threshold of 0.90 has been proposed for maize (Smith et al. 1995).

Thresholds may be established on the basis of theoretical data and empirical data. Theoretical results obtained by Wang and Bernardo (2000) were consistent with the threshold of 0.90 proposed for maize. However, publicly available empirical data needed to validate proposed thresholds in maize are limited. In 1997, the American Seed Trade Association commissioned us, in cooperation with maize seed companies in North America, to obtain

Table 1 Summary of restriction fragment length polymorphism data for maize families, developed without selection, from 7 F_2 populations

Population	Number of families	Generation of selfing	Number of RFLP loci	Population average:		
				Similarity to Parent 1 (%)	Similarity to Parent 2 (%)	Heterozygous markers (%)
1	976	$F_{4:5}$	184	47.6	48.2	13.0
2	356	Recombinant inbreds from Syn4	286	45.6	42.8	2.3
3	99	$F_{2:4}$	194	48.2	46.3	48.3
4	112	$F_{2:3}$	243	46.9	42.2	43.9
5	160	$F_{3:4}$	61	46.9	48.4	13.0
6	117	$F_{4:5}$	80	51.7	48.1	8.9
7	95	$F_{4:5}$	72	53.1	46.7	10.0

empirical data useful as benchmarks for setting thresholds in maize. The purpose of the North American study on essential derivation in maize was ''to learn, in terms of probabilities, what could be expected to be derived using common breeding procedures" (unpublished report of the Corn Variety Identification Committee, American Seed Trade Association). We present the results of this study in this article.[1] Our specific objective in this study was to determine the range of parental contribution among inbreds developed without and with selection.

Materials and methods

Inbreds developed without selection

We asked maize seed companies in North America to contribute existing proprietary data on the molecular marker similarity of about 100 or more randomly-selfed families, derived from an F_2 or BC_1 population, with each of their parents. Specifically, we solicited information on the proportion of marker loci at which each family (1) was homozygous for the marker allele found in the first parent (i.e., Parent 1), (2) was homozygous for the marker allele found in the second parent (i.e., Parent 2), (3) was heterozygous, or (4) had nonparental marker alleles (i.e., present in the family but not in either parent). The companies contributed data for 7 F_2 populations, each with 95–976 random families (Table 1). Unfortunately, none of the companies contributed data for BC_1 populations. The families had data for 61–286 restriction fragment length polymorphism (RFLP) markers that were polymorphic between their parents. The choice of marker alleles being scored as individual bands or as banding patterns was left to the discretion of each company. Because we had no means of assessing the quality of the marker data, we took the data at face value.

Inbreds developed with selection

We asked maize seed companies in North America to send seed samples of elite inbreds, derived by selection from F_2 or BC_1 populations, for molecular marker analysis by Biogenetic Services (Brookings, S.D., USA). The entries comprised the (1) two parental inbreds, (2) two testers used in testcross selection, (3) three or more sister inbreds (F_6 or later) selfed from the same population, and (4) early-generation families, if available, from which the elite inbreds were derived. The companies contributed entries for 14 F_2 populations. Again, none of the companies contributed data for BC_1 populations. The entries comprised 97 elite inbreds and 23 early generation families. The elite inbreds were developed by testcross selection for yield and per se selection for plant and ear characteristics (Hallauer 1990), although detailed breeding procedures (e.g., generation of testcrossing, number of environments, selection intensity, etc.) probably differed among the companies.

Molecular marker analysis of inbreds developed with selection

The elite inbreds, the available early generation families, and testers were analyzed with about 60 RFLP and 20 simple sequence repeat (SSR) marker loci that were polymorphic between the respective parents. The parents of the 14 F_2 populations differed in their level of polymorphism; consequently, the final number of marker loci differed among populations. Different sets of RFLP and SSR markers were used for each F_2 population. The RFLP and SSR markers were randomly distributed across all ten maize chromosomes. A list of the RFLP and SSR marker loci used in this study is available from Biogenetic Services.

Ten to fifty seeds representing each sample were planted in a greenhouse. Leaf tissue of 3-week-old plants was harvested, freeze-dried, and ground into fine powder. Total cellular DNA was isolated from each sample with 35 ml 2% CTAB/0.2% BME extraction buffer that was prewarmed to 65°C. The DNA was purified using methods reported by Ausbel (1987) with slight modifications by Biogenetic Services (unpublished).

