Theor Appl Genet (2004) 108:1017–1023
DOI 10.1007/s00122-003-1521-8

ORIGINAL PAPER

D. L. Auger · T. S. Ream · J. A. Birchler

A test for a metastable epigenetic component of heterosis using haploid induction in maize

Received: 15 April 2003 / Accepted: 27 October 2003 / Published online: 21 November 2003

Abstract We conducted a test to detect if there is a heritable epigenetic component to hybrid vigor and/or inbreeding depression. The impetus for this work was a classical study of the effect of homozygosis on the expression of the maize *red color* (*r1*) locus. It had been shown that maintaining *R1* mottling alleles in the homozygous state over several generations produces a progressive decrease of their paternally imprinted expression. This effect is reversed by *R1/r1* allele heterozygosity. If this behavior were characteristic of many regulatory genes, then such a phenomenon could contribute to inbreeding depression and heterosis. To examine this question, inbreds of Mo17 and B73 and the two reciprocally produced hybrids were crossed by Stock 6 to generate four classes of maternal haploids. The mature haploid plants were measured for several quantitative traits. If inbreeding depression results from an accumulating heritable effect that is reversed by the hybrid state, one would expect the haploids derived from the hybrids to perform better than those derived from the inbred lines. The hybrid-derived haploids did not exhibit greater average performance than the inbred-derived haploids. These data fail to support the hypothesis that inbreeding depression and heterosis have a metastable epigenetic component.

Introduction

Heterosis refers to the phenomenon in which hybrids between two inbred varieties or lines exhibit greater biomass and other superior characteristics than those of the better parent (Shull 1914). The phenomenon has been known for centuries, but there is still no consensus as to the genetic basis, and even less is known about the molecular parameters associated with heterosis. One proposed basis of heterosis is dominance, i.e., slightly deleterious alleles, which are homozygous in the respective parents, are complemented in the hybrids with superior alleles (Bruce 1910; Jones 1917). Another potential basis for heterosis is overdominance. In this case, unlike alleles result in a stimulating effect, that is, that genetic heterozygosity per se results in greater genetic activity (East 1936; Hull 1945). The reverse concept to heterosis is inbreeding depression, in which quantitative trait values decrease as homozygosity increases in successive generations, even though the allele frequency as a whole for the successive populations remains unchanged. Inbreeding depression is often attributed to the fixation of deleterious alleles. The elucidation of heritable epigenetic phenomena in recent years raises the possibility that epigenetic effects contribute to heterosis and/or inbreeding depression.

Some transcription factor loci in maize show differential expression, depending upon whether they are present as a homozygote or heterozygote. A classic example involves the *red color* (*r1*) locus in maize. It is a member of the regulatory complex that controls the anthocyanin pigment pathway in the aleurone layer of the endosperm, as well as in other tissues. Many dominant *R1* alleles, when transmitted through the female parent, condition full color in the aleurone. However, when transmitted through the male, there is a mottled phenotype in which it functions only in some cells and variably when it does (Emerson 1918; Kempton 1919). This phenomenon is referred to as "gene imprinting" (Kermicle 1970). Maintenance of many *R1* alleles in the homozygous condition over several generations results in progressively fewer aleurone cells expressing color when transmitted as a male onto recessive *r1* females (Styles and Brink 1966; Styles 1967). Upon crossing the *R1*/*r1* heterozygote onto a recessive *r1* female, the number of pigmented aleurone cells in *R1*/*r1* heterozygotes tends to be greater than in the previous generation. These experiments were performed

Communicated by D. Hoisington

D. L. Auger · T. S. Ream · J. A. Birchler (✉)
Division of Biological Sciences,
University of Missouri,
117 Tucker Hall, Columbia, MO 65211-7400, USA
e-mail: birchlerj@missouri.edu
Tel.: +1-573-8824905
Fax: +1-573-8820123

in highly inbred lines, and so other modifiers seem an unlikely explanation for this finding. Rather, the determining factor seems to be the association in the same nucleus of like alleles versus unlike alleles.

