

Mapping QTLs for Popping Ability in a Popcorn × Dent Maize Genetic Cross

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Summary

Corn (maize, *Zea mays* L.) is one of the world's most economically important grain crops. When exposed to heat, its starch-filled kernels pop and cook into puffs. The high demand for popcorn has spurred research to discover traits that dictate its idiosyncratic popping abilities. The purpose of this investigation is to study favorable popping traits and locate the regions on the maize chromosomes that show affiliation with such traits. Traits of interest include the kernel popping efficiency, the kernel popping expansion volume, the popping phenotype, and the average kernel size. In this study, kernel popping characteristics were assessed on 112 recombinant inbred corn lines from a large field population made by crossing B73 dent corn with popcorn line HP301. With a genetic marker linkage map available for the ten maize chromosomes, an analytical procedure called quantitative trait locus (QTL) mapping was done to determine chromosomal regions in the maize genome that may be important in the popping phenotype, based on the traits analyzed. QTL computer analysis revealed two QTLs for kernel popping efficiency on chromosomes 7 and 8, two additional QTLs for popping expansion volume on chromosomes 1 and 3, two QTLs for average kernel size on chromosomes 1 and 3, and one popping phenotype QTL on chromosome 2. The results of the QTL and maize phenotype correlation studies have relevance for better understanding the molecular basis of the popping phenotype. Furthermore, they could have practical applications in enhancing marketable popcorn.

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Introduction

Teosinte, considered by scientists to be the ancient wild ancestor of domesticated corn, has historic records that date back 4,000 years (1). Besides being a major food and feed crop, maize serves as a model organism for studying genetics (2). Through the lengthy process of natural and artificial selection, modern corn has become

a highly cultivated diploid domesticated crop plant that contains 20 chromosomes (n=10), and a total of 2.3 gigabases coding for an estimated 32,000 individual genes (3). According to the maize genetic map, the combined length of the chromosomes is about 1,500 centiMorgans (cM).

Popcorn is a unique line of flint corn with a dense, starchy interior and hard pericarp (exterior shell). The trait that distinguishes popcorn from other types of corn is its ability to form large flakes when heated (4). The popping ability of a popcorn kernel involves many heritable polygenic traits such as pericarp thickness and endosperm starch type in the seed kernel (5). Previous studies suggest that a more crystalline arrangement of cellulose and higher packing of fibrils in the pericarp, as well as the ratio of hard to soft endosperm, affect the explosion and flake formation of popcorn (6). When heated, the water inside the kernel attempts to expand into a gaseous phase, but cannot escape the kernel during this expansion period due to the tough pericarp. Upon reaching an internal temperature of 180 °C (356 °F), the pressure inside the kernel reaches about 135 psi. This pressure is sufficient for the exterior shell of the kernel to rupture. The rupture event instantly releases pressure and cooks the proteins, oils, and starches that make up the interior to create soft flakes (7). The morphology of the popped kernel can show distinct variations. Some kernels form a butterfly shape characterized by large 'wing-like' flakes, some form a mushroom shape characterized by a spherical expansion, and others form a combination of the two morphologies.

Today, popcorn is a very popular consumable good whose high demand has invited investigation and research in discovering ways to enhance its popping abilities for further consumer satisfaction. Using the genomic data and linkage maps currently available for corn and applying a molecular genetics approach, a fast recovery time for developing elite popcorn lines should be possible in corn breeding programs. One approach called Quantitative Trait Locus (QTL) mapping uses computer-based statistical programs to map important trait genetic loci in the genome to expedite marker-assisted breeding and selection, and to allow a better understanding of the actual genes that contribute to the phenotypic traits of interest. The process involves a program that uses information from a molecular marker

