Mapping the *mop3* gene

By

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ABSTRACT

Mutations in the *mop3* gene interfere with epigenetic regulation of gene silencing and cause pleiotropic effects including reduced fertility, shortened stature, and increased anthocyanin pigmentation. Analysis of *mop3-1/mop3-1* plants yielded conflicting results about the identity of the mutation. Homozygous *required to maintain repression6-1* (*rmr6-1*) mutant plants have a similar phenotype to *mop3-1* mutants, but complementation testing failed to produce a phenotypically wild type plant; this suggests that the mutations are in the same gene. Molecular analysis of the two mutants showed that while siRNA levels were decreased in *rmr6-1* mutants, levels were unchanged in *mop3-1* mutants, indicating that the mutations occur in different genes. Simple sequence repeats (SSRs) were used in linkage analysis to classify *mop3-1* as a mutation separate from rmr6-1, and to define the *mop3* interval as a 2.5Mb sequence containing 32 BACs on the short arm of chromosome 1.

INTRODUCTION

Paramutation

Paramutation is the directed, heritable alteration of the expression of one allele when heterozygous with another allele (Dorweiler, et al., 2000). This is in contrast to mutations that arise from altered DNA sequence, as paramutation is the interaction between epialleles. Epialleles are alleles of a gene that have identical DNA sequences, but differ in DNA methylation and chromatin compaction. Epialleles differ in levels of gene transcription, and are distinguished as paramutagenic, paramutable, or neutral. Paramutagenic epialleles have the ability to induce an altered expression state in the susceptible paramutable epialleles. Neutral epialleles are also called non-paramutagenic, and they neither cause nor respond to paramutation.

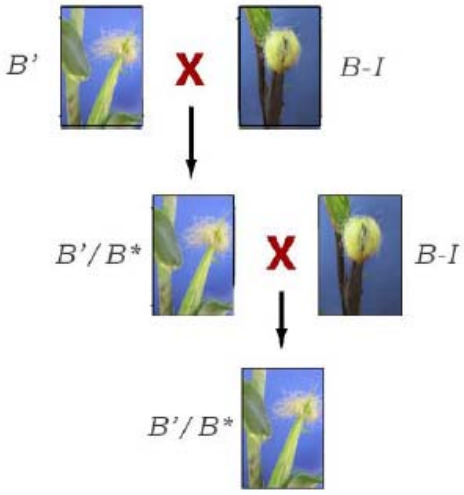
The heritability of an altered expression state is based on two distinct mechanisms of paramutation: establishment and maintenance. Establishment is the ability of the paramutagenic allele to change the expression of the paramutable allele when heterozygous with each other. Maintenance is the persistence of the altered expression in subsequent generations.

Paramutation is described in maize for five loci (reviewed extensively in Chandler et al, 2000). Four of the loci affect pathways responsible for flavonoid biosynthesis. Flavonoids are water-soluble plant pigments; the two studied in maize are anthocyanin, a purple pigment, and phlobaphene, which is red. The intensity of the pigment is determined by the amount of transcription factors produced from genes at these loci. The first locus with paramutation is *r1* (*red1*), which encodes a transcription factor that results in the production of red pigment expressed mainly in the seeds. The mechanism of the

second gene, *b1* (*booster1*), is identical, but results in purple pigmentation in the stalk, leaves, and other vegetative tissues. A transcription factor encoded at a third locus, *pl1* (*purple plant1*), activates the anthocyanin pathway by interaction with transcription factors from *b1* and *r1*. The pigment is expressed in the anthers as well as the tissues affected by *b1* and *r1* (reviewed in Chandler et al, 2000). The fourth gene participating in paramutation is *p1* (*pericarp color1*), which activates biosynthesis of phlobaphene in mature ears (Sidorenko and Peterson, 2001).

At the *b1* locus, paramutation occurs between the two epialleles *B'* and *B-I*. The weakly expressed *B'* produces light purple plant pigment, whereas *B-I* transcription is 10-20 times greater than that of *B'* and results in a solid purple plant color (reviewed in Chandler et al, 2000). When heterozygous, the paramutagenic *B'* permanently alters the expression of the paramutable *B-I.* The silenced allele is indistinguishable from *B'* in its ability to paramutate *B-I* alleles in subsequent generations, but is denoted as *B'** to indicate its paramutant state.

Figure 1. Paramutation at the *b1* locus



B' paramutates *B-I* to produce *B'**. The newly paramutagenic *B'** is able to paramutate *B-I* alleles in future generations.