A more recent example that implicates an epigenetic contribution to heterosis involves the *pl1* transcription factor that controls the anthocyanin pathway in plant tissue. Hollick and Chandler (1998) showed that a paramutated *mahogany* allele of *pl1* (*Pl1′-mah*) gave higher plant color expression in the heterozygous state than as a homozygote. It is conceivable that both inbreeding depression and heterosis are the phenotypic manifestation of such epigenetic events. If partial gene silencing results from continued homozygosis, then this would contribute to inbreeding depression. If genes increase in activity upon outcrossing, this would contribute to the heterotic response. The *r1* locus encodes a helix-loop-helix DNA-binding regulatory protein (Ludwig et al. 1989). The regulatory protein encoded by *pl1* has homology to *myb* transcription factors (Cone et al. 1993). These types of transcription factors are common among higher eukaryotes. Therefore, it is possible that the phenomenon exhibited by *r1* is common to other such regulatory factors.

Indeed, a study of inbreeding of doubled haploids showed that there were heritable changes in quantitative traits over several generations (Sprague et al. 1960). Because the progenitor lines were presumably completely homozygous, it was believed that the underlying cause was an accumulation of new mutations. However, in light of the results with the *r1* locus described above, it is also possible that continued homozygosity of regulatory genes resulted in accumulating epigenetic changes.

To investigate this possibility, we conducted a test of whether there is a heritable epigenetic component to inbreeding depression and/or heterosis. If this behavior were characteristic of many regulatory genes with a concordant modulation of target gene expression, then such a phenomenon should be reflected in quantitative traits. The experiment determined whether heritable epigenetic changes were induced by heterosis by eliminating the confounding effects of heterozygosity in the subsequent generation. The most direct way to completely relieve heterosis is haploidy. Although smaller, the physical traits of haploids correlate well with their diploid counterparts (Chalyk and Ostrovsky 1993).

We examined quantitative traits in haploids that were derived from hybrids and their inbred parents. From the hybrids, each of the haploid plants was the product of the segregation and assortment of alleles from its hybrid maternal parent. This procedure allowed for one generation of heterozygosity and then immediately removed that state in order to test for any heritable parental effect. Because the traits of the resulting plants were affected not only by the alleles received but also by the effects of haploidy, per se, the controls for this experiment were haploids that were derived from the same inbreds that were used to produce the hybrids. If an epigenetic change in gene expression is a cause for the higher performance of hybrids, then it should be expected that these changes should be reflected in a subsequent generation where there is no heterozygosity. In addition, we report some observations on haploid induction.

Materials and methods

The starting materials were the inbred lines B73 and Mo17. Hybrids were made by reciprocally crossing the two lines. The hybrids in which B73 was the female are designated "B73/Mo17," whereas the hybrids in which Mo17 was the female are designated "Mo17/B73." Samples of kernels from both inbreds and the reciprocal hybrids were planted and crossed as females with pollen from Stock 6. Pollinations using Stock 6 as the male parent result in a significant increase in the rate of embryo haploidy where the single genome is of maternal origin (Coe 1959).

The Stock 6 plants possessed all the color factors necessary for kernel color expression. Notably, they were homozygous for the *R1-scm2* allele, which conditions color in both the aleurone and the embryo. The inbred and hybrid stocks were colorless due to recessive *r1* and *c1* (*colored aleurone 1*) alleles. Crosses of Stock 6 onto the inbreds and the hybrids produced colored aleurone on every kernel, but haploid embryos possess only the maternal complement of genes, so they were colorless. Generally, the diploid embryos displayed poor color saturation and, in addition, aleurone color often extended over both the haploid and diploid embryos. Consequently, extra care had to be taken to score haploid embryos. This was accomplished by scraping the pericarp with a scalpel just at the margin of the scutellum. In addition, haploid embryos tend to have a dorsal-pointed appearance that aids in scoring. Kernels that putatively possessed haploid embryos were pooled into four classes based upon genotype. Two classes were derived from inbred lines B73 and Mo17, respectively. The other two classes were from the reciprocal hybrids. In the field, each row possessed up to 20 plants from one of the four groups, but the order of rows was randomized.

