Epigenetic Allelic States of a Maize Transcriptional Regulatory Locus Exhibit Overdominant Gene Action

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ABSTRACT

Using alleles of the maize *purple plant* locus (*pl*), which encodes a transcriptional regulator of anthocyanin pigment synthesis, we describe a case of single-locus heterosis, or overdominance, where the heterozygote displays a phenotype that is greater than either homozygote. The *Pl-Rhoades* (*Pl-Rh*) allele is subject to epigenetic changes in gene expression, resulting in quantitatively distinct expression states. Allelic states with low-expression levels, designated *Pl′-mahogany* (*Pl′-mah*), are dominant to the high-expression state of *Pl-Rh*. *Pl′-mah* states retain low-expression levels in subsequent generations when homozygous or heterozygous with *Pl-Rh*. However, *Pl′-mah* alleles frequently exhibit higher expression levels when heterozygous with other *pl* alleles; illustrating an overdominant allelic relationship. Higher expression levels are also observed when *Pl′-mah* is hemizygous. These results suggest that persistent allelic interactions between *Pl′-mah* and *Pl-Rh* are required to maintain the low-expression state and that other *pl* alleles are missing sequences required for this interaction. The *Pl-Rh* state can be sexually transmitted from *Pl′-mah*/*pl* heterozygotes, but not from *Pl′-mah* hemizygotes, suggesting that fixation of the high-expression state may involve synapsis. The existence of such allele-dependent regulatory mechanisms implicates a novel importance of allele polymorphisms in the genesis and maintenance of genetic variation.

HERITABLE changes in gene activity can, in some cases, be influenced by allelic interactions. Several examples of such interactions at loci affecting pigment production have been described in *Zea mays* (Hollick *et al.* 1997). The *pl* gene encodes a *myb*-like transcriptional regulator of the anthocyanin biosynthetic pathway, making the amount of visual pigment produced an excellent indicator of *pl* gene expression (Cocciolone and Cone 1993; Patterson 1993). The *Pl-Rhoades* (*Pl-Rh*) allele is highly expressed, producing strong pigment in most tissues of the juvenile and adult maize plant. However, the allele is unstable and subject to paramutation, a process in which one allele promotes a heritable alteration in the expression of another allele in the heterozygote (Hollick *et al.* 1995, 1997). At various frequencies (typically ~16% in our W23 inbred stocks), lighter pigmented progeny arise from crosses between *Pl-Rh* homozygous plants. This reduction in pigment is easily quantified in anther tissues, where we have defined a graded series of anther color scores (ACS; Hollick *et al.* 1995). Collectively, these lighter pigmented variants represent a continuum from virtually no color in the anthers (ACS of 1) to slightly less than that of *Pl-Rh* (ACS of 7). These reduced pigment phenotypes are due to the heritable alteration of *Pl-Rh* to states expressing lower levels of *pl* RNA (Patterson 1993) that are collectively designated *Pl′-mahogany* (*Pl′-mah*) (Hollick *et al.* 1995).

The behavior of different *Pl′-mah* states is distinct in subsequent crosses with homozygous *Pl-Rh* plants. *Pl′-mah* states with virtually no color (ACS of 1–2) are meiotically very stable and have a strong ability to change *Pl-Rh* alleles into *Pl′-mah*; in crosses of *Pl′-mah* plants to *Pl-Rh* homozygotes, only weakly pigmented phenotypes of *Pl′-mah* plants (ACS of 1–4) are recovered (Hollick *et al.* 1995). *Pl′-mah* states with intermediate levels of pigment (ACS of 3 or 4) frequently change to lower expression states, but changes to higher expression states (ACS of 5–7) have not been observed. The intermediate states are nonetheless capable of changing *Pl-Rh* to *Pl′-mah* with 100% efficiency. *Pl′-mah* states that confer slightly less color than *Pl-Rh* (ACS of 5 or 6) are metastable; they can change to lower expressed *Pl′-mah* states (ACS of 1–4), or they can change to fully expressed *Pl-Rh* states (ACS 7) that are unable to change *Pl-Rh* to *Pl′-mah* in subsequent generations. Although the molecular basis underlying the distinct expression states is not known, current evidence favors an epigenetic mechanism, perhaps involving heritable alterations in chromatin structure (Hollick *et al.* 1997).

The spontaneous instability of *Pl-Rh*, and the exclusive transmission of *Pl′-mah* alleles from heterozygotes, suggests that an open-pollinated population of homozygous *Pl-Rh* plants would change to exclusively *Pl′-mah* plants within several generations. The persistence of the highly

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expressed *Pl-Rh* allele in culture is undoubtedly due in large part to the stringent artificial selection imposed by maize geneticists. Additionally, the maintenance of the *Pl-Rh* state in maize stocks may be facilitated by *pl* allele polymorphisms or other genetic factors affecting the stability of the *Pl-Rh* or *Pl′-mah* states. Herein, we show that the stability of the *Pl′-mah* state is sensitive to allelic interactions and that *Pl′-mah* can change back to *Pl-Rh* when it is heterozygous with polymorphic *pl* alleles. In heterozygous combination, two weakly expressed *pl* alleles produce more than additive *pl* gene activity, thus illustrating an example of overdominant gene action. Potential implications of such allelic interactions to studies on quantitative genetic variation are discussed.