Mutations that affect paramutation

There are several types of mutations that affect paramutation. These mutations were identified in specialized mutagenesis screens as preventing the establishment and/or maintenance of silencing associated with paramutation. One group of these mutations is the *mediator of paramutation* (*mop*). Mutations in three *mop* genes have been identified. The *mop1* (*mediator of paramutation1*) gene encodes an RNA-dependent RNA polymerase. Mutations in *mop1* cause reactivation of silenced *Mutator* elements (Alleman et al, 2006). The *Mop2* gene encodes the second largest subunit of a DNA-dependent RNA polymerase (Sidorenko et al, 2008). The third gene, *mop3*, is not yet cloned and is the focus of this thesis.

A second group of mutations, called *required to maintain repression* (*rmr*), was isolated using the *pl1* gene paramutation system and the effects on anther color. *Required to maintain repression1*, or *rmr1*, is an SNF2 chromatin remodeler. Another gene, *rmr6*, encodes the largest subunit of PolIV, a DNA-dependent RNA polymerase (Erhard et al, 2009).

mop3-1 mutation affects paramutation at b1

The *mop3-1* mutation was isolated in an EMS (ethyl methane sulfonate) mutant screen. Pollen from a *B-I* plant was treated with EMS suspended in paraffin oil (Neuffer, 1994) and placed on the silks of *B'* plants. The resulting F_1 plants were light colored indicating that paramutation had occurred normally. The F_1 plants were self-pollinated and a family that segregated ¼ dark plants was recovered in F_2. This family was designated as carrying the *mop3-1* mutation (Chandler, unpublished data).

Figure 2. Phenotype of the *mop3-1* mutation

The plant on the left is *B'/B';mop3-1/mop3-1*. The plant on the right is *B'/B';mop3-1/+*. Photo credit: Lyudmila Sidorenko, 2004

Genetic tests demonstrated that *mop3-1* is a recessive mutation, (not shown, Chandler Lab) and that it segregates at the expected Mendelian ratios in test crosses with *b*, a neutral allele (Sidorenko, unpublished data). The *mop3-1* mutation affects both the establishment and maintenance of paramutation at the *b1* locus (Chandler Lab, unpublished data). Plants that are *B-I*/*B';mop3-1/mop3-1* are darkly pigmented, indicating that the establishment of paramutation, or the ability of *B'* to paramutate *B-I*, has been prevented by *mop3-1*. When dark *B'/B';mop3-1/mop-3-1* plants are crossed with wild type plants (*B'/B';+/+*), the progeny are lightly pigmented. The reappearance of the light phenotype indicates that the *B'* allele was not heritably altered to *B-I*, but that wild type *Mop3* is required for the maintenance of the decreased expression levels in paramutated plants.

Initial genetic experiments showed that the *mop3-1* mutation fails to complement the *required to maintain repression6-1* (*rmr6-1*) mutation (Hollick and Chandler, personal communication). *Rmr6* encodes the largest subunit of Polymerase IV, a plant-specific RNA polymerase (Erhard et al, 2009). When *mop3-1* homozygotes were crossed with *rmr6-1* homozygotes, the progeny displayed the mutant phenotype, which suggests that mutations may have occurred in the same gene. However, further molecular analysis revealed that the two mutations had different effects on global levels of short interfering RNA (siRNA). Previous experimentation showed that siRNAs, which are 20-25 nucleotide long double-stranded RNA molecules, are involved in transcriptional gene silencing in Arabidopsis thaliana (Kanno et al, 2005). The overall levels of siRNA were reduced in *rmr6-1* mutants (Erhard et al, 2009). Similar experiments with *mop3-1*, showed that no significant changes in total siRNA levels are observed (Arteaga-Vazquez, unpublished data).

To conclusively determine the relationship between *rmr6-1* and *mop3-1*, linkage analysis was performed using simple sequence repeats (SSRs) as markers. An SSR is a sequence of 2 to 6 nucleotides that are tandemly repeated a variable number of times. These regions are often polymorphic between backgrounds, making them an invaluable resource for genetic mapping. The distance between the polymorphic region, which serves as a co-dominant DNA marker, and an unknown gene is determined by recombinant analysis. PCR amplification of the region produces distinct fragment sizes between the mutant and wild type parentals based on the number of repeats of the SSR. The differences in fragment sizes are distinguishable using agarose gel electrophoresis, followed by ethidium bromide staining and ultraviolet transillumination. Linkage

between the marker and the gene of interest varies based on the location of the marker relative to the gene. As the distance between the marker and the gene increases, so does the probability of recombination. When the marker is very close to the gene, a high proportion of individuals possess the fragment size associated with the mutant parent, because recombination is less likely to occur between closely linked sequences. If the marker and gene were on separate chromosomes, an independent segregation would be observed between the plant phenotype and molecular genotype determined by the marker.