Mature plants were scored for haploidy. Haploid plants are short in stature, have distinctively narrow leaves, and are sterile (Chase 1964). Sterility was determined by the lack of fertile florets in the tassel. (Occasionally, a few fertile anthers extrude due to small sectors of tissue that have spontaneously doubled to the diploid state.) Within the planting, two exceptional diploid classes were eliminated from consideration. The first resulted from incorrectly scored kernels. These plants were fertile, produced colored kernels, and tillered heavily, which is characteristic of Stock 6. The second consisted of diploids that resulted from spontaneous chromosome doubling early in development, i.e., doubled haploids. These plants had fertile tassels and were self-pollinated to confirm that they did not possess kernel color factors.

Haploid plants were measured for several quantitative traits. Measurements were made after flowering to ensure complete maturation. Height was measured from the ground to the highest point of the uppermost sheath. Circumference was measured at the top of the internode that was immediately above the primary ear. Leaf length was measured from the ligule to the tip of the blade of the leaf subtending the primary ear. Leaf width was measured at the widest point of the same leaf. Also recorded for each plant were the number of tassel branches including the main spike, the number of tillers, and the number of nodes above the ground. The occurrence of at least one fertile floret on the tassel was noted, which indicated sectors of spontaneously doubled tissue. The node location and the cob length of the primary ear were recorded. Analyses of differences between the haploid classes were made on a pair-wise basis using the *t*-test with two exceptions: data for tillering and fertile tassel sectors were subjected to chi-square tests for homogeneity.

Results

Haploid induction

Maize (*Zea mays* L.) is typically a diploid plant (2*n*=20), but haploid individuals arise at a rate of 1 per 1,000 kernels (Chase 1949). Stocks have been identified that, when used as the pollen parent, increase the rate of haploidy at least tenfold (Coe 1959; Aman and Sarkar 1978; Deimling et al. 1997). Because the mechanism mediated by haploid inducing lines remains unknown, we are including observations about haploid induction.

The results of pollinating inbred and hybrid plants with Stock 6 are summarized in Table 1. Although the overall rate of putative haploid kernels was 1.27%, the rates for the four genotypes were not homogeneous ($P<0.001$, chi-square). The differences between the two inbreds were not significantly different from each other, nor were the differences between the reciprocal hybrids (both $P>0.05$, chi-square homogeneity). The inbreds yielded a higher combined rate of haploid recovery (1.98%) than the hybrids (1.13%). It has previously been shown that maternal factors can affect the rate of haploid induction (e.g., Eder and Chalyk 2002).

While screening for putative haploids, two additional classes of kernels were noted: germless and aborted. The aborted kernels were not counted in figuring the rates of putative haploid recovery in Table 1. If they were, the putative haploid recovery rates were substantially reduced (Table 2). The occurrence of both of these classes appeared to be more common than normal, but no control crosses were made for comparison. It is not clear how they may relate to haploid induction. The incidence of putative haploid, germless, and aborted kernels was recorded for each ear. Within each of the four classes of females, correlations were made between the haploid and germless rates found on each ear, as well as between the haploid and aborted rates (Table 2). The rates of putative haploids and germless kernels correlated on the Mo17 and B73/Mo17 ears, but not on B73 and Mo17/B73. The rates of putative haploids and aborted kernels were significantly correlated on the B73/Mo17 ears.

One maternal factor affecting the differences in haploid recovery may have been ear length. The hybrid ears were typically much longer than the inbreds. Sarkar and Coe (1966) found a negative correlation between ear length and haploid induction rate (i.e., shorter ears yield a greater rate of haploids). The opposite correlation was observed by Chase (1969), but in neither case were the correlations significant. One consequence of variation in ear length is differential pollen tube growth. To examine this issue, 14 hybrid ears were broken approximately in half, and each half was scored separately for putative haploids (Table 2). The total recovery rate of putative haploids from these 14 ears was 0.76%. The distal portions of the 14 ears had a haploid recovery rate of 1.01%, whereas the recovery rate on the proximal portions was 0.46% ($P<0.05$, chi-square homogeneity). The recovery rate of distal portions on the hybrid ears was still less than the haploid recovery rate on the inbred ears. It is unlikely, therefore, that the different rates of haploidy are explained solely by ear length. The incidence of germless and aborted kernels was also similar (Table 3). The rates of putative haploid, germless, and aborted kernels increase in parallel from the proximal to the distal portions of the ear. It may be that the mechanism that induces haploidy also causes both embryo and endosperm failure or, alternatively, whatever causes the distal embryo sacs to be more susceptible to haploidy makes them more susceptible to embryo and endosperm abortion.