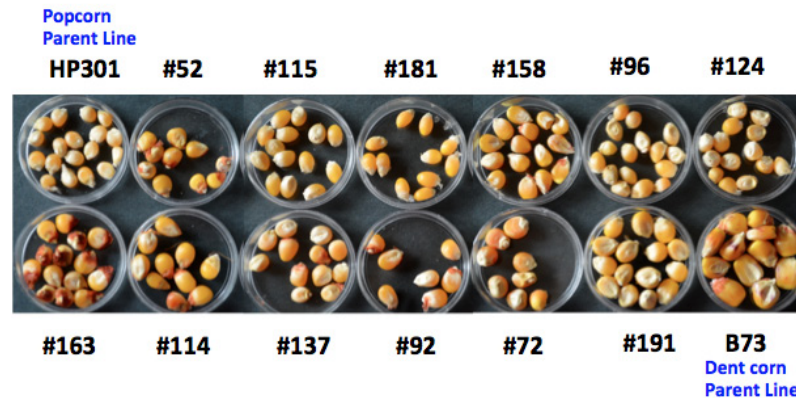


Figure 1: Comparison of the phenotypic appearance of kernels from the parent popcorn line HP301 and parent dent corn line B73 used to generate the recombinant inbred line (RIL) population studied, with a sampling of RIL intermediates. Note that HP301 kernels are small and rounded, while B73 kernels are larger and flattened.

linkage map coupled with the parent genotypes of the recombinant lines used for analysis and a quantitative account of variation in specific traits of interest. These are used to compute statistical probabilities of likelihood for existence of genetic loci responsible for the expression of traits of interest at certain chromosomal locations associated with the variance in phenotype for a set of recombinant inbred lines. The molecular marker linkage map used in this study was based on single nucleotide polymorphisms (SNPs).

In order to identify genes that may be important for a particular heritable trait (kernel size, starch structure, or popping expansion volume (PEV)) in a popcorn line, a population of plants that are polymorphic for such genes is essential. Recombinant inbred lines, or RILs, provide the genetic variation that is needed to conduct molecular genetic studies. A RIL is made by crossing two inbred

parent lines to produce an F1 generation. F1 seeds are planted; the seeds are collected and re-sown, and then used to make an F2 generation of plants. These plants are then repeatedly self-pollinated to generate a population of new isogenic lines whose genomes are a mosaic of the two original parental genomes (8). Because the plants are mosaic, multiple phenotypes can be generated across the highly variable RIL population (variability generates better QTL approximations), creating a permanent resource for phenotypic analysis and trait mapping, particularly quantitative trait loci.

The purpose of the current investigation is to study certain favorable popping traits and find the regions on the maize chromosomes that show affiliation with such traits. For this work, a RIL population of 112 recombinant lines made from the popcorn parent line HP301 crossed with a dent corn parent line B73 was studied for the

Trait Name	Chromosome	Positions on		LOD Score ¹	R ²	Permutation		Nearest Marker
		Chrom (cM)				Sum of R ²	Threshold ²	
AKS	1	57.56	10.16423389	0.2198467	0.5392854	2.891843298	2.891843298	PZA03561
AKS	3	47.90	6.172983364	0.1281434	0.5392854	2.891843298	2.891843298	PZB02044
MinFerret	9	20.46	6.532027591	0.1226684	0.5257117	2.983766072	2.983766072	PZA03058
MinFerret	1	57.56	8.83005483	0.1822534	0.5257117	2.983766072	2.983766072	PZA03561
FeretX	1	120.16	5.139468076	0.169578	0.169578	2.745093015	2.745093015	PZA03001
Mass per Seed Popped	1	63.09	5.863753977	0.1658037	0.5437437	2.944716482	2.944716482	PZA01267
Mass per Seed Popped	8	78.34	5.515256983	0.1542109	0.5437437	2.944716482	2.944716482	PHM1834
Total Mass of Popped Seeds	5	9.89	6.180231544	0.2575051	0.2575051	2.815542097	2.815542097	PZA01294
UnPopped Mass	6	78.41	6.218110645	0.1842108	0.5945611	2.544509758	2.544509758	PZA01468
Total Popped Volume	2	8.54	5.607800776	0.1610288	0.2751887	2.879418132	2.879418132	PZA03747
Volume per Seed Popped	2	13.63	5.163050984	0.1418053	0.3777932	2.87266051	2.87266051	PZB00901
PEV	3	128.51	5.241494407	0.1381418	0.62298	2.9844566	2.9844566	PZA02668
PEV	1	59.09	7.227781472	0.1710085	0.62298	2.9844566	2.9844566	PZA03561
KPE	7	24.13	9.301232723	0.2600441	0.5032281	2.886742509	2.886742509	PHM12830
KPE	8	50.24	6.632007765	0.1712396	0.5032281	2.886742509	2.886742509	PZA00908
PP	2	96.45	6.286228956	0.1581058	0.4931621	2.601986463	2.601986463	PHM16125