Recombinant analysis was employed to determine whether *mop3-1* is distinct from *rmr6-1*.

Methods

Mapping Population and Sample Collection

The mapping population was created as diagrammed in Figure 3 (Chandler et al, 2008). The initial cross of homozygous [K55] *mop3-1* with [A188] wild type was followed by a series of crosses between segregating siblings to develop the BC3 mapping population. The population segregated ¼ dark plants, ½ light plants, and ¼ green plants.

The mapping population was planted in mid-March and sampled in June of 2008. Leaf tissue was collected when the phenotype of the plant was fully evident, and only those expressing dark purple pigment over the majority of their stalk were sampled. The healthiest leaf tissue from each plant was cut, labeled, and transported to the lab in water. The samples were promptly processed for DNA extraction, and the remaining leaf tissue was put into a labeled envelope and stored at -80°C.

DNA Isolation

A 3mm carbide bead was put into each 2 mL well of a 96-well plate which was partially submerged in liquid nitrogen. Approximately 100 mg of leaf tissue was put into each well. The samples were ground in a GlenMills Mixer Mill two times at 25,000 shakes per minute, and put back into liquid nitrogen between and following the grinding. The plate was warmed slightly at room temperature without allowing the tissue to melt. 600 μL of CTAB (cetyltrimethyl-ammonium bromide) buffer with 1% β-mercaptoethanol was added to each sample and the plate was shaken gently to mix. The samples were incubated in a 65°C water bath for twenty minutes, and were removed and inverted gently several times to ensure mixing. After cooling for ten minutes, 300 μL of 24:1 chloroform:octanol was added to each sample. The plate was shaken in the Mixer

Mill at 5,000 shakes per minute two times for 3 minutes, rotating the plate between. Immediately following shaking, the plate was spun at 3000 rotations per minute (rpm) for 15 minutes in a B4i centrifuge. Using a multi-channel pipette, the supernatant was transferred to a new 96-well plate with 300 μL of chilled isopropanol in each tube. After transferring the liquid, the tubes were capped and inverted gently to precipitate out the DNA. The plate was immediately spun in a B4i centrifuge at 3000 rpm for 5 minutes. The liquid from each tube was poured off, leaving the pellet at the bottom. The samples were washed with 400 μL of 70% ethanol. The plate was shaken gently, and then centrifuged at 3000 rpm for 5 minutes, and the liquid was poured off. The wash was repeated with 100% ethanol. After decanting the ethanol, the DNA pellets were loosely covered with a KimWipe and allowed to dry overnight.

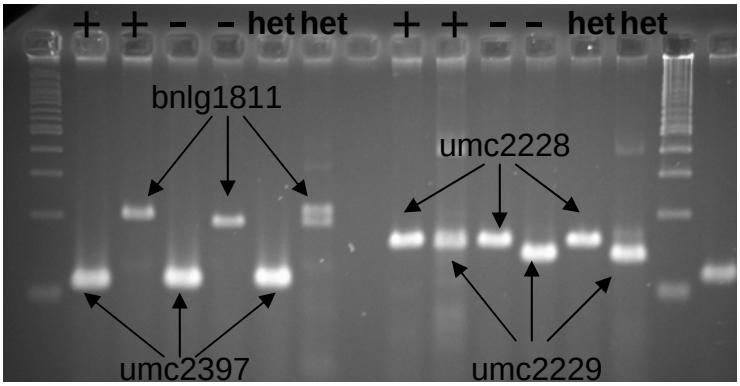
The DNA pellets were resuspended in 100 μL 10:1 Tris EDTA (TE) pH8 buffer. After addition of the buffer, the samples were warmed in a 65°C water bath and gently shaken until the pellets were no longer visible. The concentration of each sample was determined using a NanoDrop spectrophotometer. A 50 ng/μL DNA working stock was prepared by diluting the appropriate amount of the DNA in 10:1 TE pH8 up to a total volume of 100 μL. Both the original DNA sample and the working stock were stored at 4°C.

Primers used

The primers were selected from the database on MaizeGDB (www.maizegdb.org), and ordered from Bioneer. A BLAST (Basic Local Alignment Search Tool) was used to verify the position of the primer pair within the high-throughput genomic DNA and to ensure specificity (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Primers

that were not unique, or whose location could not be precisely determined were not used. The primers were resuspended in 10:0.1 TE to a concentration of 100 pmol. After determining the concentration with the NanoDrop, a 50 ng/μL working stock of each primer was made by diluting accordingly with sterile water. The SSR primer pairs were tested in our genetic backgrounds for scorable polymorphisms using four controls: [A188] *b/b;+/+* wild type, [K55] *B'/B'; mop3-1*/*mop3-1* mutant, *B'b;mop3-1/+* heterozygote, and a no DNA negative control. In this experiment, the amplified region had to produce distinct bands of different sizes in each of the parental controls, both bands had to be present in the *mop3-1/+*heterozygote, and the genotypes had to be easily scored on the gel. The no DNA control was used to confirm absence of contamination in the PCR, and to ensure that the primers were not forming extensible primer-dimers close to the fragment size of interest. Information on all primers used is provided in Table 1.