MATERIALS AND METHODS

Genetic stocks: *Pl-Rh* was maintained in the W23 inbred background and in four other genetic backgrounds of mixed parentage. The original *Pl-Rh sm* stock derives from an unknown genetic background (Hollick *et al.* 1995). *Pl′-mah* alleles arose spontaneously in three different genetic backgrounds and were subsequently propagated by self- or sib crossing. Self- or sib crossing propagated *pl-CO159* in a CO159 × Tx303 recombinant inbred line. The TB-6Lc stock was provided by the Maize Genetics Cooperation Stock Center, accession no. 614C (Urbana, IL). All stocks used in these experiments were also homozygous for a functional allele of the *r* locus required for anther pigmentation, *r-r*.

Genetic crosses: Hand pollinations were performed for all experiments. For the cosegregation experiment shown in Figure 2, three of the six families used *Pl′-mah sm*/*Pl′-mah sm* individuals as pistillate parents while the other three used *Pl-Rh sm*/*pl-CO159 Sm* individuals as pistillate parents. *Sm*/*sm* individuals have yellow silks, whereas *sm*/*sm* individuals have distinct salmon-colored silks. All heterozygotes contain at least one copy of the *P-rr* allele, which is required to score the *sm*/*sm* phenotype. All the *Pl′-mah sm*/*Pl′-mah sm* parental individuals used in this experiment were siblings from the same ear. The *Pl-Rh sm*/*pl-CO159 Sm* individuals derive from three closely related ears. Silk color was scored ~2 days after silk emergence from the husks.

For the segmental aneuploidy experiment in Figure 3, normal diploid individuals (*Pl′-mah*/*Pl′-mah*) were crossed as female with pollen from *Pl-Rh* individuals that carried two TB-6Lc *B-A* chromosomes (hyperploids) in which 90% of 6L is now attached to the supernumerary *B* chromosome. Over four successive growing seasons, progeny plants were scored for anther color on the 1–7 scale. Using a ×50 magnification pocket microscope, these plants were further classified, using frequency of pollen grain abortion as an indicator of 6L ploidy; hypoploids, euploids, and hyperploids have 50, 25, and <10% aborted pollen, respectively. For the initial crosses, hyperploid plants were identified by their low levels of pollen abortion, and these were confirmed to be hyperploids by crosses with recessive *pl* testers. Progeny of these testcrosses were primarily hyperploid (low pollen abortion) and hypoploid (~50% pollen abortion) individuals, and in 10/10 cases, the hyperploid individuals had a pigment phenotype like that of *Pl-Rh* plants. Following exposure of these *B-A* chromosomes to *Pl′-mah*, they now conferred phenotypes like that of *Pl′-mah* plants and they were fully capable of causing naive *Pl-Rh* alleles to change to *Pl′-mah* in subsequent crosses. Although the original hyperploid parents also have a normal chromosome *6* that carries a recessive, neutral *pl* allele, experimentally this chromosome is only rarely transmitted through a hyperploid male. Because meiosis will result in the segregation of a *B-A* chromosome with the normal chromosome, such pollen grains have a duplication of 6L genetic material. The failure to transmit the normal chromosome is presumed to reflect a selective growth, or fertilization, disadvantage for such pollen grains (Beckett 1991). We found no evidence in any subsequent generations that the *B-A* chromosomes carried neutral *pl* alleles. However, because low levels of recombination are expected to place a neutral *pl* allele onto a *B-A* chromosome, it is possible that a few of the hyperploids represented in Figure 3 are (*Pl′-mah*/*pl*/*pl*).

RESULTS

***Pl′-mah* alleles exhibit higher expression levels when heterozygous with other *pl* alleles:** Most *pl* alleles are "neutral" with respect to paramutation; they are unable to cause *Pl-Rh* to change to *Pl′-mah*, and their expression levels or their ability to paramutate *Pl-Rh* is unaffected by exposure to *Pl′-mah*. Previously we showed that when the *Pl′-mah* allele is heterozygous with the neutral *pl* allele, *pl-W23*, the *Pl′-mah* allele exhibits higher expression levels (Hollick *et al.* 1995). In some cases, a fully expressed *Pl-Rh* state was observed, and this state was sexually transmitted from such *Pl′-mah*/*pl-W23* heterozygotes (Table 1). However, in such crosses we could not distinguish between allele interactions leading to instability or the involvement of nonallelic genetic factors. To examine this further, we have determined the stability of *Pl′-mah* when heterozygous with other neutral alleles in

TABLE 1

Stability of *Pl′-mahogany*

Genotype	Frequency of *Pl-Rhoades* types
Pl′-mah/Pl-Rh[a]	0/>67,000
Pl′-mah/pl-W23[b]	8/356
Pl′-mah/plCO159[c]	4/41
Pl′-mah/pl-606B[d]	6/21

Pl-Rh anther color phenotpyes were noted from the progeny of matings using weakly colored *Pl′-mah* (ACS 1 or 2) homozygous plants as the female parent and either homozygous *Pl-Rh* or homozygous *pl-neutral* plants as the male parent.