Table 3 Frequency of abnormal kernels relative to ear position

	Kernels screened	Haploid	Germless	Aborted
Proximal	2,840	0.46%	0.56%	17.12%
Distal	3,570	1.01%	2.02%	37.32%
Total ear	6,410	0.76%	1.37%	28.37%

Table 1 Summary of haploid production

Maternal parent	Ears pollinated	Kernels produced	Putative haploids	Rate of putatives	Rows planted	Haploid plants
B73	24	3470	67	1.93%	4	32
B73/Mo17	53	13722	145	1.06%	7	78
Mo17/B73	51	14962	179	1.20%	10	97
Mo17	21	2182	45	2.06%	2	18
Total	149	34336	436	1.27%	23	225

Table 2 Frequency of exceptional kernel classes including aborted kernels

Female	Rates of exceptional kernels			Correlation with haploids	
	Germless	Aborted	Haploids	Germless	Aborted
B73	3.25%	26.3%	1.42%	–0.163	–0.262
B73/Mo17	1.20%	31.0%	0.73%	0.578*	0.415*
Mo17/B73	0.81%	28.9%	0.85%	0.240	0.253
Mo17	1.33%	32.5%	1.39%	0.585*	–0.172

Significance level: * $P<0.01$

Table 4 Means of quantitative traits for four classes of haploids. *B* B73-derived haploid, *BM* B73/Mo17-derived haploid, *MB* Mo17/B73-derived haploid, *M* Mo17-derived haploid, *SD* standard deviation, *SE* standard error

Quantitative trait	B			BM			MB			M		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
Plant height (cm)	119.3	6.5	1.2	104.1	21.2	2.5	109.4	21.6	2.1	109.1	3.9	1.0
Circumference (cm)	4.6	0.5	0.1	4.6	0.7	0.1	4.5	0.6	0.1	4.5	0.4	0.1
Leaf length (cm)	53.6	2.9	0.6	53.1	6.7	0.9	53.6	6.1	0.7	54.2	2.4	0.7
Leaf width (cm)	5.4	0.7	0.1	5.4	1.1	0.1	5.4	1.0	0.1	5.9	0.4	0.1
Tassel branches	10.8	2.1	0.4	8.9	3.5	0.4	9.3	3.9	0.4	5.2	0.8	0.2
Fertile tassel sectors (%)	22.6	42.5	7.8	3.8	19.4	2.3	6.1	24.0	2.4	5.3	22.9	5.6
Number of nodes	12.7	1.0	0.2	11.3	1.5	0.2	11.8	1.6	0.2	10.4	0.6	0.1
Ear node	7.2	0.9	0.2	6.2	1.4	0.2	6.7	1.3	0.1	6.2	0.9	0.2
Ear length (cm)	7.7	1.2	0.2	8.9	3.4	0.5	9.0	2.9	0.3	11.1	1.5	0.4
Tillers per plant	0.1	0.3	0.1	0.2	0.6	0.1	0.2	0.7	0.1	0.0	0.0	0.0

Table 5 Significant differences between haploid classes. *Bold letters* indicate the higher scoring haploid for a particular trait

Quantitative trait	Haploid			
	B	BM	MB	M
Height	BM, MB, M	**B**	**B**	**B**
Circumference	-	-	-	-
Leaf length	-	-	-	-
Leaf width	**M**	**M**	**M**	B, BM, MB
Tassel branches	BM, MB, M	**B**, M	**B**, M	**B**, **BM**, **MB**
Fertile sectors*	BM, MB, M	**B**	**B**	**B**
Total nodes	BM, MB, M	**B**, **MB**, M	**B**, BM, M	**B**, **BM**, **MB**
Ear node	BM, MB, M	**B**, **MB**	**B**, BM	**B**
Ear length	**BM**, **MB**, **M**	B, **M**	B, **M**	B, BM, MB
Tillering[a]	-	-	-	-

[a] Per chi-square; all others per *t*-test. $P < 0.05$

Plant measurements

The measurements for all of the examined quantitative traits are summarized in Table 4. The traits are listed with the mean measurements, standard deviations (SD) and standard errors (SE) for each haploid class. Haploids derived from B73 and Mo17 are designated "B" and "M," respectively. Those derived from B73/Mo17 and Mo17/B73 hybrids are designated as "BM" and "MB." Significant differences between each of the measurements are summarized in Table 5.