For the RFLP assays, restriction enzyme digestion with either *Eco*RI, *Bam*HI, or *Hind*III for each RFLP probe was performed according to the manufacturer's protocols (Life Technologies, Rockville, Md.). Gel electrophoresis was conducted on 0.7% agarose gels + EtBr in 1 × TAE. Southern hybridization was according to the methods described by Budowle and Baechtel (1990). Membranes were washed three times to remove unhybridized probe solution; the first wash was with 2 × SSPE/0.1%SDS/0.02% sodium azide for 15 min at room temperature, the second with 0.2 × SSPE buffer for 15 min at room temperature, and the third with 0.1 × SSPE for 20 min at 65°C. The membranes were then placed on Kodak® AR Safety Film and incubated according to known optimal exposure times for each probe.

For the SSR assays, DNA amplification was performed on a PTC-100 programmable thermal controller (MJ Research, Waltham, Mass.). The polymerase chain reaction (PCR) procedure was conducted on 30 ng DNA template in a final reaction volume of 10 µl as described by Akkaya et al (1992). A total of 3 µl of loading buffer was added to each amplified product. Denatured PCR products were loaded onto 7% polyacrylamide gels, with the electrophoresis conditions as described by Akkaya et al (1992). The amplified fragments were visualized by autoradiography or silver staining (Promega, Madison, Wis.).

Simple matching coefficients (Sneath and Sokal 1973) were calculated between an inbred and each of its parents. Suppose Parent 1 had the *AA* genotype, whereas Parent 2 had the *BB* genotype. At this locus, the parental contribution of Parent 1 was 1 if

[1] Our views and interpretation of the results do not necessarily reflect those of the American Seed Trade Association.

Table 2 Number of transgressive segregants among maize families, developed without selection, from 7 F_2 populations. The expected numbers oftransgressive segregants, for a simple *t*-distribution, are in parentheses

Population	Number of families	Maximum similarity to:		Number of families:							
		Parent 1	Parent 2	>0.60 similarity to:		≥ 0.65 similarity to:		≥ 0.70 similarity to:		≥ 0.75 similarity to:	
				Parent 1	Parent 2	Parent 1	Parent 2	Parent 1	Parent 2	Parent 1	Parent 2
1	976	0.74	0.72	71 *(67.3)*	88 *(78.4)*	28 *(24.4)*	22 *(29.4)*	5 *(5.2)*	5 *(6.5)*		
2	356	0.68	0.64	14 *(24.2)*	6 *(13.9)*	5 *(10.1)*	0 *(5.3)*	0 *(2.8)*	0 *(1.3)*		
3	99	0.67	0.68	5 *(5.9)*	5 *(3.0)*	1 *(1.9)*	1 *(0.8)*	0 *(0.3)*	0 *(0.1)*		
4	112	0.64	0.56	2 *(3.3)*	0 *(0.7)*	1 *(0.8)*	0 *(0.1)*	0 *(0.1)*	0 *(0.0)*		
5	160	0.69	0.72	14 *(17.9)*	24 *(22.5)*	7 *(9.0)*	9 *(11.9)*	0 *(3.3)*	2 *(4.6)*		
6	117	0.78	0.75	25 *(26.2)*	17 *(16.2)*	16 *(15.2)*	6 *(8.6)*	8 *(6.6)*	1 *(3.4)*	3 *(2.5)*	1 *(1.1)*
7	95	0.79	0.68	16 *(22.8)*	5 *(6.2)*	4 *(10.4)*	1 *(2.2)*	2 *(3.0)*	0 *(0.4)*	1 *(0.6)*	0 *(0.1)*

the inbred had the *AA* genotype, 0.5 if the inbred had the *AB* genotype, and 0 if the inbred had the *BB* genotype. Because the loci were polymorphic between the parents, the simple matching coefficient averaged across loci was a direct estimate of parental contribution.

Results and discussion

Observed versus expected parental contribution

Among the families developed without selection, the average parental contribution was close to the expected value of 0.50 for each F_2 population (Table 1). However specific families within 2 of the F_2 populations received up to 0.79 of their marker alleles from one parent (Table 2). Random mating an F_2 population prior to selfing (i.e., Population 2) decreased the frequency of such transgressive segregants. We therefore speculate that the converse would be true: doubled haploid inbreds, which are not subject to repeated recombination between loci during meiosis, would increase the range of parental contribution and, consequently, the probability of essential derivation. The average proportion of heterozygous marker loci within families was generally consistent with the generation of selfing.