[a] Data pooled from a series of experiments representing five distinct genetic backgrounds.

[b] Data originally presented in Hollick *et al.* (1995). All eight plants were subsequently confirmed to be *Pl-Rh/pl-W23* through testcrosses to *Pl-Rh/Rh-Rh* individuals.

[c] *pl-CO159* is the resident *pl* allele found in the CO159 inbred line. Seeds from two ears gave rise to four plants with pigment phenotypes of *Pl-Rh* plants; all four were confirmed by testcrosses. The distribution of anther color score phenoytpes seen in this family is presented in Figure 1.

[d] *pl-606B* is the resident "sun-red" *pl* allele found in the maize coop stock 606B. Six of 21 *Pl′-mah/pl-606B* plants scored over the span of two generations had pigment phenotypes of *Pl-Rh* plants. All six plants were confirmed to be *Pl-Rh/pl-606B* by testcrosses.

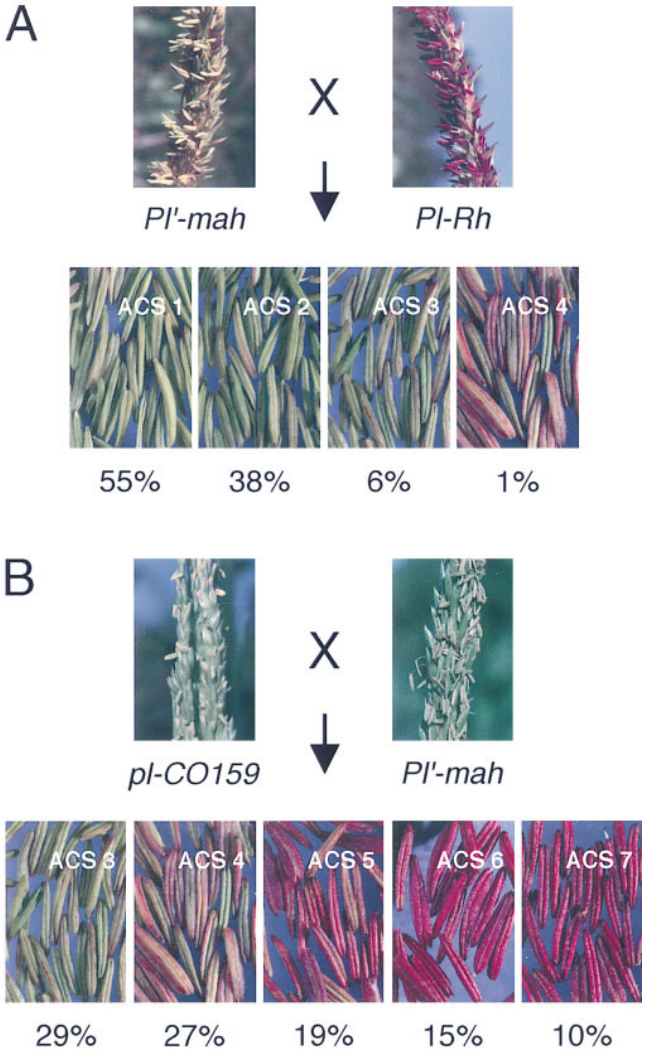

Figure 1.—Epigenetic behavior of *Pl′-mah*. (A) Crosses between *Pl′-mah* (*Pl′-mah*/*Pl-Rh*) and *Pl-Rh* (*Pl-Rh*/*Pl-Rh*) individuals exclusively produce progeny with pigment phenotypes of *Pl′-mah* plants. Percentages of progeny with distinct anther pigment phenotypes (ACS) are indicated for 678 plants derived from the seeds of 14 ears (data from Hollick *et al.* 1995). (B) Crosses between *pl-CO159* (*pl-CO159*/*pl-CO159*) and *Pl′-mah* (*Pl′-mah*/*Pl′-mah*) individuals produce more progeny with greater anther pigmentation than either parent. Percentages of progeny with distinct anther pigment phenotypes are indicated for 41 plants derived from the seeds of two ears.