Plant height: The B haploids were significantly taller (119.3 cm) than the other three haploid classes. The other three haploid classes were not significantly different from each other (BM = 104.1 cm, MB = 109.4 cm, M = 109.1 cm).

Circumference: There were no significant differences among the four haploid classes. All were from 4.5 cm to 4.6 cm.

Leaf length: There were no significant differences. Leaf lengths ranged from 53.1 cm for the BM haploids to 54.2 cm for the M haploids.

Leaf width: The leaves of the M haploids averaged significantly wider (5.9 cm) than the other haploids. The other three haploids averaged 5.4 cm.

Tassel branches: The B haploids had a significantly higher average number of tassel branches (10.8) than the other haploids, while the M haploids were significantly lower (5.2) than the others. The hybrid-derived haploids were indistinguishable from each other (BM = 8.9, MB = 9.3).

Fertile tassel sectors: Because fertile tassel sectors were scored simply for their presence or absence, differences among the four haploid classes were judged using chi-square. The four haploid classes were not homogeneous ($P<0.02$). Nearly the entire chi-square statistic was because 23% of the B haploids had fertile sectors. The remaining three haploid classes were homogeneous with 4% to 6% of plants having at least one fertile sector.

Number of nodes: All four classes of haploids were significantly different from each other for the average number of nodes ($P<0.05$). The B haploids had the highest average number of nodes (12.7), while the M haploids had the lowest (10.4). It is interesting that the MB haploids had a significantly higher average number of nodes (11.8) than their BM counterparts (11.3), even though they are genetically equivalent.

Ear position: The average node position of the B haploid ears (7.2) was significantly higher than the other haploids. The average node position of the M haploid ears (6.2) was not significantly different from either of the hybrid-derived haploids. However, the MB haploid ears had a significantly higher average nodal position (6.7) than the BM-derived haploids (6.2).

Ear length: The average ear length of the M haploids (11.1 cm) was significantly longer than the other haploids, while the ears of the B haploids (7.7 cm) averaged the shortest. The ears of the BM-derived haploids (8.9 cm) and the MB-derived haploids (9.0 cm) were not significantly different from each other.

Tillers: Although the BM and MB haploids appeared to have a greater average number of tillers per plant (0.18 and 0.17, respectively) than B and M haploids (0.09 and 0.00, respectively), the four classes were not significantly heterogeneous ($P>0.05$).

Because the inbreds are essentially homozygous at all loci, all the haploids derived from them should be genetically identical. The haploids that were derived from the hybrids are expected to possess segregated

alleles of numerous genes. These expectations parallel the general observation that except for fertile tassel sectors, the standard deviations for the measurement of the hybrid-derived haploids exceeded the standard deviations for the inbred-derived haploids (Table 3).

Correlations of quantitative traits

Each plant was measured for up to ten different quantitative traits, and correlations were computed between the various traits for each of the four classes of haploids (Table 6). Positive correlations of quantitative traits among the hybrid-derived haploids may reflect pleiotropy of assorted alleles. The inbred-derived haploids are genetically homogeneous so they cannot display similar effects. Correlations of traits in either the B and M inbreds likely indicate that these two traits respond similarly to environmentally induced variation. Correlations between two traits of hybrid-derived haploids might also be a response to environment. The average of all correlations for the inbred-derived haploids was 0.095, whereas the average correlation of all of the hybrid-derived haploids was 0.237. This suggests that pleiotropy was involved in the variation of quantitative traits in the haploids.

