

Identification of QTL Controlling Thermal Properties of Maize Starch

M. P. Scott^{1,2} and S. A. Duvick³

ABSTRACT

Cereal Chem. 82(5):546–553

Starch has many uses and some of these uses would be facilitated by altering its thermal properties. Genetic manipulation of starch thermal properties will be facilitated by a better understanding of the genetic control of starch gelatinization. We used differential scanning calorimetry to characterize the gelatinization parameters of maize (*Zea mays* L.) kernel starch prepared from two populations of recombinant inbred lines, an intermated B73xMo17 population (IBM) and an F_{6,7} Mo17xH99 population. The traits examined were the onset and peak temperatures of gelatinization and the enthalpy of gelatinization. These traits were measured for both native starch and for gelatinized starch allowed to

recrystallize, a process called retrogradation. Substantial variation in these traits was found in spite of the narrow genetic base of the populations. We identified several quantitative trait loci (QTL) controlling traits of interest in each population. In the IBM population, a significant QTL for the peak temperature of gelatinization of retrograded starch colocalized to a molecular marker in the *Wx1* gene, which encodes a granule bound starch synthase. The major QTL identified in this study explain, on average, ≈15% of the variation for a given trait, underscoring the complexity of the genetic control of starch functional properties.

Starch accumulates in the endosperm of maize kernels as an insoluble granule composed of the glucose polymers amylose and amylopectin. These polymers are highly organized, to the extent that parts of the granule are considered to be crystalline. This structure is determined by the concerted action of many starch biosynthetic enzymes. Starch structure, in turn, determines the physical properties of starch. Thus, to make designed genetic modifications to the physical properties of starch, it is necessary to understand both how genes control starch structure and how starch structure determines its physical properties.

Starch is an important component of many pastes, gels, adhesives, and thickeners used in food and industry. Many uses of starch require that the granule structure be disrupted before use. This is often accomplished by heating the starch, which results in a noncrystalline gel. The thermal parameters related to this gelatinization process are important functional properties that vary between different native starches and determine how suitable a particular starch is to a given use. In addition to this functional variation in native starches, the functional properties of gelatinized starches vary as well. One property of gelatinized starch is its tendency to recrystallize upon storage at low temperature, a process called retrogradation. This process affects the ability to store starch-containing products. Thus, the thermal properties of both native and gelatinized starches determine the usefulness of a starch for a given purpose.

Because of the impact of starch thermal properties on the usefulness of starch, there is great interest in developing starches with altered thermal properties. This is frequently done with chemical modification; however, methods that are less expensive and more environmentally friendly are desired. In this regard, the use of plant breeding to develop crop cultivars that produce starch with altered thermal properties is an attractive alternative to chemical modification.

To this end, thermal properties of maize (*Zea mays* L.) kernel starch have been studied in many different maize populations. These studies have included open pollinated populations (White et

al 1990), a chemically mutagenized population (Yamin et al 1999), Corn Belt and exotic inbreds (Campbell et al 1995; Ng et al 1997a; Pollak and White 1997), and Argentinean landraces (Seetharaman et al 2001). The impact of agronomic practices on maize kernel starch properties has been examined as well (Campbell et al 1994; Ng et al 1997; Krieger et al 1998). Collectively, these studies show that variation in thermal properties of maize kernel starch is abundant in maize germplasm.

While genetic variation in starch thermal properties is well established, the molecular mechanisms controlling these traits are poorly understood. Many genes involved in starch biosynthesis have been isolated and characterized (reviewed by Kossmann and Lloyd 2000). Mutations in some of these genes have affected the thermal properties of starch (Wang et al 1993; Ng et al 1997a); however, there are very little data linking genes to the thermal properties of wild-type starch. Establishing these linkages is critical to breeding and biotechnology efforts directed to developing starch with improved thermal properties.

An important step toward the goal of linking genetic loci to starch thermal properties has been taken in rice. Quantitative trait loci (QTL) responsible for controlling gelatinization temperature in a population of recombinant inbred lines have been identified. An interval containing the *waxy* gene, encoding a granule bound starch synthase, controlled ≈50% of the phenotypic variance for the onset, peak, and concluding temperatures of gelatinization (Tan et al 2001).

The objective of this study was to identify QTL in maize responsible for controlling starch thermal properties related to starch gelatinization and retrogradation. To do this, we prepared starch from two sets of recombinant inbred lines and characterized the gelatinization and retrogradation with differential scanning calorimetry. These data, together with molecular marker data from the populations studied, allowed us to identify QTL responsible for controlling several parameters of gelatinization and retrogradation of starch. The results provide insight into the genetic control of starch biosynthesis in maize. In addition, this information is valuable to breeders interested in developing strategies to produce starch with novel gelatinization and retrogradation parameters.

MATERIALS AND METHODS

Plant Material

Seed from 94 recombinant inbred lines derived from the intermated progeny of a cross of the inbred lines B73xMo17 were obtained from the Maize Genetic Stock Center (Urbana, IL). These lines are referred to as the IBM (Intermated B73xMo17) population. Each line was planted at the ISU Agronomy Farm near Boone, IA, in the spring of 2001 (year 1) and in the spring of

¹ USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, IA 50011. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

² Corresponding author. Phone: 515-294-7825. Fax: 515-294-9359. E-mail: pscott@iastate.edu

³ USDA-ARS North Central Region Plant Introduction Station, Ames, IA 50011.