¹An LOD Score is a number assigned that depicts a favoring to the presence of genetic linkage with the nearest marker.

An LOD Score higher than 1.5 times the Permutation Threshold for each QTL was a determining factor of individual significance for a QTL

²A Permutation threshold is used as a standard for comparison to the LOD Score

Table 1: Significant QTLs. The logarithm of odds LOD score referred to is a statistical estimate of whether 2 gene loci (for genes, traits or markers) are likely to be located near one another on a chromosome, and therefore, likely to be inherited together. It is a measure of statistical certainty that also indicates the likelihood a specific marker is associated with a trait of interest. An LOD score of 3 implies that a gene and marker for a specific trait are located close enough so that the odds of them being linked is 1,000:1.

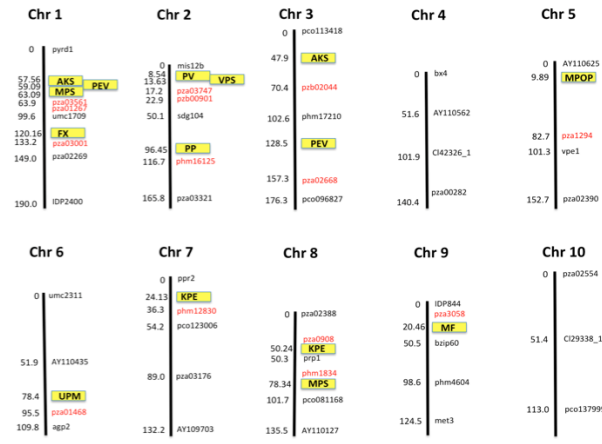


Figure 2: Molecular Marker Linkage Map showing 49 different SNP markers from the 154-marker basket used in the QTL analysis performed. QTLs for PEV, KPE, AKS, PP, MF (minFerret), UPM (UnPopped Mass), FX (FerretX), MPS (Mass per Seed), MPOP (Mass per Seed Popped), PV (Popped Volume), and VPS (Volume per Popped Seed). Numbers to the left of the chromosomes indicate cM locations of markers/QTLs. Numbers to the right of the chromosomes indicate the name of the marker or trait of interest.

expression of important popcorn traits that dictate a kernel's ability to pop favorably, including the average kernel size (AKS), the kernel popping efficiency (KPE), the kernel popping expansion volume (PEV), and the popping phenotype (PP). Upon conducting QTL analysis, the significance of each of the traits to the overall popping phenotype was determined, as were the relationships between certain popping traits.

Results

The purpose of the current investigation is to study certain favorable popping traits and find the regions on the maize chromosomes that show affiliation with such traits.

Kernel appearance

A range of kernel shapes and sizes were observed across the RIL samples analyzed. A survey of kernel types is shown in **Figure 1**. Most obvious are the differences in size and shape of the parent lines, HP301 and B73 (**Figure 1**).

QTL analysis

The results of the QTL mapping analysis are given in **Table 1**. The major traits (PEV, KPE, AKS, PP) are described in detail below. More minor traits are introduced in the discussion section.