Leaf width correlated positively with a number of other traits among the hybrid-derived haploids, but much less so with the inbred-derived haploids. This is most apparent for the correlations of leaf width with plant height, circumference, leaf length, and ear length. Other dimensional traits show trends that are more modest, e.g., plant height and ear length, leaf length, and ear length. Circumference, interestingly, did not correlate well with most of the other dimensional traits. It is likely that any underlying pleiotropic effect is related to cell number since plant height, leaf length, leaf width, and ear length also correlate significantly with total node number in the hybrid-derived haploids. The strongest correlations were between total node number and node position of the primary ear. While it may seem intuitive that these two

Table 6 Correlations of quantitative traits for each class of haploid. *n/d* Not determined

Trait		Circum-ference	Leaf length	Leaf width	Tassel branches	Tassel sectors	Nodes	Ear node	Ear length	Tillering
Height	B	0.292	0.057	0.266	0.069	0.152	0.445*	0.302	–0.011	–0.109
	BM	0.272*	0.508**	0.485**	0.273*	0.052	0.680**	0.524**	0.332**	0.090
	MB	0.246*	0.402**	0.470**	0.260*	0.307**	0.561**	0.466**	0.326**	–0.060
	M	0.288	0.582**	0.336	0.268	0.127	0.048	–0.132	0.017	n/d
Circumference	B		–0.282	0.296	0.418*	0.274	0.291	0.033	0.027	0.200
	BM		0.254	0.587**	0.054	–0.025	0.142	–0.103	0.185	0.064
	MB		0.496**	0.478**	0.224*	0.145	0.248*	0.233*	0.159	0.141
	M		0.452*	–0.067	0.183	0.043	–0.077	–0.332	–0.603*	n/d
Leaf length	B			–0.074	–0.238	0.313	0.063	–0.030	0.291	–0.286
	BM			0.415**	0.130	–0.095	0.464**	0.360**	0.481**	0.145
	MB			0.526**	0.220*	–0.039	0.313**	0.459**	0.300**	0.225*
	M			–0.259	0.114	0.055	–0.387	–0.408	–0.398	n/d
Leaf width	B				0.012	0.327	0.392	0.538**	–0.031	0.111
	BM				0.385**	0.076	0.441**	0.371**	0.278*	0.138
	MB				0.299**	–0.032	0.366**	0.377**	0.413**	0.078
	M				0.584**	0.292	0.087	0.352	0.247	n/d
Tassel branches	B					0.086	0.280	0.340	–0.090	0.089
	BM					0.108	0.291*	0.332**	0.132	0.134
	MB					0.122	0.206	0.228*	0.198	–0.113
	M					0.262	–0.330	0.316	–0.065	n/d
Tassel sectors	B						0.333	0.243	–0.110	0.084
	BM						0.103	0.066	0.132	–0.064
	MB						0.048	0.001	–0.224*	0.001
	M						0.251	0.225	n/d	n/d
Total nodes	B							0.870**	–0.436*	0.083
	BM							0.882**	0.362**	0.119
	MB							0.843**	0.270*	0.051
	M							–0.122	0.112	n/d
Ear node	B								–0.293	0.051
	BM								0.187	0.064
	MB								0.218*	0.117
	M								0.339	n/d
Ear length	B									–0.206
	BM									0.230
	MB									–0.252*
	M									n/d

Significance levels: * $P<0.05$, ** $P<0.01$

parameters are closely related, Mo17-derived haploids showed no correlation for these two traits. Tassel branching, fertile tassel sectors, and tillering showed no trends to suggest pleiotropy.

Discussion

To examine the question of whether inbreeding depression and heterosis have an accumulating heritable epigenetic component, inbreds of Mo17 and B73 and the two reciprocally produced hybrids were used to generate four classes of maternal haploids. The hybrid condition was established for one generation and then eliminated for all genes in the subsequent generation in order to assay for any heritable effect. The haploids derived from the hybrids were expected to show more variance than the inbred-derived haploids, because each hybrid-derived individual has a unique genotype (see Table 4). Even so, the trait means of the hybrid-derived haploids should be at the midpoint value of the two inbred-derived haploids. If inbreeding depression results from an accumulating heritable effect that is reversed by the hybrid state, one would expect the haploids derived from the hybrids to perform better than those derived from the inbred lines.

The hybrid-derived haploids demonstrated no advantage over the inbred-derived haploids as revealed by quantitative traits. For three traits (circumference, leaf length, and tillers per plant), there were no significant differences among any of the classes (Table 5). For the seven other traits, the inbred-derived haploids were significantly different from each other. In all seven cases, the hybrid-derived haploids were significantly below the higher scoring haploid, but in only three of those cases (tassel branches, total nodes, and ear length) were the hybrid-derived haploids greater than the lower scoring inbred-derived haploid (Table 5). The hybrid-derived haploids were not distinguishable from the lower scoring inbred-derived haploid for plant height, leaf width, fertile tassel sectors, and ear position (Table 5). For these four traits, hybrid-derived haploids appear to be under-performing.