distinct genetic backgrounds (Table 1). One particular *pl* allele (*pl-CO159*) produces no detectable pigment or RNA and is thought to be nonfunctional (Cone *et al.* 1993). Most *pl-CO159*/*Pl′-mah* heterozygotes showed increased pigment levels relative to their parents, and nearly 10% of these had *Pl-Rh*-like levels of pigment (Figure 1B, Table 1). Crosses of these *Pl-Rh*-like plants with a *Pl-Rh*/*Pl-Rh* tester resulted in 47/52 progeny with the phenotype of *Pl-Rh* plants. Although the frequency of the *Pl′-mah* exceptions is the same as that typically seen spontaneously among *Pl-Rh* homozygotes, it is possible that the exceptions represent the transmission of some *Pl′-mah* gametes. These results confirm that *Pl′-mah* had changed to *Pl-Rh* in ~10% of the *Pl′-mah*/*pl-CO159* heterozygotes, and this change was, for the most part, heritable. We have also observed and confirmed these changes when *Pl′-mah* is heterozygous with one other neutral *pl* allele, *pl-606B* (Table 1). All three neutral *pl* alleles tested have distinct pigment phenotypes, and restriction fragment analyses demonstrate that both *pl-CO159* and *pl-W23* are structurally polymorphic from each other and from *Pl-Rh* (Cone *et al.* 1993; K. Cone, J. Hollick, and V. Chandler, unpublished results). The DNA structure of *pl-606B* has not yet been determined.

Increased gene activity cosegregates with the presence of a neutral *pl* allele: As several different genetic backgrounds were used in these experiments, it seemed likely that the neutral *pl* alleles facilitated the instability of *Pl′-mah*. However, it was formally possible that other loci, rather than the neutral alleles, were responsible. As such, we tested whether the ability of *Pl′-mah* to change to higher expression states cosegregated with a neutral allele between individuals from single crosses. A linked morphological marker (*salmon silks*, *sm*), which is 10 cM distal to *pl* on chromosome *6*, was used to indicate the *pl* allele combination. Homozygous recessive *sm* individuals have salmon-colored silks, while *Sm*/*sm* heterozygotes have yellow silks. Anther color scores were determined for the plants derived from six ears produced by the crossing scheme illustrated in Figure 2A. Scoring for the *sm* marker allowed classification by *pl* allele based on linkage to *sm*. The *sm*/*sm* (*Pl-Rh*/*Pl′-mah*) class had lower ACS and no potential *Pl-Rh* plants relative to the *Sm*/*sm* (*pl-CO159*/*Pl′-mah*) class (Figure 2B). All six families gave the same result; a higher frequency of individuals with increased *pl* gene expression cosegregated with the *Sm* allele. The average ACS values between the *sm*/*sm* and *Sm*/*sm* classes were significantly different from one another ($P \ll 0.01$; two-sample *z*-test). These results indicate that the ability to cause increased gene expression of the *Pl′-mah* allele is genetically linked to the homologous region of chromosome *6* containing a neutral *pl* allele. Furthermore, the observation that most heterozygotes (*Pl′-mah*/*pl-CO159*) have higher gene expression levels than either homozygote represents an overdominant allelic relationship. Overdominance is defined as an allelic relationship whereby the heterozygote displays a phenotype exceeding that of either homozygote.

Hemizygosity of *Pl′-mah* leads to increased gene expression: Two simple explanations for this overdominant relationship are that either the neutral allele actively promotes the instability of the *Pl′-mah* state, or allelic interaction between *Pl′-mah* and *Pl-Rh* are required to maintain the reduced expression states. To address these possibilities, we used B-A translocation stocks to create a 6L dosage series with zero, one, or two doses of *Pl-Rh* combined with one dose of *Pl′-mah*

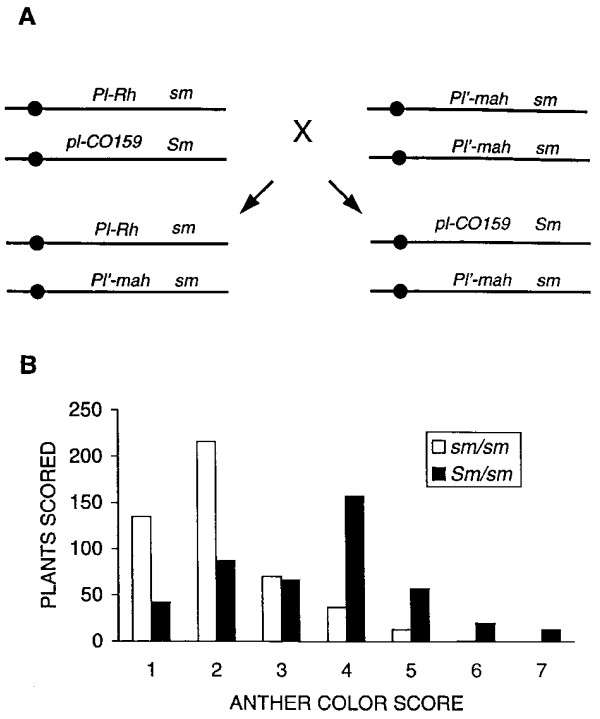

Figure 2.—Segregation analysis shows that a *pl* allele exhibits its overdominant gene action. (A) Schematic diagram of parental and segregant chromosomes with the relevant genetic markers. (B) Histogram representing *pl* gene expression among the segregant progeny derived from six ears. Open bars, *sm*/*sm* individuals; solid bars, *Sm*/*sm* individuals.