A total of 11 out of 97 (11%) elite inbreds, which were developed with selection, had observed parental contributions that deviated significantly ($P = 0.05$) from the expected value of 0.50 (Table 3). The largest deviations were for Inbred 2 in Population G (i.e., 2G) and Inbred 1 in Population K (i.e., 1 K), wherein one parent contributed 0.73 of its genome and the other parent contributed 0.27.

This study aimed to answer the question ‘‘What is the range of parental contribution among inbreds developed without and with selection during inbreeding?’’ The answer from this study is that inbreds with 70% to nearly 80% of their genome derived from one parent can be obtained from an F_2 population. In other words, it is possible to obtain inbreds from an F_2 population that, in terms of parental contribution, are similar to inbreds from a BC_1 population. These results agreed with those of Bernardo et al. (1997). Also, the observed proportion of transgressive segregants, among families developed without selection, agreed well with the proportions expected from a simple *t*-distribution (Table 2). For a given threshold, the expected proportion of essentially derived inbreds can therefore be calculated if the variance of marker estimates of parental contribution is known. This variance has been derived by Wang and Bernardo (2000) for inbreds that are (1) fully homozygous, (2) developed without selection, and (3) selfed directly, without random mating, from an F_2 or a BC_1 population. Unfortunately, none of the families in this study met these criteria. The families in Population 2 were highly inbred (i.e., only 2.3% heterozygous markers) but were selfed after four generations of random mating. The families developed without selection in the 6 other F_2 populations were far from being fully inbred (i.e., 8.9–48.3% heterozygous

Table 3 Marker estimates of parental contribution to elite maize inbreds, developed with selection, from 14 F_2 populations

Population	Number of markers		Inbred	Parental contribution[a]		Sum of parental contributions
	RFLP	SSR		Parent 1	Parent 2	
A	71	22	1 A	0.47	0.53	1.00
			1A-F_3[b]	0.51	0.45	0.96
			2 A	0.44	0.56	1.00
			2A-F_3	0.44	0.56	1.00
			3 A	0.47	0.52	0.99
			3A-F_3	0.53	0.47	1.00
			Average	0.48	0.51	
B	66	22	1B	0.64	0.35	0.99
			1B-F_3	0.48	0.46	0.94
			2B	0.61	0.39	1.00
			3B	0.56	0.43	0.99
			4B	0.53	0.38	0.91
			5B	0.62	0.38	1.00
			2–5B-F_3	0.56	0.43	0.99
			6B	0.44	0.56	1.00
			6B-F_3	0.49	0.51	1.00
			7B	0.49	0.49	0.99
			7B-F_3	0.53	0.46	0.99
			Average	0.54	0.44	
C	33	20	1 C	0.61	0.39	1.00
			2 C	0.62	0.38	1.00
			1–2C-F_3	0.53	0.40	0.92
			3 C	0.61	0.39	1.00
			3C-F_3	0.65	0.35	1.00
			4 C	0.52	0.44	0.96
			5 C	0.58	0.41	0.98
			6 C	0.61	0.37	0.98
			4–6C-F_3	0.47	0.43	0.91
			7 C	0.51	0.47	0.98
			7C-F_3	0.58	0.41	0.98
			8 C	0.50	0.48	0.98
			8C-F_3	0.56	0.41	0.96
			Average	0.57	0.41	
D	52	22	1D	0.59	0.40	0.99
			2D	0.57	0.42	0.99
			1–2D-F_3	0.59	0.40	0.99
			3D	0.42	0.58	1.00
			4D	0.41	0.59	1.00
			5D	0.45	0.53	0.99
			3–5D-F_3	0.41	0.59	1.00
			6D	0.53	0.47	1.00
			6D-F_3	0.50	0.47	0.97
			Average	0.50	0.50	
E	64	21	1 E	0.69*	0.30*	0.99
			1E-F_3	0.67*	0.33*	0.99
			2 E	0.63	0.36	0.99
			2E-F_3	0.64	0.35	0.98
			3 E	0.68*	0.31*	0.99
			3E-F_3	0.65*	0.33*	0.98
			4 E	0.59	0.38	0.98
			4E-F_3	0.60	0.38	0.98
			5 E	0.38	0.45	0.84
			5E-F_3	0.55	0.44	0.98
			Average	0.61	0.36	
F	61	20	1F	0.65*	0.34*	0.99
			2F	0.62	0.36	0.99
			3F	0.44	0.56	1.00
			4F	0.37	0.62	0.99
			5F	0.51	0.46	0.96
			6F	0.46	0.52	0.98
			7F	0.54	0.44	0.99
			8F	0.41	0.59	1.00
			9F	0.44	0.53	0.96
			10F	0.52	0.48	1.00