It is not clear why, for plant height, leaf width, fertile tassel sectors, and ear position, the means of the hybrid-derived haploids would fail to exceed the lower performing inbred-derived haploid. It is unlikely to be an artifact of the haploid induction process. Although there may be segregating genetic factors in the hybrids that make some gametophytes more susceptible to haploid induction, it is difficult to imagine how these factors may be associated with less favorable adult traits except through random linkage. If the traits were associated with survivability of the haploids, then differences in survival rates could result in differences in means of physical traits. In order for this to be a factor, the association of trait-to-haploid-plant survival would have to be exceedingly strong, since the survival rate of the inbred-derived haploid plants (45%) is not greatly different from the hybrid-derived haploid plants (54%). Another possible explanation is epistasis. If favorable epistatic combinations of alleles at different loci were specifically selected in each of the two inbreds, then random assortment would tend to break up these favorable combinations. This assortment should result in lowering trait averages in the hybrid-derived haploids. Shaw et al. (1997) demonstrated that the detection of epistasis is facilitated in haploids, although that study examined moss gametophytes.

It can be reasoned that the breakup of favorable epistasis confounds the ability to observe metastable epigenetic changes that result from heterosis. However, for this to be true, epistatic effects would have to contribute more to vigor than epigenetic effects. Many studies detect little significant contribution of epistasis to variability (e.g., Stuber and Moll 1974; Silva and Hallauer 1975). Thus, even if metastable epigenetic changes are masked by a breakup of favorable epistatic conditions, they must not be a major factor contributing to heterosis and inbreeding depression.

One other possibility is that the inbred-derived haploids may have profited from a transient increase in gene expression. Hollick and Chandler (1998) observed that in the hemizygous state, *Pl1′-mah* resulted in higher expression, but that in the next generation the allele itself was not heritably affected. Such a non-metastable change would affect both the inbred-derived haploids as well as the hybrid-derived haploids. Thus, specific comparisons could be obscured by a general increase in quantitative trait values among all the haploids.

The epigenetic behavior of *R1* indicates that the effects of hemizygosity should not be confounding, because this state behaves like the heterozygote. The increase in color expression induced by hemizygosity is cumulative with epigenetic changes in previous generations and should be detectable (Styles and Brink 1969). Furthermore, unlike *Pl1′-mah*, changes in *R1* due to hemizygosity are metastable. If haploidy causes metastable changes in genes that affect quantitative traits, they should be seen in a subsequent generation. However, work by Kato (2002) on doubled haploids indicates that the haploid state does not invoke any obvious change in the quantitative characteristics of an inbred line, suggesting an absence of metastable heritable effects.

Whether transient or metastable, there appears to be no general up-regulation of gene expression in haploids, especially as it relates to quantitative traits. Guo et al. (1996) compared the steady state levels of 18 different RNA transcripts in haploid, diploid, triploid, and tetraploid maize plants. They found that the modal expression levels in the various ploidies remained equal relative to the gene dosage. For example, haploids tended to have approximately half the transcript levels per cell relative to diploids. There were exceptions, and more of them resulted from increased gene expression levels. Both triploids and tetraploids experienced a nearly identical array of exceptions (Guo et al. 1996). It is clear that these exceptions do not correlate with effects upon quantitative traits, because any deviation from diploidy in maize is

detrimental (Randolph et al. 1944). Specifically, haploid plants are smaller than what would be predicted, given the expectation that cell size in haploids is one-half that of diploids (Chase 1964). Taken together, the results suggest that there is no metastable epigenetic component to heterosis.