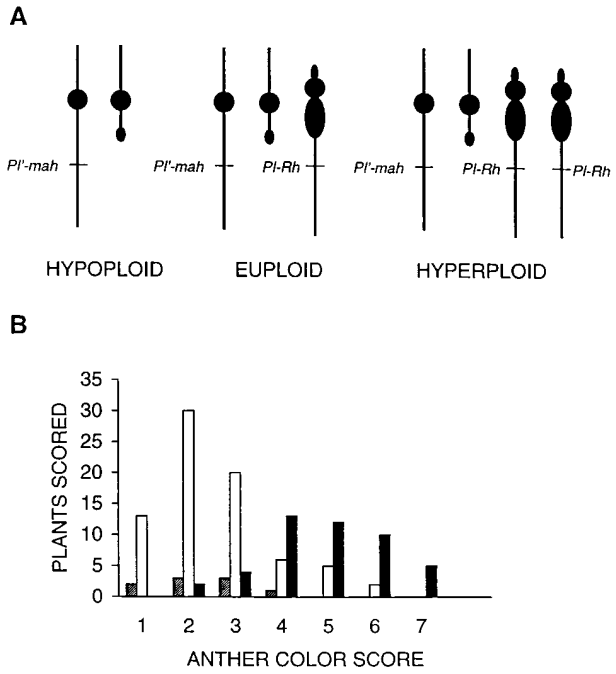

Figure 3.—Hemizygosity leads to increased *pl* gene expression. (A) Schematic diagram of the chromosome 6L dosage series generated through the use of *B-A* translocation chromosomes. The *B* chromosome segments are enlarged relative to the normal chromosome *6* regions. (B) Histogram representing *pl* gene expression among hyperploid (open bars), euploid (crosshatched bars), and hypoploid (solid bars) individuals. Data represent individuals derived from two ears scored over four growing seasons. Higher frequencies of hyperploid *vs.* hypoploid individuals are typically observed with *B* chromosomes (Beckett 1991).

and then examined the anther pigment phenotypes of the resulting progeny. *Pl′-mah*/*Pl′-mah* individuals were crossed with pollen from an individual that carried *B-A* translocations consisting of most of chromosome 6L, carrying *Pl-Rh*, linked to the supernumerary *B* chromosome centromere (see materials and methods). Due to a high frequency of B centromere nondisjunction during the second mitotic division of the male gametophyte (Roman 1947), progeny are produced that are hypoploid (*Pl′-mah*/−) or hyperploid (*Pl′-mah*/*Pl-Rh*/*Pl-Rh*). Euploid progeny (*Pl′-mah*/*Pl-Rh*) are also produced when nondisjunction fails to occur. Figure 3A illustrates the relevant chromosomes in these different individuals. These three classes are distinguishable by different percentages of pollen abortion, which can be scored with a hand-held pocket microscope. Anthers of progeny from the above-mentioned cross were scored on the 1–7 ACS scale, and the amount of pollen abortion was then used to discriminate among the various 6L classes.

More plants in the hemizygous (hypoploid) class had higher expression levels than did plants in either the euploid or hyperploid classes (Figure 3B). The average ACS value of the hypoploid class was significantly different from the average ACS values of both the hyperploid ($P \ll 0.01$; two-sample *z*-test) and euploid ($P = 0.01$; two-sample *z*-test) classes. In addition, the plants with the highest expression levels were *Pl′-mah*/− hemizygotes. The increase in expression observed in the hemizygote is unlikely to be due simply to gene dosage. If gene expression were inversely proportional to gene dosage, one would predict that the euploid class (*Pl′-mah*/*Pl-Rh*) would have pigment values intermediate to those seen in the hypoploid and hyperploid classes, which is not observed. The average ACS score of the euploid class (2.3 ± 0.72; 95% confidence interval is ± 2 SE, calculated with d.f. = 8) was not statistically different from the average ACS score of the hyperploid class (2.55 ± 0.28; 95% confidence interval is ± 2 SE). However, there are examples where dosage compensation upregulates some hemizygous genes (Guo and Birchler 1994). We think this is unlikely in our examples because dosage compensation usually results in 2- to 3-fold changes in expression, and the phenotype of the *Pl′-mah* hemizygote suggests >10-fold increase in expression (Patterson 1993). In addition, we would expect to see increased expression in every hemizygous plant, which is not observed.

Even though the hypoploid class had many plants with a phenotype like that of *Pl-Rh* plants, it was unknown whether *Pl′-mah* had heritably changed to *Pl-Rh*. An

additional cross was performed to determine whether or not the *pl* allele in the *Pl′-mah*/− hemizygotes was capable of causing paramutation. Three of the ACS 7 hypoploids and one of the ACS 6 hypoploids were crossed to homozygous *Pl-Rh* plants. Progeny with an ACS of 7 would be expected if *Pl′-mah* had changed to *Pl-Rh* in the *Pl′-mah*/− individuals. This was not observed; all of the progeny (95/95) from these crosses had phenotypes like that of *Pl′-mah* plants. This indicates that these hypoploids with strongly pigmented anthers transmitted only *Pl′-mah* alleles. Thus, when hemizygous, the expression levels of *Pl′-mah* increase to those of *Pl-Rh* in some plants, but these alleles are not transmitted in the *Pl-Rh* state, as they can still promote paramutation of naive *Pl-Rh* alleles. These results contrast with the *Pl-Rh* examples found among *Pl′-mah*/*pl-neutral* heterozygotes (Table 1), in which plants with an ACS 7 transmitted *Pl-Rh*.