Popping expansion volume (PEV)

Two QTLs were identified for PEV, with one locus on chromosome 1 and another locus on chromosome 3

Trait	Chromosome	Percent Contribution	Sum Contribution of each QTL
Popping Expansion Volume (PEV)	1	27.5%	49.8%
	3	22.2%	
Kernel Popping Efficiency (KPE)	7	51.7%	85.7%
	8	34.0%	
Average Kernel Size (AKS)	1	40.8%	64.6%
	3	23.8%	
Popping Phenotype (PP)	2	32.0%	32.0%

Table 2: The contributions of each individual QTL to phenotype studied. In each case, the QTLs detected that failed to reach significance, when summed, accounted for the remainder of the phenotypic variance.

(**Figure 2**). The QTL on chromosome 1, located closest to molecular marker PZA03561 at 59.09 cM in the linkage map, accounted for 27.4% of the phenotypic variance of PEV. Percent variance accounted for was calculated in all cases as a percentage of each R^2 value for each individual QTL in relation to the sum of all R^2 values of all QTLs identified for each trait analyzed. The QTL on chromosome 3, located closest to marker PZA02668 at 128.51 cM, accounted for 22.2% of the phenotypic variance for the PEV trait. Both of the QTLs detected for PEV accounted for 49.8% of the total PEV phenotypic variance, with the QTL on chromosome 1 having a more significant association with phenotypic variance than the QTL on chromosome 3 (**Table 2**). The remainder of the total variance of PEV could be accounted for by the sum of three other less significant QTLs that failed to meet the established threshold for significance.

Kernel popping efficiency (KPE)

For KPE, two QTLs were identified out of the 66 total QTLs (including both significant and insignificant QTLs; 66 QTLs were identified in total). One QTL was on chromosome 7 and the other on chromosome 8 (**Table 1**) (**Figure 2**). The QTL on chromosome 7 was located closest to marker PHM12830 at 24.13 cM and was found to be more significant than the one on chromosome 8, accounting for 51.7% of the phenotypic variance. The QTL on chromosome 8 was located closest to marker PZA01294 at 50.24 cM and accounted for 34% of the total KPE variance. Together, both QTLs accounted for 85.6% of the phenotypic variance (**Table 2**). The remainder of the total variance of KPE can be accounted for by one other less significant QTL that failed to meet the established threshold for significance.

Average kernel size (AKS)

Two QTLs for AKS were found on chromosomes 1 and 3 (**Table 1 and Figure 2**). One QTL, located closest to marker PZB02044 at 47.9 cM on chromosome 3, accounted for 23.8% of the phenotypic variance of AKS. Another QTL was located for AKS closest to marker PZA03561 at 57.56 cM on chromosome 1 and

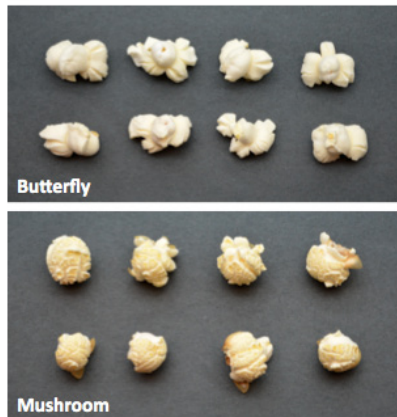


Figure 3: Popped kernels showing the “butterfly” (upper panel) and “mushroom” (lower panel) flake morphologies used to score the popping phenotype (PP) trait in the study.

accounted for 40.8% of the phenotypic variance of the trait. Both of the QTLs detected for AKS together accounted for 64.5% of the phenotypic variance of the AKS trait with the QTL on chromosome 1 being more significant than the one on chromosome 3 (**Table 2**). The remainder of the total variance for AKS could be accounted for by the sum of three other less significant QTLs that failed to meet the established threshold for significance.