References

Aman MA, Sarkar KR (1978) Selection for haploidy inducing potential in maize. Indian J Genet 38:452–457

Bruce AB (1910) The Mendelian theory of heredity and the augmentation of vigor. Science 32:627–628

Chalyk S, Ostrovsky V (1993) Comparison of haploid and diploid maize (*Zea mays* L.) plants with identical genotypes. J Genet Breed 47:77–80

Chase SS (1949) Monoploid frequencies in a commercial double-cross hybrid maize, and its component singlecross hybrids and inbred lines. Genetics 34:328–332

Chase SS (1964) Monoploids and diploids of maize: a comparison of genotypic equivalents. Am J Bot 51:928–933

Chase SS (1969) Monoploids and monoploid derivatives of maize (*Zea mays* L.). Bot Rev 35:117–167

Coe EH (1959) A line of maize with haploid frequency. Am Nat 93:381–382

Cone KC, Cocciolone SM, Burr FA, Burr B (1993) Maize anthocyanin regulatory gene *pl* is a duplicate of *c1* that functions in the plant. Plant Cell 5:1795–1805

Deimling S, Röber F, Geiger HH (1997) Methodik und genetic der in-vivo-haploideninduktion bei mais. Vortr Pflanzenzüchtg 38:203–224

East EM (1936) Heterosis. Genetics 21:375–397

Eder J, Chalyk S (2002) In vivo haploid induction in maize. Theor Appl Genet 104:703–708

Emerson RA (1918) A fifth pair of factors, *Aa*, for aleurone color in maize, and its relation to the *Cc* and *Rr* pairs. Cornell Univ Agric Exp Stn Mem 16:231–289

Guo M, Davis D, Birchler JA (1996) Dosage effects on gene expression in a maize ploidy series. Genetics 142:1349–1355

Hollick JB, Chandler VL (1998) Epigenetic allelic states of a maize transcriptional regulatory locus exhibit overdominant gene action. Genetics 150:891–897

Hull FH (1945) Recurrent selection for specific combining ability in corn. J Am Soc Agron 37:134–135

Jones DF (1917) Dominance of linked factors as a means of accounting for heterosis. Genetics 2:466–479

Kato A (2002) Chromosome doubling of haploid maize seedlings using nitrous oxide gas at the flower primordial stage. Plant Breed 121:370–377

Kempton JH (1919) Inheritance of spotted aleurone color in hybrids of Chinese maize. Genetics 4:261–274

Kermicle JL (1970) Dependence of the *R*-mottled aleurone phenotype in maize on mode of sexual transmission. Genetics 66:69–85

Ludwig SR, Habera LF, Dellaprorta SL, Wessler SR (1989) *Lc*, a member of the maize *R* gene family responsible for tissue-specific anthocyanin production, encodes a protein similar to transcriptional activators and contains the *myc*-homology region. Proc Natl Acad Sci USA 86:7092–7096

Randolph LF, Abbe EC, Einset J (1944) Comparison of shoot apex and leaf development and structure in diploid and tetraploid maize. J Agric Res 69:47–77

Sarkar KR, Coe EH (1966) A genetic analysis of the origin of maternal haploids in maize. Genetics 54:453–464

Shaw AJ, Weir BS, Shaw FH (1997) The occurrence and significance of epistatic variance for quantitative characteristics and its measurement in haploids. Evolution 51:348–353

Shull GH (1914) Duplicated genes for capsule form in *Bursa pastoris*. Z Indukt Abstammungs Vererbungsl 12:97–149

Silva JC, Hallauer AR (1975) Estimation of epistatic variance in Iowa Stiff Stalk Synthetic maize. J Hered 66:290–296

Sprague GF, Russell WA, Penny LH (1960) Mutations affecting quantitative traits in the selfed progeny of doubled monoploid maize stocks. Genetics 45:855–866

Stuber CW, Moll RH (1974) Epistasis in maize (*Zea mays* L.). IV. Crosses among lines selected for superior intervariety single-cross performances. Crop Sci 14:314–317

Styles ED (1967) The metastable nature of paramutable *R* alleles in maize. III. Heritable changes in level of *R* action in heterozygotes carrying different paramutable *R* alleles. Genetics 55:411–422

Styles ED, Brink RA (1966) The metastable nature of paramutable *R* alleles in maize. I. Heritable enhancement in level of standard *R-r* action. Genetics 54:433–439

Styles ED, Brink RA (1969) The metastable nature of paramutable *R* alleles in maize. IV. Parallel enhancement of *R* action in heterozygotes with *r* and in hemizygotes. Genetics 61:801–811