DISCUSSION

Our results demonstrate that the stability of *Pl′-mah* depends on the other allele. *Pl′-mah* is very stable when homozygous or when heterozygous with *Pl-Rh*. In contrast, the expression of *Pl′-mah* frequently increases when it is heterozygous with a neutral allele or when hemizygous. The stronger expression phenotype of the *Pl′-mah*/*pl-CO159* heterozygote when compared to the phenotype of either homozygote represents an example of overdominance or single-locus heterosis. The presence of *pl* allelic interactions results in a repression of gene activity, whereas the absence of allelic interactions results in increased gene activity.

Gene activity is increased when *Pl′-mah* is heterozygous with a neutral allele or when hemizygous: While the expression of *Pl′-mah* frequently increases when it is heterozygous with a neutral allele or hemizygous, increases in *Pl′-mah* expression do not occur in every instance. One possibility is that the changes in state are stochastic and that absence of allelic interactions increases the frequency that a change to a higher expression state can occur but does not dictate that it will occur in every individual. It is also possible that changes occur at high frequency in all allele combinations, but in the absence of interacting DNA sequences, the higher expression states are more stable and, thus, more frequently observed. Additionally, or alternatively, there may be intrinsic differences between individual *Pl′-mah* alleles following meiotic segregation, or there may be modifier loci that play a role in stabilizing higher expression states.

The observations that gene expression is increased when *Pl′-mah* is hemizygous and when *Pl′-mah* is heterozygous with neutral *pl* alleles suggests that neutral alleles do not interact with *Pl′-mah* and that the low-expressing *Pl′-mah* state is destabilized by the absence of allelic interactions. Direct interactions between *Pl′-mah* alleles or between *Pl′-mah* and *Pl-Rh* alleles are thus presumed to favor the lower expression state. In this interpretation, neutral alleles are hypothesized to lack specific sequences that are found in *Pl-Rh* and *Pl′-mah* alleles. One prediction from these results is that fully expressed *Pl-Rh* alleles would be more stable, less likely to undergo spontaneous changes to *Pl′-mah*, when maintained in heterozygous association with a neutral allele. Long-term experiments are in progress to address this prediction.

When *Pl′-mah* is hemizygous, its expression levels increase to those of *Pl-Rh* in some plants, but these alleles are not transmitted in the *Pl-Rh* state as they can still promote paramutation of naive *Pl-Rh* alleles. These results contrast with the *Pl-Rh* examples found among *Pl′-mah*/*pl-neutral* heterozygotes, which transmitted *Pl-Rh*. Unlike the heterozygotes, the hypoploids do not have homologous-pairing partners. Thus, one very intriguing possibility is that fixation of the *Pl-Rh* state for meiotic transmission may depend on homologue synapsis during meiotic prophase. These results further suggest that the establishment of the *Pl-Rh* expression states may be mechanistically separable from the heritable maintenance of the expression states through meiosis.

The fact that none of the hyperploid individuals (*Pl′-mah*/*Pl-Rh*/*Pl-Rh*) had a phenotype like that of *Pl-Rh* plants (Figure 3B) demonstrates that paramutation can occur between multiple *pl* alleles within the span of a single generation. However, unlike diploid (*Pl′-mah*/*Pl-Rh*) plants from the related W23 inbred line, which all had anther color scores between 1 and 4 (Hollick *et al.* 1995), the hyperploid class did have a number of plants with anther color scores of 5 and 6. More euploid individuals will need to be scored to determine if these ACS 5 and 6 plants are due to two *Pl-Rh* alleles being less efficiently paramutated than one.

Comparison to other allele-dependent regulatory mechanisms: Paramutation interactions have been detailed for three of the four maize loci that encode transcriptional activators of the anthocyanin biosynthetic enzymes. While certain features are similar, each case of paramutation has unique properties, implicating either distinct molecular mechanisms or intrinsic differences in how alleles at a particular locus interface with common regulatory machinery (Hollick *et al.* 1995, 1997). Two of these three examples, *pl* and *r*, show an increased frequency of enhanced expression when heterozygous with neutral alleles or when hemizygous. The pigment levels of both the *R-r* allele and its weakly pigmented, paramutant derivative *R-r′*, are heritably increased by exposure to either a neutral *r* allele or to a small chromosomal deficiency spanning the *r* locus (Styles and Brink 1968). These changes in *r* gene expression are meiotically heritable. By repeated exposure to a neutral allele or a small deficiency over several generations, *R-r′* will change back to the original *R-r* level of pigment. Changes of *Pl′-mah* to *Pl-Rh* can occur within the span

of a single generation. In contrast to the *r* and *pl* examples, the paramutant *B′* allele does not change to higher expression states when carried over neutral *b* alleles (Coe 1966; Patterson *et al.* 1995). While large numbers of plants hemizygous for *B′* have yet to be generated, no increases in *B′* expression levels have been observed among the 40 hemizygotes examined to date (V. Chandler, unpublished results). Thus, the *B′* state is much more stable than either the *Pl′-mah* or *R-r′* states.