Figure 4. An example of screening markers to be used for mapping



The genotype of the controls is shown on each lane; **+** for wild type, **-** for mutant, and **het** for the heterozygote. The markers umc2397 and umc2228 produce identical bands in all three controls and are not polymorphic. The marker bnlg1811 gives distinct bands in the two parental controls, both of which are seen in the heterozygote; this marker is appropriate for mapping. umc2229 shows distinct bands in the parental controls, but the faint upper band from the wild type parent is difficult to see in the heterozygote. This marker, although polymorphic, would be hard to use in high throughput mapping.

PCR conditions

The PCR protocol was as follows: each reaction contained 2 μL (100 ng) genomic DNA, 1 μL (50 ng) each forward and reverse primer, 1.5 μL 1 mM dNTPs, 1.5 μL 10X PCR Buffer (Applied BioSystems), 1.5 μL 25 mM $MgCl_2$ solution (Applied BioSystems), and 0.3units AmpliTaq Gold (Applied BioSystems). Sterile water was added to increase the final volume to 15 μL.

The PCR was performed in a GeneAmp thermocycler (Applied BioSystems) using the Touchdown method. The protocol consists of an initial 8-minute dwell at 95°C to activate the AmpliTaq Gold, followed by ten cycles consisting of a 60 second denaturing step at 95°C, a 90 second annealing step at 65°C, and a 90 second extension step at 72°C. The annealing temperature was decreased by 1C each cycle. This was followed by 35 cycles of denaturing, annealing, and extension at 95°C, 55°C, and 72°C, respectively, a 7-minute hold at 72°C, and a final dwell at 4°C. The number of cycles at 55°C annealing temperature was increased by five cycles from the original protocol, again in an effort to maximize PCR product. After amplification, the product was mixed with 2.5 μL 6X loading dye, and a total of 10 μL was used for gel electrophoresis.

Agarose Gel Electrophoresis

The PCR product and loading dye were loaded into the wells of a 4% agarose gel. The gel was made by adding 12 g agarose powder (Super Fine Resolution blend, Amresco) to 300 mL 1X TBE. The mixture was boiled in a microwave until homogeneous, then put on a hot plate at 75°C with a magnetic stir bar, and stirred slowly to remove bubbles trapped in the agarose. After the bubbles had been removed, the liquid agarose was poured into a gel mold and allowed to polymerize. The gel was covered with

1X TBE buffer to a depth of approximately 3 mm. The sample was loaded and the gel was run at 120V for 1.5 to 2.5 hours. Specific run times varied among markers and depended on the size of the amplified fragment and the difference in size of the two parental bands. Following electrophoresis, the gel was placed in the staining solution ethidium bromide and 1X TBE buffer. The solution was made by mixing 10 mL of 10 ng/mL ethidium bromide stock solution with 90 mL buffer. After staining, the gel was rinsed briefly in fresh buffer, and then visualized under ultraviolet light.

Polyacrylamide Gel Electrophoresis

To aid in visualizing faint or close bands, denaturing poly-acrylamide gel electrophoresis (PAGE) was used. To make a 10% denaturing solution, 8μg urea was dissolved in 20 mL 5X TBE, 50 mL 40% 19:1 acrylamide:bis-acrylamide, and water to 200 mL total volume. The mixture was warmed (but not boiled) to dissolve the urea completely, sterile filtered, and stored at 4°C protected from light. To make the gel, 20 mL of the 10% denaturing solution was added to 9 μL of tetramethylethylenediamide (TEMED), and 172.5 μL of 1% ammonium persulfate (APS). After mixing to ensure homogeneity, the gel was cast immediately. A syringe was used to transfer the liquid into the vertical gel mold. After the gel had polymerized, the comb was removed and each well was flushed using the 0.5X TBE buffer surrounding the gel to remove excess urea. The PCR product was mixed in equal volumes with a loading buffer. The buffer was made with 20 μL 10 mM EDTA, 10 μL 2% bromophenol blue, 10 μL 2% xylene cyanol FF (XCFF), and 960 μL 80% deionized formamide (DIF). The loading buffer and PCR product were heated at 65°C for 5 minutes to denature secondary structures, chilled on ice, and loaded onto the gel. A total of 30 μL (15 μL PCR product, 15 μL loading buffer)

was loaded into each well. The gel was run at 100V for 2 hours, then stained with EtBr and visualized under ultraviolet light.