2002 (year 2). Plants were self-pollinated and seed from one ear of each line was used in this study. Six lines failed to set seed in year 1 and 11 lines failed to set seed in year 2, so 88 and 83 lines from years 1 and 2, respectively, were used in this study. A second set of recombinant inbred lines used in this study was derived from $F_{6,7}$ lines from the cross Mo17xH99. (Austin and Lee 1996a,b). Grain from the Mo17xH99 population was produced in the same field as the IBM population in year 1. One hundred sixty-four lines set seed and were used in this study.

All of the IBM lines were phenotypically uniform, but seven of the Mo17xH99 lines appeared to contain individuals resulting from outcrosses, judging by the plant height and morphology relative to other plants in the same row. The data from these lines was dropped from the study.

Analysis of Starch Thermal Properties

Starch was purified from two kernels from each recombinant inbred line using a small-scale method designed to mimic the industrial wet-milling process (White et al 1990) as modified by Krieger et al (1997). Starch (4 mg) was analyzed using differential scanning calorimetry (DSC-7 Perkin-Elmer) at a rate of 10°C/min (White et al 1990). After analysis, the gelatinized starch was stored at 4°C for one week to allow retrogradation to occur. The resulting retrograded starch was then analyzed by differential scanning calorimetry again, yielding a second endotherm. For the IBM population, starch samples were analyzed in duplicate and the average of these values was used for subsequent analyses. A single analysis was performed on one ear from each line in the Mo17xH99 population. This was done to allocate our analytical capability to the largest possible number of samples.

Statistical Analysis

All statistical analysis was conducted using the software JMP 5.0 (SAS Institute, Cary, NC). Pearson product-moment correlations between each trait were determined. Examination of the distributions of the differential scanning calorimetry data suggested that the Mo17xH99 data contained outlying data points. The differential scanning calorimetry data from the Mo17xH99 population was therefore subjected to multivariate analysis, and outliers were defined as those samples with Jackknife distances of ≥ 6 . Five such individuals were identified by this analysis, and these individuals were excluded from analyses.

Because the IBM population was produced in two environments, data from this population were analyzed differently than the data from the Mo17xH99 population. This analysis allowed estimation of the environmental effects on trait values. Mean values of the two measurements taken on each IBM line in both years were analyzed using a generalized linear model with a standard least squares method to estimate the contribution of lines, years, and error to the total variance of the measurements. The distribution of values for each trait was tested for normality using the Shapiro-Wilk test. With the exception of T_{PR} in the Mo17xH99 population and T_{PG} in the year 2 IBM population, the distributions of values for all traits in both populations were not significantly different from normal ($P = 0.05$).

Identification of QTL

Molecular marker data for the two populations are publicly available (maizeGDB: <http://www.maizegdb.org/>). We used data from 940 markers for the IBM population and 141 markers for the Mo17xH99 population. Using these data, QTL were identified using the composite interval mapping procedure (Zeng 1994). The trait data for the Mo17xH99 population was the value of the single measurement taken on each line. For the IBM population, three maps were constructed: one using the trait data from year 1, a second using the trait data from year 2, and the third using the least squares mean value for each line generated by the generalized linear model described above. The walk speed was 2 cM.

Five background markers were used. To determine the threshold of statistical significance for a QTL, the trait data were randomly permuted and the highest likelihood ratio generated by analysis of the randomly permuted data was noted. This process was repeated 1,000 times. The peak likelihood ratio of each potential QTL was then compared with the maximum likelihood ratios identified using the same trait data in each of the 1,000 random permutations. For each QTL, the significance probability was calculated as the fraction of randomly permuted data sets with maximum likelihood ratios lower than the peak likelihood ratio of the QTL in question.

RESULTS

Populations Examined

To identify genetic factors controlling gelatinization and retrogradation parameters of maize starch, we sought to identify sets of well-characterized recombinant inbred lines. A survey of the starch thermal properties of a number of inbred lines has been reported (Campbell et al 1995). Statistically significant variation between B73 and Mo17 and between Mo17 and H99 was observed for onset and peak temperatures of gelatinization of kernel starch, suggesting that variation for these traits could be expected in recombinant inbred lines derived from crosses of these inbred lines.

One set of recombinant inbred lines that we selected for this study is derived from the intermated progeny of the cross between inbred lines B73 (female) and Mo17. This population is called IBM for Intermated B73xMo17. This set of lines is particularly well suited to QTL mapping because the population was intermated for several generations, providing more opportunities for recombination than would occur in a population of F_2 -derived lines. The result of these extra recombinations is that the genetic map has been expanded nearly fourfold relative to F_2 -derived recombinant inbred lines from the same cross (Lee et al 2002). A second attractive feature of this population is that the genetic map is populated with an abundance of molecular markers. Nine hundred and forty-one molecular markers were used in this study, giving an average marker density of 6.6 cM/marker.

The second population used contains recombinant inbred lines derived from $F_6:F_7$ lines resulting from a cross between Mo17 (female) and H99. This population has been used to identify QTL for several traits including plant height and flowering (Austin and Lee 1996a,b) and grain yield and yield components (Austin and Lee 1998). The genetic map for this population is populated with 150 markers with an average marker density of 11.3 cM/marker.