Popping phenotype (PP)

Overall examination of the popping morphologies of the 112 RILs indicated that only a few of the RILs had almost all mushroom or butterfly morphologies. However, most lines showed a mixture of the two. Examples of these two popping phenotypes (PP) are shown in **Figure 3**. One QTL for popping phenotype was detected on chromosome 2 at 96.45 cM and was closest to genetic marker PHM16125 (**Table 1 and Figure 2**). By itself, it accounted for 32% of the phenotypic variance of the trait, making it the most significant QTL for PP identified in the study (**Table 2**). The remainder of the total variance of PP could be accounted for by the sum of five other less significant QTLs that failed to meet the established threshold for significance.

A summary of the contributions of individual QTLs to each phenotype studied can be found in **Table 2**, and a summary of the QTL mapping results from the study is schematically shown in **Figure 2**.

Correlation Analysis

By creating numerous plots comparing and identifying the relationships between the quantified traits measured and listed in the materials and methods section, several significant correlations amongst certain traits were observed. The plot between PEV and KPE yielded a positive correlation of 0.7117, indicating a positive

relationship between these two measured variables. A negative relationship was found between PEV and AKS, with a correlation of -0.5674 between the two. The plot between PEV and mass per seed popped displayed a correlation of -0.4653, indicating a significant (negative) relationship between PEV and mass per seed popped. Other correlations include PEV vs. Mass per seed not popped (-.5149) and PEV vs. Percent of Seeds Popped (.3446). Plots between PEV and Mass per Seed, Percent of Seeds Popped and AKS, Percent of Seeds Popped and KPE, and Percent of Seeds Popped and Mass per Seed all yielded plots with no correlations. For a summary of all significant correlations, refer to **Table 3**.

Discussion

Experimental procedure and results

Composite interval mapping using QTL Cartographer resulted in the identification and localization of at least sixteen significant QTLs associated with kernel popping. Loci were identified on all maize chromosomes except on chromosomes 4 or 10 (**Table 1**). The chromosome mapping of the most significant QTLs detected is shown in **Figure 2**. Two QTLs were identified for PEV on chromosomes 1 and 3. The QTL on chromosome 1 matched that of another QTL reported by a study on popcorn line A-1-P crossed with the flint corn line V273 (5). The QTL identified in the current study was located at 59.09 cM on chromosome 1 and accounted for 27.4% of the phenotypic variance for PEV, while one of the four QTLs for PEV detected in another study was located at 67.0 cM and accounted for 30% of the phenotypic variance, very similarly (5). Such similarities indicate that this region in chromosome 1S appears to be a very significant region in the overall expression of PEV.

Significance of the QTL analysis results and correlations analyses

A centiMorgan is a unit of recombination frequency for measuring genetic linkage. Therefore, genetic markers that are closer in terms of centiMorgans are more likely to exhibit genetic linkage and co-segregation than markers that are located farther apart on a chromosome. The results from the QTL analysis revealed that two of the QTLs on chromosome 1 (one from PEV at 59.09cM, and the other from mass per seed popped at 63.09 cM) are very close (within 5 cM) to one another (**Table 1**). This result indicates a genetic linkage amongst various genes on chromosome 1 that contributes to the favorable expression of traits for PEV and mass per seed popped. Given the accuracy of QTL mapping, these QTLs for PEV and mass per seed popped could be driven by the same gene, or by two genes that are close to each other. It was also noted that a plot between PEV and mass per seed popped yielded a negative correlation of -0.4653

Trait X	Trait Y	Correlation
AKS	PEV	-0.5674
Mass Per Seed not Popped	PEV	-0.5149
Mass Per Seed Popped	AKS	0.7237
KPE	PEV	0.6833
Mass per Seed	PEV	-0.0219
Mass Per Seed Popped	PEV	-0.4653
Percent Popped	PEV	0.3446
AKS	Percent Popped	-0.0939
KPE	Percent Popped	0.0759
Mass Per Seed	Percent Popped	0.0462

Table 3: Summary of correlation analyses. Correlation coefficients of each plot were computed and displayed for all relevant relationships.