Genotyping/ Scoring/ Interpretation of gel results

Individuals with a single band similar to [A188] wild type were scored as "BB", and those with a band like the [K55] *mop3-1* mutant as "AA". Individuals presenting both bands as the *mop3-1*/+ heterozygote control were scored as "AB".

RESULTS

Recombinant mapping demonstrated that mop3-1 is not rmr6-1

The *mop3-1* mutation fails to complement the *required to maintain repression6-1* (*rmr6-1*) mutation, and both mutations result in dark purple pigmentation. Only plants that appeared phenotypically mutant (intense purple color with very little green showing through) were used for mapping. In a region unlinked to the phenotypic trait of interest, the expected genotypic segregation ratio is 1:2:1, AA:AB:BB. Different ratios, specifically more AA individuals and less AB and BB individuals, indicate linkage of the marker to the mutation. As the distance between the mutation and a marker decreases, recombination frequency is lower, and this results in fewer AB individuals. The plants were scored for each marker as homozygous mutant (AA), homozygous wild-type (BB), or possessing one copy of each allele (AB). Linkage was determined by analyzing the number of plants in each class.

Only one out of 23 tested primer pairs from the *rmr6* region, bnlg1598, was polymorphic. Due to the close proximity to *rmr6*, linkage of bnlg1598 to *mop3-1* would show linkage of the mutations. This marker produced a thick band in *mop3-1* and a faint band in the wild type that is difficult to score. However, because it was the only

polymorphic marker near *rmr6*, it was used despite this complication. Examination of SFR agarose gel showed that the single faint band representing [A188] wild type appeared in 1 plant (BB), the single band of the [K55] mutant appeared in 29 individuals (AA), and 56 individuals showed both bands (AB). If *mop3-1* were *rmr6-1*, very few AB and BB individuals would have been observed. The large number of these individuals (66%) indicates that *mop3-1* is not *rmr6-1*. To ensure that the scoring of the polymorphism was not affected by visibility of the faint band, a subset of samples was reanalyzed using poly-acrylamide gel electrophoresis (PAGE). PAGE allows better resolution for small PCR products, and the bands are much sharper. When 45 samples were tested on PAGE, there were 27 AB, 1 BB, and 17 individuals (not shown). This confirmed the results from the agarose gel, clearly indicating that the *mop3-1* mutation is not linked to the *rmr6* gene.

mop3-1 is not linked to several tested candidate genes on chromosomes 3 and 10

The few of the most likely candidate genes were tested as well by using polymorphic markers linked to each. These genes were of interest because they encode subunits of DNA-dependent RNA polymerases known to affect paramutation (Erhard, 2009). The results of each are listed in supplementary material, and indicate that none of these genes are linked to *mop3-1*.

mop3-1 is located on chromosome 1S, contig 14

The absence of linkage between *mop3-1* and any of the tested candidate genes led to a reevaluation of mapping strategy, and a systematic approach utilizing SSR markers was chosen. Experiments unrelated to this thesis failed to obtain a combination of *mop3-1* and a certain allele of the *p1* gene (located on the short arm of chromosome 1), which led

to a suspicion of possible linkage between *p1* and *mop3-1* (Sidorenko, unpublished data). This indication, combined with the results of the linkage analysis, prompted us to begin screening each chromosome for *mop3-1* linkage beginning with chromosome 1.

Eighteen primer pairs were ordered for SSRs on chromosome 1. The markers were selected based on their location, specificity, and functionality. Of the 18 markers, four showed scorable polymorphisms in the controls and were used to screen a larger group of individuals. The marker umc2227 showed significant linkage; 70% of individuals were scored as AA. The marker umc1403 showed linkage as well, with 39% scored as AA. The higher than expected proportion of AA individuals of these two markers indicates closeness to the *mop3* gene. The other two markers were unlinked. Twenty-five markers flanking umc2227 were screened, and one polymorphic marker from each side was tested for linkage. Markers umc1452 and bnlg1811 were linked to *mop3*, but less so than umc2227. Loosely linked markers are still valuable, as they direct the search back toward the area of a tightly linked marker providing boundaries of the region of interest. Five more markers inside the boundaries defined by umc1452 and bnlg1811 were tested. Two of these, bnlg2238 and umc1917, decreased the interval significantly. Marker bnlg2238 has 62% linkage, while umc1917 is the most tightly linked to *mop3-1* with 75% of individuals scored as AA. Several primers were designed in unique sequences of the 5' UTR of genes in the interval (B. Echalier, 2009). One of these, VC5907Q/R, was polymorphic with slightly less linkage than umc1917, indicating that it is farther from the *mop3* gene. The results for each marker used to define the *mop3* interval are shown in the table. Figure 5 shows the region of interest as defined by the markers described above.