Thermal Properties of Maize Starch from Recombinant Inbred Lines

To assess the potential for identifying QTL controlling starch thermal properties in these populations, we analyzed purified, native starch from each line in the study by differential scanning calorimetry. In this process, the starch is gelatinized by heating while the energy input is monitored. The gelatinized starch that resulted from this analysis was allowed to retrograde and the gelatinized, retrograded starch was analyzed by differential scanning calorimetry again. The parameters used in this study were extracted from the endotherms resulting from analysis of native and retrograded starch. These parameters were the onset and peak temperatures of gelatinization (representing temperatures of the start and highest point of the thermogram peak) of native starch and retrograded starch (T_{OG} , T_{PG} , T_{OR} , T_{PR}) and the enthalpy of gelatinization of native starch and retrograded starch (ΔH_G and ΔH_R) as determined by the area under the peak in the thermogram. The percentage of the starch that underwent retrogradation (%R) was calculated by dividing ΔH_R by ΔH_G . These data are summarized in Table I. Comparing the IBM and Mo17xH99 populations, the IBM population had a greater range in trait values with the exception of T_{OG} and T_{PG} . The trait values of the IBM population were similar in both years. The variation in trait values

was significant for some traits in the IBM population. It was not possible to determine whether this was the case for the Mo17xH99 population because only one measurement was conducted on each sample.

Several of the trait values exhibited high correlations with each other (Tables II and III). The majority of the traits that were correlated in one population were correlated in the other population. Thus, in both populations, the onset and peak temperatures of gelatinization of native starch had correlation coefficients of ≥ 0.6 , while the onset and peak gelatinization temperatures of retrograded starch had correlation coefficients of ≥ 0.8 . In addition, the correlation coefficient was >0.7 when comparing %R and ΔH_R in both populations. It should be noted that %R and ΔH_R are mathematically related and would therefore be expected to be correlated. In most cases, traits that were correlated in the IBM population in year 1 were also correlated in year 2. The Kwt measured in the IBM population and did not have any significant correlations with any of the starch thermal property traits.

Because the DSC parameters of the IBM population were determined in two years, it was possible to extract information about the environmental effect on the traits. We did this in two ways. First, correlations between the traits in year 1 and year 2 were determined. These correlations are presented in Table II. Only T_{oG} , T_{pG} , and ΔH_R had significant correlations with the same trait measured in a different year. Similarly, 10 kernel weight was not significantly correlated between years. The lack of correlations between years suggests that there are substantial environmental effects on these traits.

As a second method to characterize the effect of different environments on starch thermal properties in the IBM population, the significance of the year and line effects were determined by least-squares fitting of the data using a generalized linear model. These results are presented in Table IV. Kernel weight is included to provide more information about the variation in the lines. Line and year effects were significant in the majority of the traits measured.

By pooling the environmental and error variance terms, it was possible to estimate the proportion of the total variance attributable to genetic variance (7.6–43.6%). Most of the variance observed for all traits is attributable to error or environmental effects, emphasizing the importance of multi-environment testing.

Identification of QTL Controlling Starch Thermal Properties

The distributions and ranges of the traits measured, when considered in light of the population structure, suggest that these traits are subject to control by multiple genetic loci. To characterize the genetic control of these traits, we conducted a QTL analysis to associate trait variation with chromosomal regions defined by molecular markers. We used a composite interval mapping procedure (Zeng 1994) to identify QTL for T_{oG} , T_{oR} , T_{pG} , T_{pR} , ΔH_G , ΔH_R , and %R in the IBM and Mo17xH99 populations. To determine the extent of the environmental effect, data from the IBM population produced in two years were analyzed, with each year treated independently (two analyses), and a third analysis was conducted on the averaged data.

TABLE I
Thermal Properties of Maize Kernel Starch from Recombinant Inbred Line Populations^{a,b}

Population	T_{oG} (°C)	T_{oR} (°C)	T_{pG} (°C)	T_{pR} (°C)	ΔH_G (J/g)	ΔH_R (J/g)	%R
IBM year 1 $n = 88$							
Min	65.0	37.7	69.8	50.9	11.3	4.5	33.8
Max	70.0	44.6	73.2	54.0	15.0	6.6	50.0
Avg	67.7	41.5	71.6	52.2	12.9	5.6	43.4
SD	1.04**	1.18	0.62**	0.58	0.66	0.42	3.47
IBM year 2 $n = 83$							
Min	65.7	38.2	69.7	48.5	11.7	3.9	26.0
Max	69.9	44.1	72.6	53.4	14.7	6.2	47.3
Avg	68.3	40.4	71.2	50.5	13.0	5.5	42.7
SD	0.92**	1.12**	0.63**	0.96**	0.62	0.38**	3.12
H99xMo17 $n = 153$							
Min	65.9	41.1	71.2	52.2	10.4	4.3	36.4
Max	72.3	44.8	74.5	54.7	13.3	6.1	51.6
Avg	69.2	42.9	72.7	53.2	12.0	5.2	43.6
SD	1.00	0.70	0.67	0.53	0.57	0.35	2.44

^a T_{oG} , Temperature of gelatinization onset of native starch; T_{oR} , temperature of gelatinization onset of gelatinized starch following retrogradation; T_{pG} , temperature of endotherm peak for gelatinization of native starch; T_{pR} , temperature of endotherm peak for gelatinization of gelatinized starch following retrogradation; ΔH_G , enthalpy of gelatinization of native starch; ΔH_R , enthalpy of gelatinization of gelatinized starch following retrogradation; %R, percentage of starch undergoing retrogradation.

^b **, Indicates significant ($P < 0.01$) variation among sample means determined by ANOVA in the intermated B73xMo17 population (IBM). This test was not run on the H99xMo17 population because measurements were not made in duplicate so that more individuals could be measured for quantitative trait loci (QTL) identification.