(Table 2), meaning that higher popping expansion volume is associated with lower mass per seed for seeds that popped. Because of the relationship between PEV and mass per seed popped and the observed results from the QTL analysis that indicate similar chromosomal loci for each trait, it is possible that the mass of a kernel for seeds that pop is a component trait for PEV.

A significant positive correlation was found between PEV and KPE, which suggests that a kernel's popping efficiency is largely related to its flake volume. A strong negative correlation was observed between PEV and AKS, coupled with an almost identical QTL location for the QTLs for each trait on maize chromosome 1. Therefore, a smaller kernel size, with its more compact dense starch and reduced volume, apparently allows for better kernel explosion, improved starch expansion, and attainment of maximum flake volume when exposed to heat during the popping process. This supports the assertion that AKS is an important component trait of quantitative PEV.

Multiple significant correlations were detected amongst traits associated with PEV and Percent Popped for each line (See Table 3). For example, the plot between PEV and mass per seed popped yielded a correlation of -0.4653. This means that for seeds that popped, those with lower masses generally experienced higher popping expansions than those with larger kernel mass. This finding makes sense when one considers the correlation between PEV and AKS (-0.5674), which indicates that a lower seed size is associated with a higher popping expansion. This is confirmed by the observation that a plot of AKS vs. mass per seed popped yielded a strongly positive correlation of 0.7237. However, a plot between PEV and overall average kernel size (including those of seeds which did not pop) displayed no correlation between the two variables, indicating that a higher PEV is associated with seeds that have a lower average size and mass only holds true for seeds that pop and not necessarily for all seed samples (including the ones that did not pop) as a whole.

Significance of the popping phenotype and experimental observations

Prior to this study, research to map chromosomal regions associated with the popping morphology (PP, popping phenotype) of popcorn had not yet been performed or published. The results from this study involving a kernel's tendency to form a mushroom-type flake expansion versus a butterfly-like expansion is of interest and could hold relevance in understanding how the structure of the endosperm starch (or pericarp) influences the flake morphology, and possibly lead to the discovery of new genes (and gene functions) that could be used in popcorn breeding programs to further select for favorable popping phenotypic traits.

Pitfalls and alternative approaches

One weakness of the current study was that using volume-based samples of each RIL might not have been as accurate as using number-based sampling techniques. Measuring out two-tablespoon volumes of seeds for each RIL could have resulted in volume gaps between measures because seed could have been collected at different levels in the measuring cup. In the future, a distinct number of seeds could be counted out from each line and then measured for volume to produce more accurate data.

Another potential concern with the experiments is that not all seeds had the optimal 13.5% moisture content during experimentation (6). Our kernels had a relative humidity of 20%, which may have accounted for some discrepancy in the amounts of seeds that popped. That said, a QTL analysis is based upon relative correlations. I argue that a small difference in the seeds' moisture content from the optimal standard would be negligible in determining relative correlations and QTLs.

Future implications

The sample of 112 RIL families used in the present study was relatively small and could be doubled for more accuracy in the QTL and correlations analyses. In addition, it is often the case that the RIL populations are planted out in at least two significantly different field environments, so the ultimate QTL analysis can include (or exclude) QTL's that might be dependent upon environmental effects. By this growing method, false positives can also be eliminated if they are due to environmental effects (5). It is important to bear in mind that the observable (or testable) phenotype of any organism is a function of its genotype and the environment. It is conceivable that growing the RILs in a more arid field environment could result in seeds that give different popping profiles, and consequently, different QTL results. In future studies, it would be good