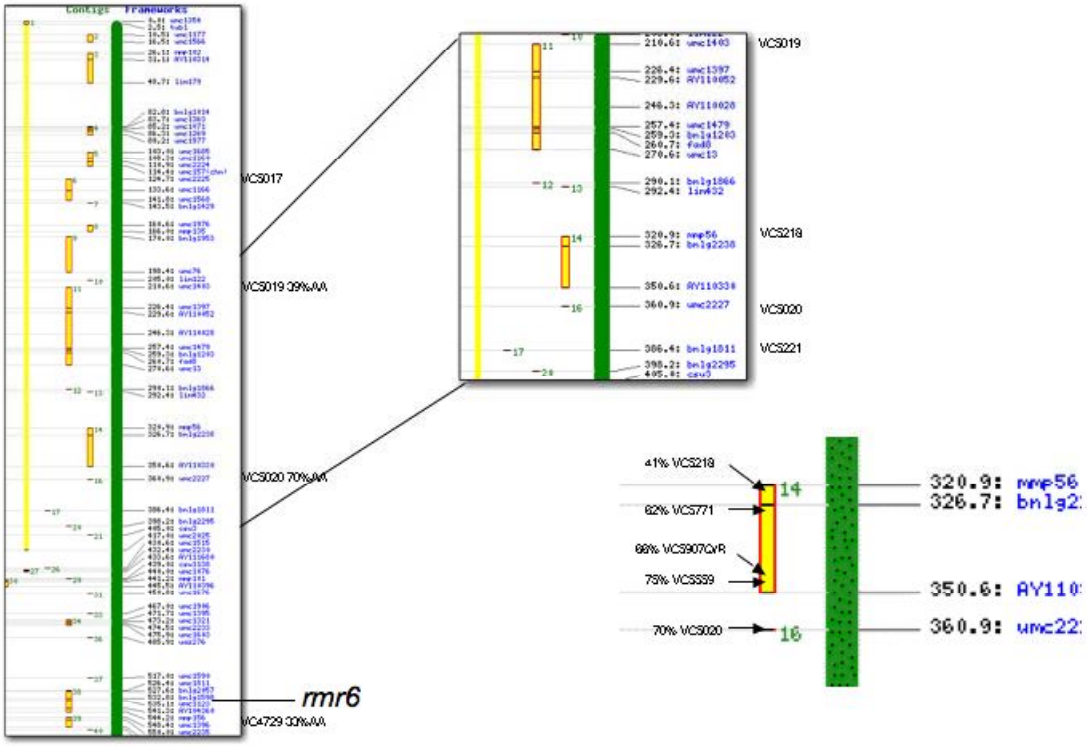
A small percentage of the plants used did not appear as AA with any of the markers used, although they were darkly pigmented like the homozygous mutant plants. One hypothesis for the disparity between genotype and phenotype is that these plants are heritable revertants of *B'* when this allele is heterozygous with neutral to paramutation *b* (Sidorenko, unpublished). See Table 1.

DISCUSSION

The results presented in this thesis define the *mop3* interval as the region between umc1917 and umc2227, on the short arm of chromosome 1. This region is approximately 2.5Mb, and contains 32 BACs.