TABLE II
Pearson Product-Moment Correlations Between Mapped Traits in the Intermated B73xMo17 Population (IBM) Population^{a-c}

	T_{oG} (°C)	T_{oR} (°C)	T_{pG} (°C)	T_{pR} (°C)	ΔH_G (J/g)	ΔH_R (J/g)	%R
T_{oG}	0.443**	0.087	0.639**	0.046	0.244**	0.328**	0.132
T_{oR}	-0.155	0.078	0.146	0.815**	-0.168	-0.575**	-0.453**
T_{pG}	0.850**	-0.158	0.456**	0.226	0.210	0.205*	0.049
T_{pR}	-0.040	0.871**	-0.044	0.136	0.004	-0.443**	-0.439**
ΔH_G	0.359**	0.110	0.220*	0.184	0.157	0.148	-0.422**
ΔH_R	0.284*	-0.402**	0.177	-0.270	0.162	0.283*	0.829**
%R	0.024	-0.425**	0.007	-0.359**	0.477**	0.786**	0.102

^a Values on the diagonal (in bold) are comparisons between year 1 and year 2; values above the diagonal are comparisons within year 1; values below the diagonal are comparisons within year 2.

^b Trait abbreviations as in Table I.

^c * Indicates a significance probability of $P < 0.05$; **, indicates a significance probability of $P < 0.01$.

The statistical significance of QTL was examined by permutation analysis. QTL with likelihood ratios higher than the maximum likelihood ratios in 95% of the permutations are reported. Eleven QTL meeting this criterion were identified in the year 1 IBM population, eleven QTL meeting this criterion were identified in the year 2 IBM population and seven QTL meeting this criterion were identified in the IBM population using the two-year averaged data (Table V). Between one and seven QTL were identified for each trait examined. Several chromosomal regions were identified that control two of the traits examined. For example, QTL controlling ΔH_R and $\%R$ co-localize on chromosome 1 and 9 and QTL controlling T_{pR} and T_{oR} are co-localized on chromosome 1. This can be explained in part by the correlation in trait values for these traits. In this population, the proportion of the phenotypic variation explained by a given QTL for a given trait had a range of 0.113–0.266. These relatively low values support the hypothesis that genetic mechanisms controlling starch thermal properties are complex.

Comparison of QTL identified in the year 1, year 2, and the two-year averaged data shows most QTL are specific to one year (Table V), indicating that single year data has little value for predicting loci that will be important in other years. Two of the significant QTL (one for ΔH_R on chromosome 7 and one for T_{pR} on chromosome 9) and QTL identified in the two-year averaged data are close to QTL identified in one of the single years, making it a slightly better predictor of loci likely to be important in a given year. In other cases, QTL identified using the two-year averaged data do not map to the locations of significant QTL identified in the single year data. This may indicate that some loci are more stable to environmental variation than others. The two-year averaged QTL are illustrated in Fig. 1.

The same procedure was used to identify nine QTL controlling six traits in the Mo17xH99 population (Table VI). One interval on chromosome 2 contains QTL for ΔH_G , T_{oG} , T_{pG} , and T_{pR} . QTL for individual traits were identified on chromosomes 3, 6, and 7 as well. On average, these QTL explain $\approx 8\%$ of the phenotypic variation for a trait.

In comparing the results obtained with the IBM and Mo17xH99 populations, it is clear that the IBM population was more useful for identifying QTL. In the IBM population, more QTL were identified in each year and they explained a greater proportion of the phenotypic variation. In addition, QTL in the IBM population were localized to a much smaller fraction of the chromosome than those in the Mo17xH99 population. These differences are likely due to the greater variation in trait values in the IBM population, the expanded genetic map produced by inter-mating the IBM population (Lee et al 2002) and the higher density of molecular markers in this population.

Starch Biosynthesis Genes Co-Localizing with QTL

Many genes involved in starch biosynthesis have been mapped genetically but very little is known about what genes control starch thermal properties in wild-type maize endosperm. It is of great interest to determine whether any of the QTL identified in this study are located in chromosomal regions containing genes known to be involved in starch biosynthesis. As shown in Figs. 1 and 2, several of the molecular markers used in this study are derived from molecularly characterized genes involved in starch metabolism. This allows an accurate determination of the location of QTL relative to these genes. The most striking example is that the peak likelihood ratio of a QTL on chromosome 9 controlling T_{pR} is at the same position as a molecular marker derived from the *Wx1* gene that encodes a granule bound starch synthase, indicating that *Wx1* may influence this trait. Caution should be used in interpreting these results because many genes involved in starch biosynthesis have been mapped in maize, and this proximity is likely to be coincidental.

Breeding and biotechnology are attractive approaches to modifying starch functional properties. To apply these approaches, it is important to understand the genetic control of starch functional properties. A great deal of progress has been made in characterizing genes encoding starch biosynthetic enzymes, and mutants identified by these studies have been useful for developing starches with different functional properties. There is still very little information about how starch biosynthetic genes work together to determine the functional properties of wild-type starch. By identifying QTL controlling starch thermal properties, we have taken an important step toward understanding the genetic control of starch functional properties.

The genetic control of thermal properties of starch is clearly complex. The distributions and ranges of variation observed in this study were comparable to those observed in previous studies (Campbell et al 1995; Pollak and White 1997). Given that the populations examined in these previous studies represent a large number of exotic or domestic inbred lines, it is likely that a variety of alleles was present at a given locus in those studies. In contrast, assuming that the three lines used to make the recombinant inbred lines in this study were homozygous at all loci, at most three alleles are present at a given locus in this study: the B73 allele, the Mo17 allele, and the H99 allele. This suggests that a large number of alleles at any single locus is not required to generate diversity in starch thermal properties. The diversity in starch thermal properties in these recombinant inbred line populations is probably derived from different combinations of alleles at several or perhaps many different loci, or from epistatic interaction between these loci. This idea is supported by our findings that on average, a single QTL explains only $\approx 16\%$ of the phenotypic variation in the IBM population (Table V) and only 9% in the Mo17xH99 population (Table VI), and that several traits are influenced by multiple QTL.