Allele-dependent regulation is not unique to these examples of paramutation. Gene regulatory systems exist in Drosophila, where the hemizygote exhibits more gene expression than the homozygote. These are cases where proper gene repression is dependent upon chromosome-pairing interactions. In the classic example of bithorax-complex transvection (Lewis 1985), proper developmental repression of the *Ubx* gene is dependent upon the ability to pair; hemizygosity leads to gene activity in ectopic positions within the developing fly. With certain *zeste* mutations, the activity of the *white* gene is reduced when the alleles can pair, yet this repression is relieved when pairing is disrupted or when the *white* allele is hemizygous (Jack and Judd 1979). Additionally, several examples of pairing-sensitive DNA elements have been identified that facilitate *Polycomb*-mediated repression. These elements have the remarkable ability to confer silencing on transgene constructs when homozygous, yet these transgenes regain activity when hemizygous (Kassis 1994; Gindhart and Kaufman 1995).

Additional examples of allele-influenced gene silencing are found among plant transgenes. In a recent study on *uidA* transgenes in tobacco (Nap *et al.* 1997), the activity of complex alleles (loci composed of multiple transgenes arranged in an unknown orientation) was observed to be enhanced when maintained in a hemizygous state. Results from several other studies have implicated a dosage-sensitive repression of transgene expression, suggesting that an RNA threshold exists beyond which homologous RNA molecules are rapidly degraded (Jorgensen 1995; Metzlaff *et al.* 1997). Hemizygotes are less likely to exceed these thresholds and would therefore not experience a high frequency of silencing.

Epigenetic sources of heritable variation: Our results demonstrate that the primary determinant of a quantitative trait (plant color) has alleles that exhibit overdominant gene action. Multiple mechanisms responsible for overdominant allelic interactions have been proposed and discussed, with most models focusing on interactions of gene products (Crow 1952). In the *pl* example, it is gene activity that is increased in the heterozygote. Some of the models for overdominance predict that a hemizygote would have the same phenotype as the heterozygote. While other models are inconsistent with this prediction, we are unaware of any arguments that exclude an overdominant relationship between two alleles, based on a similar phenotype in the hemizygote. The key feature of the *pl* example of overdominance is that allelic interactions are required to maintain repression of gene activity; the absence of such interactions results in increased gene activity.

Our example of overdominance may be relevant to the debate concerning the poorly understood phenomenon of heterosis. The term "heterosis" describes the process(es) responsible for the superiority of traits in the F_1 over that observed in their parents. The underlying mechanisms responsible for this phenomenon are likely to be diverse, and specific hypotheses have been the subject of considerable debate for over 90 yr (Schull 1908; East 1936; Sprague 1953; Crow 1993). Overdominance has been suggested as one cause of heterosis because the heterozygote would display a phenotype exceeding that of either homozygote (Hull 1945). We think it is important to consider the possibility that allele-dependent mechanisms of gene regulation could also contribute to heterosis. Interestingly, in discussing heterosis, Schull noted "that these differences [between uniting gametes] need not be Mendelian in their inheritance" (Schull 1948).

Quantitative traits are highly uniform among hybrid maize plants produced by intercrosses of elite inbred lines. In contrast, heterozygosity in the *pl* case leads to considerable variation rather than a uniform level of gene action. Due to the mixed genetic parentage of our material, it remains unclear whether the variation in our experiments is due to the segregation of additional genetic modifiers or reflects a stochastic variation solely determined by the *pl* locus itself.

Molecular genetic mapping of quantitative trait loci may be complicated by alleles subject to epigenetic regulatory mechanisms. In maize, molecular mapping experiments show that heterozygosity for certain chromosomal regions is often positively correlated with increased performance of specific traits (Stuber *et al.* 1992); although such relationships appear to be rare in rice (Xiao *et al.* 1995). However, because the molecular nature of these quantitative trait loci are mostly unknown, genetic and molecular mapping studies cannot always discriminate between apparent overdominance (cases where dominant complementation of deleterious recessive alleles at linked loci lead to increased phenotypic traits) and true overdominance (cases where single-gene heterozygosity leads to an increase in phenotypic traits). In generating the mapping populations themselves, the gene activity of some alleles may be heritably changed. In some cases, similar to the example of *Pl′-mah* changing to *Pl-Rh* in the *Pl′-mah*/*pl-CO159* heterozygote, gene activity could be heritably increased, giving the impression that recombination has uncoupled genetic linkage from a deleterious recessive allele.