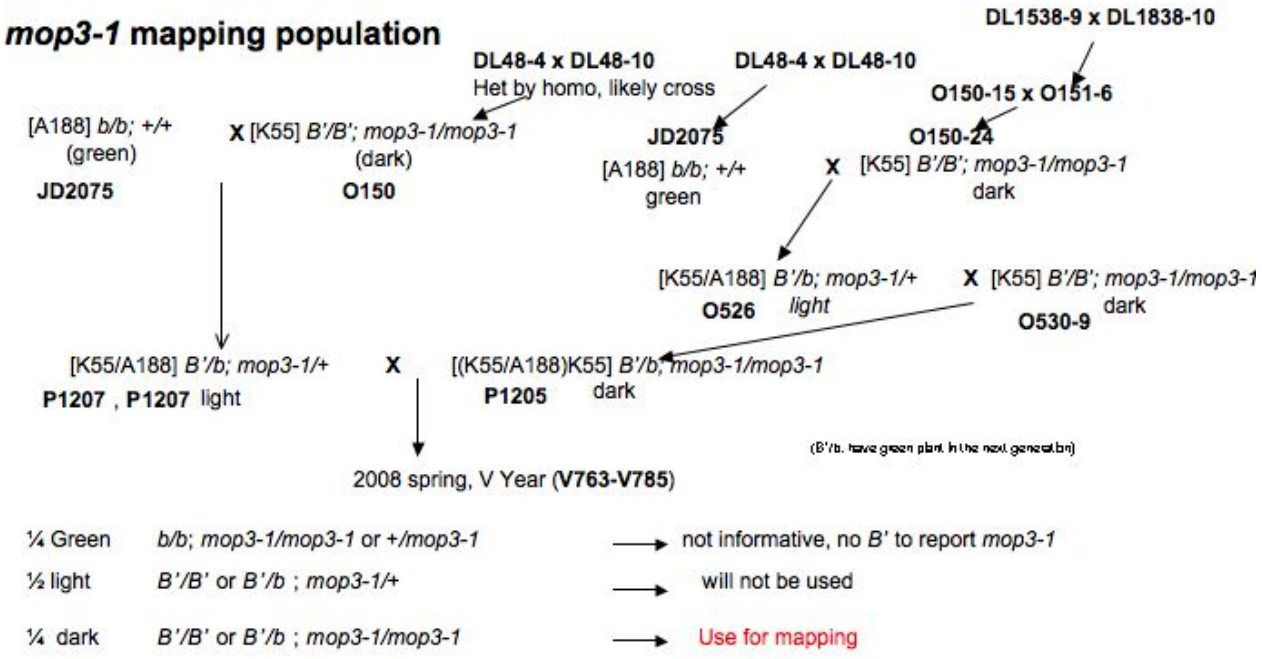
The identity of *mop3* remains unknown. Several genes have been ruled out as possibilities, but there are numerous candidate genes in the region contained by the markers. After further definition of the boundaries, the candidate genes can be screened individually. The published primers available on MaizeGDB have been exhausted as a resource for mapping *mop3*. Primers that amplify PCR fragments containing single nucleotide polymorphisms (SNPs), or large insertions or deletions (indels), will be developed to further reduce the *mop3-1* interval to 2-3 BACs. Sequences of the smaller interval will be examined for the presence of predicted genes.

Figure 5. Markers used to determine the *mop3* interval



From left to right, the region of interest is decreased using linkage analysis of the mutation to the markers shown. From MaizeGDB.