TABLE III
Pearson Product-Moment Correlations Between Mapped Traits
in the Mo17xH99 Population^{a,b}

	T_{oR}	T_{pG}	T_{pR}	ΔH_G	ΔH_R	$\%R$
T_{oG}	-0.09	0.81**	-0.04	0.66**	0.46**	0.01
T_{oR}		-0.11	0.84**	-0.15	-0.39**	-0.35**
T_{pG}			-0.04	0.43**	0.34**	0.05
T_{pR}				-0.04	-0.34**	-0.40**
ΔH_G					0.61**	-0.10
ΔH_R						0.73**

^a Trait abbreviations as in Table I.

^b *, Indicates a significance probability of $P < 0.05$; **, indicates a significance probability of $P < 0.01$.

TABLE IV
ANOVA Results of the Intermated B73xMo17 Population (IBM)
Lines Produced in Two Years^{a,b}

	Line Effect	Year Effect	Genetic Variance % ^c
T_{oG}	**	**	41.5
T_{oR}	ns	**	7.6
T_{pG}	**	**	43.6
T_{pR}	ns	**	8.4
ΔH_G	ns	*	10.9
ΔH_R	**	ns	31.0
$\%R$	ns	ns	10.4
Kwt	**	ns	35.2

^a Trait abbreviations as in Table I. Kwt, 10 kernel weight.

^b *, Indicates a significance probability of $P < 0.05$; **, indicates a significance probability of $P < 0.01$; ns, indicates not significant.

^c Variance was partitioned into the line effects and year effects plus error. The proportion of the total variance contributed by the line effect is reported as the genetic variance percentage.

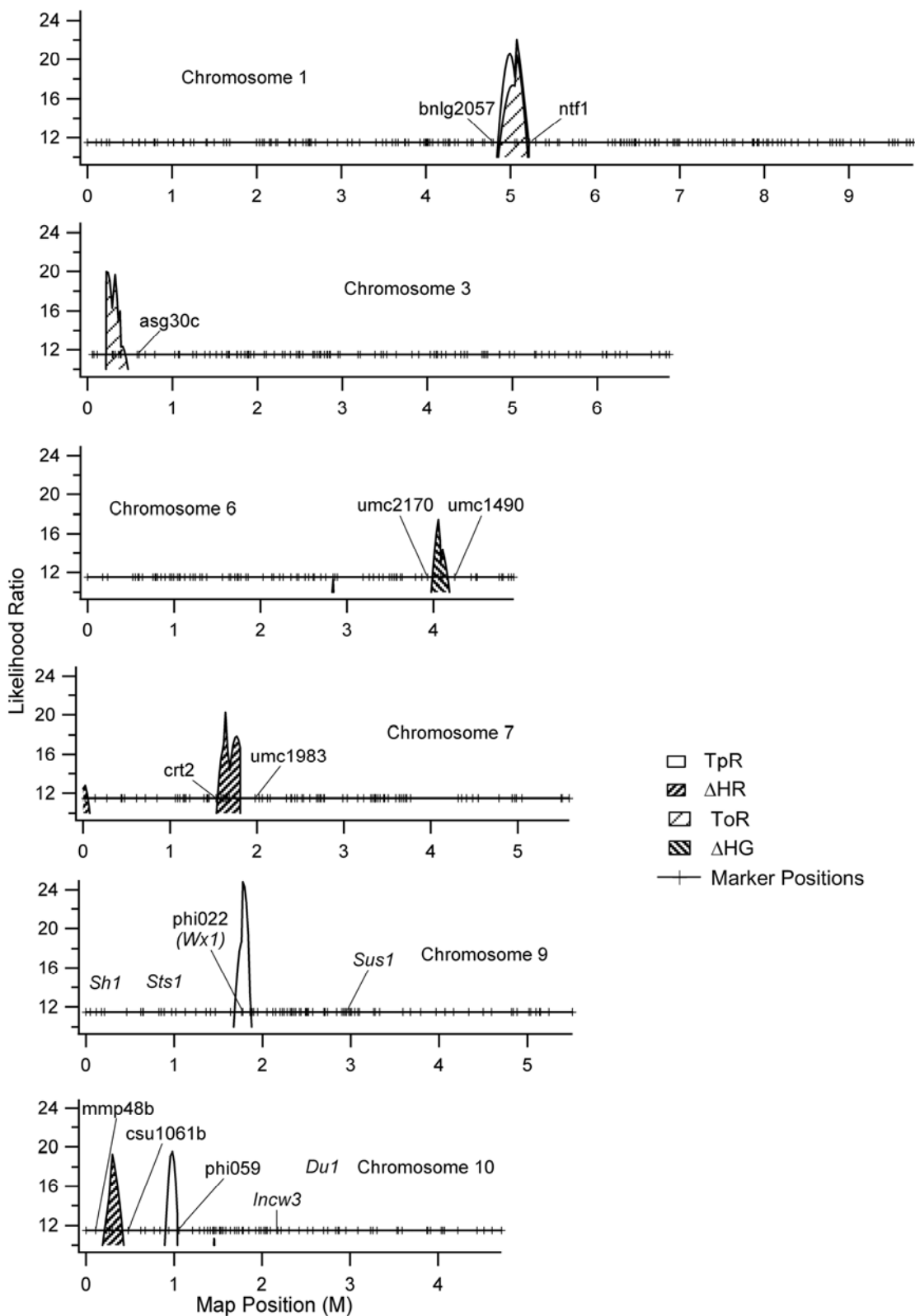


Fig. 1. Significant quantitative trait loci (QTL) identified in the intermated B73xMo17 population (IBM) population using trait data averaged over two years. The threshold line corresponds to likelihood ratio 11.5, a commonly used significance threshold. Hatches on this line indicate the positions of markers used in this study. Significance probability estimates from permutation analysis and other detailed information about each QTL are presented in Table V. Where a molecular marker is part of a known gene sequence, the name of the gene is used. Genes whose symbols are not connected to the threshold line were not used as markers in this study and therefore cannot be placed precisely on the map. In these cases, the approximate locations of the gene is shown, based on comparisons to other genetic maps. The likelihood ratio of each trait is plotted and the area under the line is filled with a pattern for each trait. Trait abbreviations are defined in Table I.