Table 3 (continued)

Population	Number of markers		Inbred	Parental contribution[a]		Sum of parental contributions
	RFLP	SSR		Parent 1	Parent 2	
F	61	20	11F	0.57	0.43	1.00
			12F	0.33*	0.65*	0.99
			13F	0.49	0.51	1.00
			Average	0.49	0.50	
G	65	21	1G	0.38	0.62	1.00
			2G	0.27*	0.73*	1.00
			3G	0.51	0.48	0.99
			4G	0.44	0.56	1.00
			5G	0.64	0.35	0.99
			6G	0.59	0.39	0.98
			7G	0.53	0.47	1.00
			8G	0.52	0.48	1.00
			9G	0.52	0.47	0.99
			10G	0.56	0.44	1.00
			11G	0.59	0.41	1.00
			12G	0.46	0.52	0.98
			13G	0.52	0.46	0.99
			14G	0.39	0.59	0.98
			15G	0.53	0.45	0.99
			16G	0.43	0.56	0.99
			17G	0.45	0.52	0.98
			18G	0.62	0.38	1.00
			Average	0.50	0.49	
H	58	22	1H	0.44	0.55	0.99
			2H	0.45	0.42	0.88
			3H	0.41	0.57	0.97
			4H	0.43	0.50	0.94
			5H	0.60	0.36	0.96
			6H	0.59	0.39	0.98
			7H	0.36	0.60	0.96
			8H	0.55	0.39	0.94
			9H	0.56	0.42	0.98
			10H	0.64	0.34	0.98
			11H	0.57	0.40	0.98
			Average	0.51	0.45	
I	48	20	1I	0.43	0.53	0.96
			2I	0.39	0.58	0.97
			3I	0.67*	0.29*	0.96
			4I	0.69*	0.31*	1.00
			5I	0.50	0.48	0.99
			6I	0.42	0.58	1.00
			7I	0.39	0.61	1.00
			8I	0.40	0.60	1.00
			Average	0.49	0.50	
J	51	22	1 J	0.50	0.47	0.97
			2 J	0.44	0.55	0.99
			3 J	0.59	0.41	1.00
			4 J	0.49	0.50	0.99
			5 J	0.66*	0.32*	0.99
			6 J	0.41	0.58	0.99
			7 J	0.40	0.56	0.96
			8 J	0.38	0.58	0.96
			Average	0.48	0.50	
K	37	20	1 K	0.27*	0.73*	1.00
			1K-F_3	0.37	0.61	0.98
			2 K	0.34	0.63	0.97
			2K-F_3	0.39	0.57	0.97
			Average	0.34	0.63	
L	52	21	1L	0.45	0.55	1.00
			1L-F_5	0.44	0.53	0.97
			2L	0.68*	0.29*	0.97
			3L	0.64	0.34	0.99
			Average	0.55	0.43	

Table 3 (continued)

Population	Number of markers		Inbred	Parental contribution[a]		Sum of parental contributions
	RFLP	SSR		Parent 1	Parent 2	
M	46	21	1 M	0.65	0.35	1.00
			2 M	0.70*	0.24*	0.94
			3 M	0.58	0.40	0.99
			Average	0.64	0.33	
N	25	21	1 N	0.50	0.50	1.00
			2 N	0.59	0.41	1.00
			Average			

* Significantly different (P = 0.05) from the expected value of 0.50. Theoretical variances of the parental contribution (Wang and Bernardo 2000) were used in z-tests for significance

[a] Parental contributions were the weighted average of RFLP and SSR estimates

[b] Elite inbreds are denoted by a number followed by the population; for example, 1 A. The early generation family from which an elite inbred was derived is denoted by the elite inbred followed by the generation of selfing; for example, 1A-F_3

Table 4 Frequency of nonparental RFLP alleles among maize families developed without selection from 7 F_2 populations

Population	Percentage of families with nonparental bands at an RFLP locus:			Percentage of families having >0.1 of markers with nonparental bands
	Average	Minimum	Maximum	
1	2.1	0	31.5	2.0
2	11.6	2.4	69.9	22.8
3	5.5	0.1	16.0	12.1
4	10.9	3.3	62.1	42.0
5	4.7	0	90.2	14.4
6	0.1	0	12.5	0.9
7	0.2	0	6.9	0.0

markers). Perhaps a follow-up study commissioned by the American Seed Trade Association (C.W. Stuber, personal communication) will enable a comparison of theoretical expectations and empirical results.