Figure 3. Creation of mapping population



Done by V. Chandler et al.,

Table 1. Scoring results for mapping markers

Plate #	well #	Plant #	VC5017	VC5019	VC5218	VC5771	VC5907Q/R	VC5559	VC5020	VC5221	VC4729	VC5024	
1002	12B	V843-22			AA	AA	AA	??	AA	AA	AA		
1002	12C	V843-15			AA	AA	AA	??	AA	AA	AB		
1002	12D	V842-104			AB	AB	AB	??	??	AA	AB		
1002	12E	V842-96			AA	AA	AA	??	AA	AA	AB		
1002	1F	V840-5	BB	AA	AB	AA	AA	AA	AA	AB	AB	AA	
1002	1G	V839-36	AB	AA	AA	AA	AA	AA	AA	AA	AB	AB	
1002	1H	V839-58	AB	AA	AA	AA	AA	AA	AA	AA	AB	AA	
1002	2A	V840-17	AB	BB	BB	AB	AA	AA	??	??	AB	AA	
1002	2D	V841-3	BB	AB	AB	AA	AA	AA	AA	??	AB	AB	
1002	2E	V841-15	BB	AA	AB	AA	AA	AA	??	??	AB	AB	
1002	2F	V841-22	BB	AA	AB	AA	AA	AA	AA	??	AB	AB	
1002	2H	V841-34	BB	AA	AB	AA	AB	AA	AA	AB	AA	AB	
1002	3A	V841-39	BB	AB	AB	AA	AA	AA	AA	??	AA	AB	
1002	3B	V841-40	BB	AA	AB	AA	AA	AA	AA	??	AB	AB	
1002	3D	V841-46	BB	AA	AB	AA	AA	AA	AA	??	AA??	AB	
1002	3F	V841-65	BB	AB	AB	AA	AA	AA	AA	??	AA	AB	
1002	3G	V841-72	BB	AB	AB	AA	AA	AA	AA	??	AB	AB	
1002	3H	V841-75	BB	AB	AB	AA	AA	AA	AA	??	AB	??	
1002	4A	V847-4	BB	AA	BB	AB	AA	AA	AA	??	AB	AB	
1002	4B	V847-48	BB	AB	AB	AA	AA	AA	AA	??	AA	AA	
1002	4C	V847-54	AB	AA	AB	AA	AA	AA	AA	??	AB	AA	
1002	4D	V847-56	AB	??	BB	AA	AA	AA	AA	??	AB	AA	
1002	4E	V830-16	AB	??	BB	AB	AA	AA	AA	??	AA	AB	
1002	4F	V830-33	AB	AA	BB	AB	AA	AA	AA	??	AA	AB	
1002	4G	V831-1	BB	AB	AB	AA	??	AA	AA	??	AA	AB	
1002	5A	V833-1	BB	AB	BB	AB	??	AA	AA	??	AB	AB	
1002	5B	V833-3	BB	BB	BB	AB	??	AA	AA	??	AB	AB	
1002	5C	V833-5	BB	AB	AB	AA	AA	AA	AA	??	AB	AB	
1002	5D	V833-10.5	BB	AA	AB	AA	AA	AA	AA	??	AB	??	
1002	5E	V833-11	BB	AB	BB	AB	AA	AA	??	??	AA??	AB	
1002	5F	V833-35	BB	AA	AB	AA	??	AA	AA	??	AB	AB	
1002	5H	V833-71	BB	AA	AA	AA	AA	AA	AA	AA	AA	??	
1002	6A	V835-55	AB	AA	AA	AA	AA	AA	AA	AA	AA		
1002	6B	V835-61	AA	AA	AA	AA	AA	AA	AA	AA	AB		
1002	6D	V836-8	AA	AA	AA	AA	AA	AA	AA	AA	AA??		
1002	6E	V836-18	AB	AA	AA	AA	AA	AA	AA	AA	AB		
1002	6F	V836-19	BB	AA	AA	AA	AA	AA	AA	AA	AB		
1002	6G	V836-36	AA	AA	AA	AA	AA	AA	AA	AA	AB		
1002	6H	V829-9	AB	??	AB	AA	??	AA	AA	??	AA		
1002	7A	V829-17			AB	AA	??	AA	AA	AB	AA		
1002	7C	V828-8			AB	AA	AA	AA	AA	AB	AA		
1002	7D	V828-16			AB	AA	AA	AA	AA	AB	AB		
1002	7E	V827-15			AA	AA	AA	AA	AA	AA	AA		
1002	7F	V827-22			AA	AA	AA	AA	AA	AA	AB		
1002	7G	V827-25			AA	AA	??	AA	AA	AA	AB		
1002	7H	V827-33			AA	AA	AA	AA	AA	AA	AB		
1002	8A	V827-34			AA	AA	AA	AA	AA	AA	AB		
1002	8B	V826-2			AA	AA	AA	AA	AA	??	AA		
1002	8C	V826-19			AA	AA	AA	AA	AA	??	AA		
1002	8D	V826-45			AA	AA	AA	AA	??	??	AB??		
1002	8E	V826-47			AA	AA	AA	AA	AA	AA	AA		
1002	8F	V826-58			AA	AA	AA	AA	AA	AA	AB		
1002	8H	V824-51			AA	AA	AA	AA	AA	AB	AB??		
1002	9B	V824-65			AA	AA	AA	AA	AA	AA	AB		
1002	10B	V838-64			AA	AA	AA	AA	AA	AA	AB??		
1002	10C	V840-1			BB	AA	AA	AA	AA	??	AA		
1002	10D	V824-44			AA	AA	AA	AA	AA	??	AB		
1002	10E	V824-83			AA	AA	AA	AA	AA	??	AB		
1002	10F	V825-45			AA	AB	AB	AA	??	??	AB		
1002	10G	V826-14			AA	AA	AA	AA	AB	BB	AB??		
1002	10H	V833-90			AA	AA	AA	AA	AA	AA	AA		
1002	11A	V833-49			AA	AA	AA	AA	AA	AA	AA		
1002	11B	V839-59			AA	??	??	AA	AA	AA	AB		
1002	11C	V839-12			AA	AA	AA	AA	AA	AA	AB		
1002	11D	V838-22			AB	AA	AA	AA	AA	AB	AB		
1002	11E	V837-23			BB	AB	AB	AA	AB	??	AB		
1002	11G	V841-60			BB	AA	AA	AA	AA	??	AB		
1002	11H	V847-19			??	AA	AA	AA	AA	??	AA		
1002	11F	V841-38			AA	??	??	??	BB	??	AA		dk, 24
1002	12A	V844-34			AA	??	AB	??	AB	AB	AB		vdk, 24
1002	12F	V842-90			AB	AB	BB	??	AB	AB	AB		vdk, 18
1002	2B	V840-35	AA	AA	AB	AB	??	AB	BB	AB	AA	AB	dk, 6
1002	9D	V824-89			AA	AA	AB	AB	BB	BB	AA		vdk, 3

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