It is interesting to compare our results with those from rice (Tan et al 2001). In rice, an interval containing the *waxy* gene explains much of the phenotypic variance for the temperature of gelatinization of native starch. In maize, the chromosomal region containing the orthologous gene *Wxl* contained a significant QTL for T_{pG} in the IBM population. The maize QTL does not explain as much of the phenotypic variation as the rice QTL. This may be because in rice the phenotypic variation for gelatinization temperature was much larger (Tan et al 2001) than the variation observed in this study.

Several genes involved in starch biosynthesis have been mapped in other populations but because they have not been mapped in the populations used in this study, it is not possible to determine their proximity to QTL identified in this study. Several such genes map to chromosomes containing QTL, including *Du1* (chromosome 10), and *Sts1* (chromosome 9), which both encode soluble

starch synthases (Gao et al 1998; Knight et al 1998). Given that the granule bound starch synthase encoded by the *waxy* gene maps near QTL controlling starch thermal properties in rice (Tan et al 2001) and maize (this study), it would be informative to map other starch synthase genes in the IBM population to determine their locations more precisely relative to QTL identified in this study.

A comparison of the QTL identified in the IBM population with those identified in the Mo17xH99 population reveals that both populations contained loci controlling starch thermal properties on chromosomes 3 and 6. Because we lack common markers between the genetic maps, we cannot determine whether these loci correspond to precisely the same chromosomal region or not. More work is needed to determine the importance of these and other regions identified in this study for controlling starch thermal properties in other maize populations. The results of this study indicate that in

TABLE V
Quantitative Trait Loci (QTL) with Significance Probabilities of $P > 0.950$ for Starch Thermal Properties in the Interbred B73xMo17 Population (IBM) Population

Trait ^a	Chromosome	Map Position (M)	Peak LOD Score	Peak Likelihood Ratio	Prob ^b	R ^{2c}	Year Identified
%R	1	10.51	3.96	18.2	0.975	0.14	1
	1	877.81	3.79	17.5	0.992	0.13	2
	8	230.91	3.09	14.3	0.963	0.11	2
	9	207.11	4.52	20.8	0.994	0.21	1
	9	219.81	6.40	29.5	1.000	0.23	1
	9	230.01	3.75	17.3	0.962	0.14	1
	10	395.51	4.84	22.6	0.996	0.19	1
ΔH_G	4	424.61	3.66	16.9	0.960	0.14	2
	6	405.51	5.15	23.7	0.999	0.18	2
	10	109.61	4.59	21.1	0.993	0.19	1
ΔH_R	1	10.51	5.20	24.0	1.000	0.17	1
	4	180.41	3.39	15.6	0.978	0.14	2
	4	198.01	3.40	15.7	0.978	0.13	2
	7	163.71	4.22	19.4	0.995	0.14	1
	7	163.71	4.40	20.2	0.998	0.13	Avg
	9	222.51	4.74	21.8	1.000	0.15	1
	10	30.11	4.19	19.3	0.997	0.13	Avg
T_{oG}	2	247.61	4.12	19.0	0.983	0.16	1
	2	407.81	4.59	21.1	0.995	0.19	2
T_{oR}	1	507.61	4.44	20.4	0.990	0.14	Avg
	3	22.01	4.34	20.0	0.997	0.14	Avg
	3	207.81	7.07	32.5	1.000	0.26	2
	4	544.01	4.49	20.7	0.995	0.16	2
T_{pG}	1	859.11	6.00	27.6	1.000	0.27	1
	1	507.61	4.79	22.1	0.994	0.14	Avg
T_{pR}	3	189.81	6.76	31.1	1.000	0.23	2
	9	177.81	5.39	24.8	0.998	0.16	Avg
	9	211.71	3.96	18.2	0.978	0.12	2
	10	98.01	4.24	19.5	0.988	0.15	Avg

^a Trait abbreviations as in Table I.

^b Probability of obtaining a QTL in the genome using permuted data with a peak likelihood ratio \leq likelihood ratio of this QTL. A value of 1 indicates that no QTL with higher peak likelihood ratios were identified in the genome in any of the 1,000 random permutations.

^c Proportion of phenotypic variation explained by a QTL.

TABLE VI
Quantitative Trait Loci (QTL) with Significance Probabilities $P > 0.950$ for Starch Thermal Properties in the Mo17 x H99 Population

Trait ^a	Chromosome	Map Position (M)	Peak LOD Score	Peak Likelihood Ratio	Prob ^b	R ^{2c}
T_{oG}	2	1.29	3.73	14.5	0.993	0.13
	3	0	1.94	10.1	0.997	0.05
	5	0.23	2.56	10.9	0.977	0.07
T_{pG}	2	1.14	2.48	13.9	1.000	0.08
T_{pR}	2	1.49	5.20	20.5	1.000	0.11
T_{oR}	7	0.41	2.74	9.20	0.967	0.06
ΔH_G	2	1.32	3.15	11.1	0.990	0.12
%R	3	0.52	2.80	20.2	1.000	0.10
	6	0.84	2.25	13.3	0.997	0.06

^a Trait abbreviations as in Table I.