Allele-dependent regulatory systems also have important implications for the maintenance of allele polymorphisms. Epigenetic changes in *pl* gene activity are clearly influenced by specific allelic combinations. In the ab-

sence of strong selection, the highly expressed *Pl-Rh* state would be driven to extinction without the presence of neutral alleles. Thus, even if a given neutral allele is selected against when homozygous, it may have a selective advantage when heterozygous with other alleles. The combination of alleles subject to epigenetic changes together with neutral alleles that influence those changes provides a diverse and dynamic source of heritable variation.

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LITERATURE CITED

Beckett, J. B., 1991 Cytogenetic, genetic and plant breeding applications of B-A translocations in maize, pp. 493–529 in *Chromosome Engineering in Plants: Genetics, Breeding, Evolution*, Vol IIA, edited by P. K. Gupta and T. Tsuchiya. Elsevier, Amsterdam.

Cocciolone, S. M., and K. C. Cone, 1993 *Pl-Bh*, an anthocyanin regulatory gene of maize that leads to variegated pigmentation. Genetics **135:** 575–588.

Coe, E. H., Jr., 1966 The properties, origin, and mechanism of conversion-type inheritance at the *B* locus in maize. Genetics **53:** 1035–1063.

Cone, K. C., S. M. Cocciolone, F. A. Burr and B. Burr, 1993 Maize anthocyanin regulatory gene *pl* is a duplicate of *c1* that functions in the plant. Plant Cell **5:** 1795–1805.

Crow, J. F., 1952 Dominance and overdominance, pp. 282–297 in *Heterosis*, edited by J. W. Gowen. Iowa State College Press, Ames, IA.

Crow, J. F., 1993 Mutation, mean fitness, and genetic load, pp. 3–42 in *Oxford Surveys in Evolutionary Biology*, Vol IX, edited by D. Futuyma and J. Antonovics. Oxford University Press, London.

East, E. M., 1936 Heterosis. Genetics **21:** 375–397.

Gindhart, J. G., Jr., and T. C. Kaufman, 1995 Identification of *Polycomb* and *trithorax* group responsive elements in the regulatory region of the Drosophila homeotic gene *Sex combs reduced*. Genetics **139:** 797–814.

Guo, M., and J. A. Birchler, 1994 Trans-acting dosage effects on the expression of model gene systems in maize aneuploids. Science **266:** 1999–2002.

Hollick, J. B., G. I. Patterson, E. H. Coe, Jr., K. C. Cone and V. L. Chandler, 1995 Allelic interactions heritably alter the activity of a metastable maize *pl* allele. Genetics **141:** 709–719.

Hollick, J. B., J. E. Dorweiler and V. L. Chandler, 1997 Paramutation and related allelic interactions. Trends Genet. **13** (8): 302–308.

Hull, F. G., 1945 Recurrent selection and specific combining ability in corn. J. Am. Soc. Agron. **37:** 134–145.

Jack, J. W., and B. H. Judd, 1979 Allelic pairing and gene regulation: a model for the *Zeste-white* interaction in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **76:** 1368–1372.

Jorgensen, R. A., 1995 Cosuppression, flower color patterns and metastable gene expression states. Science **268:** 686–691.

Kassis, J. A., 1994 Unusual properties of regulatory DNA from the Drosophila *engrailed* gene: three "pairing-sensitive" sites within a 1.6-kb region. Genetics **136:** 1025–1038.

Lewis, E. B., 1985 Regulation of the genes of the bithorax complex in *Drosophila*. Cold Spring Harbor Symp. Quant. Biol. **50:** 155–164.

Metzlaff, M., M. O'Dell, P. D. Cluster and R. B. Flavell, 1997 RNA-mediated RNA degradation and chalcone synthase A silencing in Petunia. Cell **88:** 845–854.

Nap, J.-P., A. J. Conner, L. Mlynarova, W. J. Stiekema and R. C. Jansen, 1997 Dissection of a synthesized quantitative trait to characterize transgene interactions. Genetics **147:** 315–320.

Patterson, G. I., 1993 Paramutation: a directed, meioticaly heritable change in gene expression. Ph.D. thesis, Department of Biology, University of Oregon, Eugene, OR.

Patterson, G. I., K. M. Kubo, T. Shroyer and V. L. Chandler, 1995 Sequences required for paramutation of the maize *b* regulatory gene map to a region containing the promoter and upstream sequences. Genetics **140:** 1389–1406.

Roman, H., 1947 Mitotic nondisjunction in the case of interchanges involving the B-type chromosome in maize. Genetics **32:** 391–409.

Schull, G. H., 1908 The composition of a field maize. Am. Breeders' Assoc. Rep. **4:** 296–301.

Schull, G. H., 1948 What is "heterosis"? Genetics **33:** 439–446.

Sprague, G. F., 1953 Heterosis, pp. 113–136 in *Growth and Differentiation in Plants*, edited by W. E. Loomis. Iowa State College Press, Ames, IA.

Stuber, C. W., S. E. Lincoln, D. W. Wolff, T. Helentjaris and E. Lander, 1992 Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics **132:** 823–839.

Styles, E. D., and R. A. Brink, 1968 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.

Xiao, J., J. Li, L. Yuan and S. D. Tanksley, 1995 Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. Genetics **140:** 745–754.

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