Trait Name	Definition	Units	Method of Determination
Total Count	Total number of seeds in sample	No. of Seeds	measured by Image J scanning program
Min Feret	Minimum seed diameter	cm	measured by Image J scanning program
Max Feret	Maximum seed diameter	cm	measured by Image J scanning program
Total Area	total kernel face area of seeds in sample	cm ²	measured by Image J scanning program
Percent Area	percent face area of each kernel relative to total	%	measured by Image J scanning program
Average Kernel Size	Average face area of each kernel	cm ² /kernel	measured by Image J scanning program
Total Popped Volume	Combined volume of popped kernels	mL	measured in graduated cylinder
Mass of Sample	Combined mass of all kernels in sample	g	weighed on balance before popping
UnPopped Mass	Combined mass of all kernels that did not pop	g	weighed on balance after popping complete
UnPopped Count	Total number of seeds that did not pop from sample	No. of Seeds	measured by Image J scanning program after popping
Popped Count	Total number of seeds that did pop from each sample	No. of Seeds	Total Count- UnPopped Count
Average Mass UnPopped Seeds	Average mass of each seed that did not pop	g/kernel unpopped	UnPopped Mass/UnPopped Count
Total Mass of Popped Seeds	Combined mass of the kernels that popped before popping	g	Mass of Sample- UnPopped Mass
Mass per Seed Popped	Average mass of each seed that popped	g/kernel popped	Total Mass of Popped Seeds/Popped Count
Volume per Seed Popped	Average volume taken up by each popped seed after popping	mL/kernel popped	Total Popped Volume/ Popped Count
Phenotype Rating	Popping phenotype	N/A	1-5 Scale ranking of resemblance to Mushroom (1) or Butterfly (5)
Percent Popped	Percent of total seeds in sample that popped	%	(Popped Count/Total Count)*100%
Popping Expansion Volume	Average volume expansion per kernel relative to kernel volume	mLpop/mLbefore	Total Popped Vol./[Vol. of Kernels Before Popping](Percent Popped)

Table 4: Kernel and popping traits assessed. Each trait was quantitatively calculated using methods in the “Method of Determination” column and measured in the units described in the “Units” column.

to perform the QTL mapping analysis on the same set of RILs grown in two different field locations.

For an extension of this investigation, it would also be exciting to map the specific quantitative gene loci at a higher resolution so that individual gene(s) could be identified on a physical map of the genome, rather than on a genetic map giving a relative cM chromosomal location. To do this, more advanced intercrossing between RILs with QTLs of interest or their backcrossing to a specific parent line, like B73, would be needed for higher resolution mapping power to the actual gene level. For example, this experiment could be further conducted with larger populations and more intricate RIL development to produce even more accurate and precisely measured QTLs. Such studies yielding largely precise QTL locations could reveal the exact genes that control certain traits that make popcorn pop favorably.

Materials and Methods

Population development

The cross of the original parent B73 dent corn line (a commercial processed food corn also used to feed livestock) and parent HP301 popcorn line, as well as the subsequent “selfing” (self-pollination) of the derived lines into near isogenic lines, was originally performed in Puerto Rico at the Crop Development Institute through research conducted by agricultural scientists associated with the University of Illinois and University of Missouri (8). Seed for this study came from F6 generation RILs planted in November 2012, grown under natural field conditions and harvested in April of 2013. Standard agronomic practices were used, with a single plot planted per line. Hand-pollinated ears were used to generate grain samples for this analysis.

Over 200 RILs were made available for this study, of which 112 lines were analyzed over the 6-month period of the project. The lines were designated numerically from 1 to 200 with no other descriptors so that the studies conducted could be “blind” and unbiased. The

corn ears used in the study were obtained through a research collaboration between the United States Department of Agriculture scientists at the Danforth Plant Science Center in St. Louis, the University of Missouri in Columbia, and the Boyce Thompson Plant Institute at Ithaca, New York. After receiving corn ears from the Illinois Crop Development Center in Puerto Rico (where progenitor and inbred lines were planted and processed), the ears were stored at a temperature of 40°C at 20% relative humidity at the Danforth Plant Science Center until phenomic analyses was conducted there. In this study, kernel characteristics (1) or popping traits from as many lines as possible were examined from June 2013 to December 2013.