^b Probability of obtaining a QTL using permuted data with a peak likelihood ratio \leq likelihood ratio of this QTL. A value of 1 indicates that no QTL with higher peak likelihood ratios were identified on the same chromosome in any of the 1,000 random permutations.

^c Proportion of phenotypic variation explained by a QTL.

the IBM and Mo17x H99 recombinant inbred line populations, thermal properties of endosperm starch vary sufficiently to allow us to identify QTL that influence these traits. One of these QTL mapped to an interval containing the *Wx1* gene that is involved in starch biosynthesis. This information is an important step toward understanding the genetic control of starch thermal properties. Future studies will enable better control of starch thermal properties by breeding and biotechnology methods.

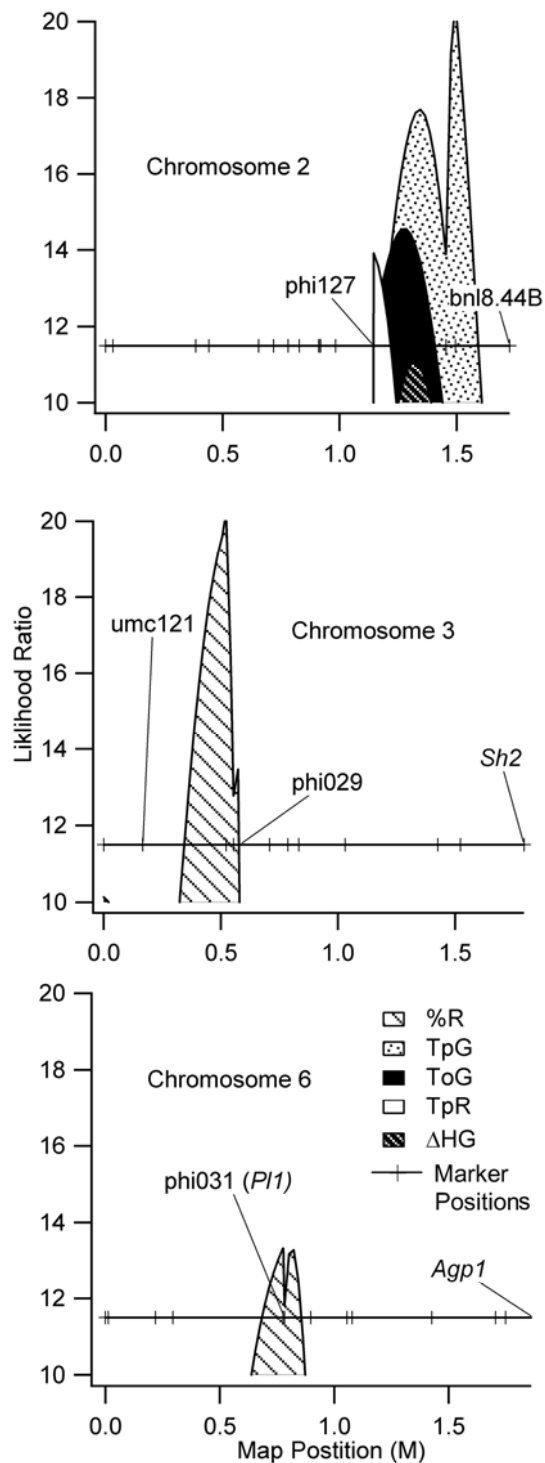


Fig. 2. Major quantitative trait loci identified in the Mo17xH99 population. Conventions as described in Fig. 1. Significance probability estimates from permutation analysis and other detailed information about each QTL are presented in Table VI.

CONCLUSIONS

Understanding the genetic control of starch thermal properties is an important step toward manipulating them with plant breeding or biotechnology. To better understand the genetic control of the thermal properties of starch, we examined the thermal properties of maize starch in recombinant inbred line populations. The variation we observed in this study is noteworthy because each population has at most two alleles at any given genetic locus, the observed variation must have been generated by combining these alleles in different ways. This is an important observation for plant breeders because it demonstrates that it is possible to generate starch with variation in thermal properties by generating different combinations of existing alleles in a population rather than introducing new alleles. This provides an alternative approach to using exotic alleles to generate variation in starch thermal properties and this approach is attractive because it could be carried out using standard, agronomically adapted germplasm.

The populations examined in this study have been mapped molecularly, allowing us to identify genetic loci influencing starch thermal properties. Statistically significant loci controlling these traits were identified. In contrast to a similar study conducted in rice (Tan et al 2001), no single locus explained a large proportion of the phenotypic variation but rather several loci with small effects were identified. This observation underscores the complexity of the genetic mechanisms controlling starch thermal properties. In addition, many of these loci did not co-localize to map positions containing genes involved in starch biosynthesis, confirming that our understanding of the molecular mechanisms controlling thermal properties of starch is incomplete.

ACKNOWLEDGMENTS

We wish to thank Kendall Lamkey for producing the grain of the Mo17xH99 population that was used in this study. This report is a joint contribution from the Corn Insects and Crop Genetics Research Unit, USDA-ARS, and project no. 3781 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA 50011.