Selection and essential derivation

Selection seemed to increase the frequency of segregants that exceeded an arbitrary threshhold. A relevant question is whether the probability of getting an essentially derived inbred is higher among a large group of inbreds developed without selection or among a smaller group of inbreds developed with selection. Suppose an essentially derived variety is arbitrarily defined as having a parental contribution greater than 0.65. With this assumption, the frequency of essentially derived families developed without selection was 96/1559=6% (Table 2). In contrast, the frequency of essentially derived inbreds developed with selection was 12/97=12% (Table 3). The difference between the frequencies was not statistically significant (P = 0.05) but nevertheless suggested that selection tends to increase the frequency of essentially derived inbreds.

Selection sometimes favored one parent over the other. In Population C, Parent 1 contributed an average of 0.57, whereas Parent 2 contributed an average of 0.41 of its genome to the 13 inbreds. In Populations K and M, the parental contributions averaged across 3 or 4 inbreds were 0.63–0.64 from one parent and 0.33–0.34 from the other parent. Not all seed companies provided seed of the testers used in selection, and of these 3 populations only Population K had the tester available. The tester in Population K was 0.40 similar to Parent 1, and 0.24 similar to Parent 2. Assuming that heterozygosity is desirable in testcrosses, we speculate whether the greater similarity between the tester and Parent 1 led to the lower contribution of Parent 1 to the inbreds in Population K.

Parental contributions were consistent between the elite inbreds and their early-generation families. In Population E, for example, the parental contributions were larger for Parent 1 than for Parent 2 in Inbreds 1 E and 1E-F_3, 2 E and 2E-F_3, 3 E and 3E-F_3, and 4 E and 4E-F_3 (Table 3). This result indicated that deviations in the parental contribution from the expected value occur early during inbreeding. Early-generation families, therefore, would be useful in determining essential derivation.

Nonparental alleles

Nonparental alleles were a major complicating factor among the families developed without selection. Across marker loci, the average percentage of families with nonparental alleles was greater than 10% in 2 F_2 populations (Table 4). Furthermore, specific families in 3 of the F_2 populations had an extremely high percentage (>60%) of marker alleles that were not present in either parent. Having each

company decide whether to score either bands or banding patterns as alleles probably contributed to the observed frequencies of nonparental alleles. For example, if an RFLP probe maps to more than one locus, then the banding patterns scored as unique alleles in the parents may segregate as individual bands in the progeny. We reiterate the absence of any means for checking the quality of the existing, proprietary data we solicited from the seed companies. This result underscored the obvious requirement of quality control when determining essential derivation.

Nonparental bands were not a complicating factor among the inbreds developed with selection. The marker analysis of these inbreds was done within this study, thereby permitting quality control in the marker analysis. The sum of parental contributions among the elite inbreds and their early-generation families was often 1 or close to 1, indicating that nonparental bands were not a major issue (Table 3). Situations in which the sum of parental contributions was less than 1 were often due to missing bands in the elite inbreds rather than nonparental bands. The low frequencies of nonparental bands may have been due to residual heterozygosity. A problem with marker analysis of inbreds and their parents is that DNA samples are taken from plants grown from remnant seed stocks. Ideally, DNA samples should be taken from the actual plants used to make the original cross from which an inbred was developed. Residual heterozygosity may therefore cause marker genotypes to differ between remnant seed stocks and the actual plants used to make the cross. We also speculate whether RFLP and SSR markers are biologically prone to low frequencies of nonparental bands (Bernardo et al. 2000). A restriction fragment comprises a long segment of DNA (4,000–20,000 base pairs), and the restriction site may change because of recombination or transposon activity. The SSR fragments comprise only 80–300 base pairs, but the number of repeats may change because of slippage of DNA polymerase during replication (Levinson and Gutman 1987). These mechanisms, however, should have little impact on the use of molecular markers for assessing essential derivation as long as the frequencies of the resulting nonparental bands remain low.

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