Laboratory procedures for analyzing kernel traits and popping characteristics

During the course of the project, 112 separate RILs representing about half of the total lines received were analyzed. For each RIL derived from the parent cross described above, kernels were physically removed from the cob of one ear, placed into labeled envelopes, and stored at room temperature until experimental analysis. A two-tablespoon volume of each recombinant inbred line (RIL) was sampled for phenotyping and data collection. First, each RIL sample was scanned using a Mustek A3 1200S scanner and the images generated of each line were uploaded to an Image J64 program with which a Summarize Scan Threshold 10 program setting was employed. This allowed the exact number of seeds in each sample to be counted and dimensional traits of interest to be measured and digitized including minFeret (smallest seed diameter), maxFeret (largest seed diameter), total area (total kernel face area of sample), percent area (the percent each individual kernel’s face area was as compared to the total area) and average area (average seed face area). The data for average size was used to determine the average kernel size for each RIL. In order to measure other popping related

traits, each sample was weighed on an Ohaus Pioneer portable electronic balance and popped on an H1 (high) setting for 2 minutes and 15 seconds in a Kenmore 1000 Watt Quicktouch microwave. The total volume of the popped seeds was measured in a 500 mL polypropylene graduated cylinder and recorded. Using this volume measurement, the popping expansion volume (PEV) was calculated for each RIL. In addition, each RIL was given an overall phenotype rating to qualitatively describe its relatedness to an either mushroom or butterfly flake shape (rating of 1 being highest resemblance to mushroom and rating of 5 being highest resemblance to butterfly) in order to quantify a measurement that would help yield data for a phenotypic assessment in the QTL analysis. Once the popping protocol was carried out for each RIL, the remaining kernels that did not pop were re-weighed and re-scanned in order to count the number of seeds that did not pop to calculate the relative popping efficiencies. The number of kernels per two-tablespoon samples of each RIL that popped in the popping process dictated kernel popping efficiency, therefore the number of kernels that popped was used as quantitative data for RIL-to-RIL comparison. A summary of the traits characterized and the analytical methods used is given in **Table 4**.

Phenomic data analysis: Molecular linkage map

The B73 maize genome was sequenced and annotated, and made available to the public in 2009 (2). The molecular markers used for the QTL analysis in this study were SNPs or single nucleotide polymorphisms. Using a core set of 1,100 NAM markers, a subset of 650 SNPs were different between B73 and HP301 (8). From this set, a subset of 154 molecular markers were selected as sufficient for mapping. (I. Baxter, personal communication). A computer map was made using the Emap function in QTL Cartographer and co-linearity was confirmed using the full map of all 1,100 markers. It is this marker panel that was used in the final QTL analysis to determine the chromosome location in cM of potential QTL candidates.

QTL mapping

Using the popping phenotypic data generated, an overnight computational analysis was conducted for QTL mapping of the traits studied. To do this, composite interval mapping (CIM) using QTL Cartographer version 1.17f (9) was set up with CIM model 6 of Zmapqtl (10), stipulating an F6 generation population. All ten chromosomes of the maize genome were scanned at a “walk speed” of 2 cM with a window of 5 cM, and 10 cofactors (the 10 maize chromosomes) using the forward and backward regression method. Empirical threshold levels for

concluding QTL significance at a Type I error rate of 0.05 were obtained using 1,000 permutations of the data derived from the RIL population examined according to the methods of another study (11). The QTL analysis itself employed both ANOVA (analysis of variance) and regression analyses to describe the genetic basis of the variance among the different polygenic traits for which data was collected. In such an analysis, the higher the number of permutations used, the more accurate the analysis. This type of QTL analysis can give information on the loci of certain chromosomal regions with very high certainty (12). The centiMorgans (cM) given in the data summary are population-dependent for the specific RIL population used in this study.

Correlation Analysis

Using LoggerPro computer software, correlation analyses were performed to analyze the possible relationships among traits involved in the popping phenotype. By creating numerous plots between the quantified traits measured in the materials and methods section, several significant correlations were revealed amongst certain traits. All possible combinations of plots involving the comparison of two traits were tried, of which several significant correlations are presented in **Table 3**.

Acknowledgments

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