LITERATURE CITED

- Austin, D. F., and Lee, M. 1996a. Genetic resolution and verification of quantitative trait loci for flowering and plant height with recombinant inbred lines of maize. *Genome* 39:957-968.
- Austin, D. F., and Lee, M. 1996b. Comparative mapping in F2:3 and F6:7 generations of quantitative trait loci for grain yield and yield components in maize. *Theor. Appl. Genet.* 92:817-826.
- Austin, D. F., and Lee, M. 1998. Detection of quantitative trait loci for grain yield and yield components in maize across generations in stress and nonstress environments. *Crop Sci.* 38:1296-1308.
- Basten, C. J., Weir, B. S., and Zeng, Z.-B. 1994. Zmap—A QTL cartographer. Pages 65-66 in: 5th World Congress on Genetics Applied to Livestock Production: Computing Strategies and Software, Vol 22. C. Smith, J. S. Gavora, B. Benkel, J. Chesnais, W. Fairfull, J. P. Gibson, B. W. Kennedy, E. B. Burnside, eds. Organizing Committee: Guelph, ON, Canada.
- Basten, C. J., Weir, B. S., and Zeng Z.-B. 2002. QTL Cartographer. Department of Statistics, North Carolina State University: Raleigh.
- Campbell, M. R., Pollak, L. M., and White, P. J. 1994. Effect of planting date on maize starch thermal properties. *Cereal Chem.* 71:556-559.
- Campbell, M. R., Pollak, L. M., and White, P. J. 1995. Genetic variation for starch thermal and functional properties among nonmutant maize inbreds. *Cereal Chem.* 72:281-286.
- Gao, M., Wanat, J., Stinard, P. S., James, M. G., and Myers, A. M. 1998. Characterization of *dull1*, a maize gene coding for a novel starch synthase. *Plant Cell* 10:399-412.
- Knight, M. E., Harn, C., Lilley, C. E. R., Guan, H., Singletary, G. W., Mu Forster, C., Wasserman, B. P., and Keeling, P. L. 1998. Molecular cloning of starch synthase I from maize (W64) endosperm and expression in *Escherichia coli*. *Plant J.* 14:613-622.
- Kossmann, J., and Lloyd, J. 2000. Understanding and influencing starch biochemistry. *Crit. Rev. Plant Sci.* 19:171-226.

- Krieger, K. M., Duvick, S. A., Pollak, L. M., and White, P. J. 1997. Thermal properties of corn starch extracted with different blending methods: Microblender and homogenizer. *Cereal Chem.* 74:553-555.
- Krieger, K. M., Pollak, L. M., Brumm, T. J., and White, P. J. 1998. Effects of pollination method and growing location on starch thermal properties of corn hybrids. *Cereal Chem.* 75:656-659.
- Lee, M., Sharopova, N., Beavis, W. D., Grant, D., Katt, M., Blair, D., and Hallauer, A. 2002. Expanding the genetic map of maize with the intermated B73 x Mo17 (IBM) population. *Plant Molec. Biol.* 48:453-461.
- Ng, K. Y., Pollak, L. M., Duvick, S. A., and White, P. J. 1997a. Thermal properties of starch from 62 exotic maize (*Zea mays* L.) lines grown in two locations. *Cereal Chem.* 74:837-841.
- Ng, K. Y., Duvick, S. A., and White, P. J. 1997b. Thermal properties of starch from selected maize (*Zea mays* L.) mutants during development. *Cereal Chem.* 74:288-292.
- Pollak, L. M., and White, P. J. 1997. Thermal starch properties in Corn Belt and exotic corn inbred lines and their crosses. *Cereal Chem.* 74:412-416.
- Scott, M. P., Jane, J. L., and Soundararajan, M. 1999. Carbon isotope ratios of amylose, amylopectin and mutant starches. *Phytochemistry Oxford* 52:555-559.
- Seetharaman, K., Tziotis, A., Borrás, F., White, P. J., Ferrer, M., and Robutti, J. 2001. Thermal and functional characterization of starch from Argentinean corn. *Cereal Chem.* 78:379-386.
- Sharopova, N., McMullen, M. D., Schultz, L., Schroeder, S., Sanchez-Villeda, H., Gardiner, J., Bergstrom, D., Houchins, K., Melia-Hancock, S., Musket, T., Duru, N., Polacco, M., Edwards, K., Ruff, T., Register, J. C., Brouwer, C., Thompson, R., Velasco, R., Chin, E., Lee, M., Woodman-Clikeman, W., Long, M. J., Liscum, E., Cone, K., Davis, G., and Coe, E. H. 2002. Development and mapping of SSR markers for maize. *Plant Molec. Biol.* 48:463-481.
- Tan, Y. F., Sun, M., Xing, Y. Z., Hua, J. P., Sun, X. L., Zhang, Q. F., and Corke, H. 2001. Mapping quantitative trait loci for milling quality, protein content and color characteristics of rice using a recombinant inbred line population derived from an elite rice hybrid. *Theor. Appl. Genet.* 103:1037-1045.
- Wang, Y. J., White, P., and Pollak, L. 1993. Physicochemical properties of starches from mutant genotypes of the Oh43 inbred line. *Cereal Chem.* 70:199-203.
- White, P., Abbas, I., Pollak, L., and Johnson, L. 1990. Intra- and interpopulation variability of thermal properties of maize starch. *Cereal Chem.* 67:70-73.
- Yamin, F. F., Lee, M., Pollak, L. M., and White, P. J. 1999. Thermal properties of starch in corn variants isolated after chemical mutagenesis of inbred line B73. *Cereal Chem.* 76:175-181.
- Zeng, Z.-B. 1994. Precision mapping of quantitative trait loci. *Genetics* 136:1457-1468.

[Received April 27, 2004. Accepted May 31, 2005.]