Fast-Flowering Mini-Maize: Seed to Seed in 60 Days

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ABSTRACT Two lines of *Zea mays* were developed as a short-generation model for maize. The Fast-Flowering Mini-Maize (FFMM) lines A and B are robust inbred lines with a significantly shorter generation time, much smaller stature, and better greenhouse adaptation than traditional maize varieties. Five generations a year are typical. FFMM is the result of a modified double-cross hybrid between four fast-flowering lines: Neuffer's Early ACR (full color), Alexander's Early Early Synthetic, Tom Thumb Popcorn, and Gaspe Flint, followed by selection for early flowering and desirable morphology throughout an 11-generation selfing regime. Lines A and B were derived from different progeny of the initial hybrid, and crosses between Mini-Maize A and B exhibit heterosis. The ancestry of each genomic region of Mini-Maize A and B was inferred from the four founder populations using genotyping by sequencing. Other genetic and genomic tools for these lines include karyotypes for both lines A and B, kernel genetic markers *y1* (white endosperm) and *R1-scm2* (purple endosperm and embryo) introgressed into Mini-Maize A, and ~24× whole-genome resequencing data for Mini-Maize A.

KEYWORDS maize; *Zea mays*; flowering time; heterosis; model system

MODEL genetic organisms are typically selected for their short generation time, small size, large number of progeny, and inexpensive maintenance. Maize began its use for genetic analysis before the concept of model organisms emerged. Nevertheless, it has continued to contribute to genetic studies despite its relatively longer generation time (typically two per year) and large stature because of the ease of conducting crosses due to its monoecious nature, large number of progeny, relatively large chromosomes, a suite of genetic markers visible at the kernel stage, several tools to readily manipulate genome or chromosomal dosage, and enormous natural variability (Nannas and Dawe 2015). Flowering time in maize landraces can vary from 2 to 11 months, and height can vary from 1 to 7 m (Kuleshov 1933), opening the possibility of selecting for small, fast-flowering varieties more suitable as model genetic systems. Previous studies have generated short-generation varieties but none are available that maximize useful model organism characteristics. Here we describe the development of Fast-Flowering Mini-Maize (FFMM), which consists of two inbred lines, FFMM-A and FFMM-B (Figure 1). FFMM has many characteristics desirable in a model system, in that it can go from seed to seed in 60 days, produces many progeny, grows to <1 m tall, and performs well under greenhouse conditions, while still being relevant to one of the most economically important cereal crops in the world.

The goal of FFMM is to provide an open-source tool for researchers to reduce the timeline of research projects and improve greenhouse capacity. FFMM successfully decreases generation time and space requirements of maize. Five generations per year are routine. The kernel genetic markers, short generation time, and small stature also have potential for FFMM to be a useful educational tool in classroom settings.

Materials and Methods

Development of FFMM

Both FFMM lines derive from a four-way cross between segregating populations. Neuffer's Early ACR (full color) is an early-flowering line obtained from M. G. Neuffer (University of Missouri; stock no. 60:8). Alexander's Early Early Synthetic was obtained from the Maize Genetics Cooperation Stock Center (http://maizecoop.cropsci.uiuc.edu/; stock #94-2-7 self). Tom Thumb Popcorn was purchased

doi: 10.1534/genetics.116.191726
Manuscript received May 16, 2016; accepted for publication July 2, 2016; published Early Online July 18, 2016.
Supplemental material is available online at www.genetics.org/lookup/suppl/doi:10.1534/genetics.116.191726/-/DC1.
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Figure 1 FFMM-A and -B. A FFMM-A plant (left), and a FFMM-B plant (right) 30 days after planting. The flag leaves of the ears have been trimmed. Silks were present on the FFMM-A plant and were cut with the flag leaves. The nursery pots are ~15 cm in diameter by ~17 cm in depth, which is smaller than what is required for a normal maize plant.

from Johnny's Selected Seeds (www.johnnyseeds.com, no longer listed; but available from North Central Regional Plant Introduction Station, Ames, IA; accession no. PI 217412). Gaspe Flint was obtained from the North Central Regional Plant Introduction Station (accession no. PI 214279).

Plants from Neuffer's Early ACR were crossed by plants from Alexander's Early Early Synthetic in 2001. Those F_1 progeny were crossed in 2008 to a single Tom Thumb × Gaspe F_2 plant that had been selected for early flowering. The lineage was selfed for 11 generations, keeping individuals each generation while selecting for fast flowering, short

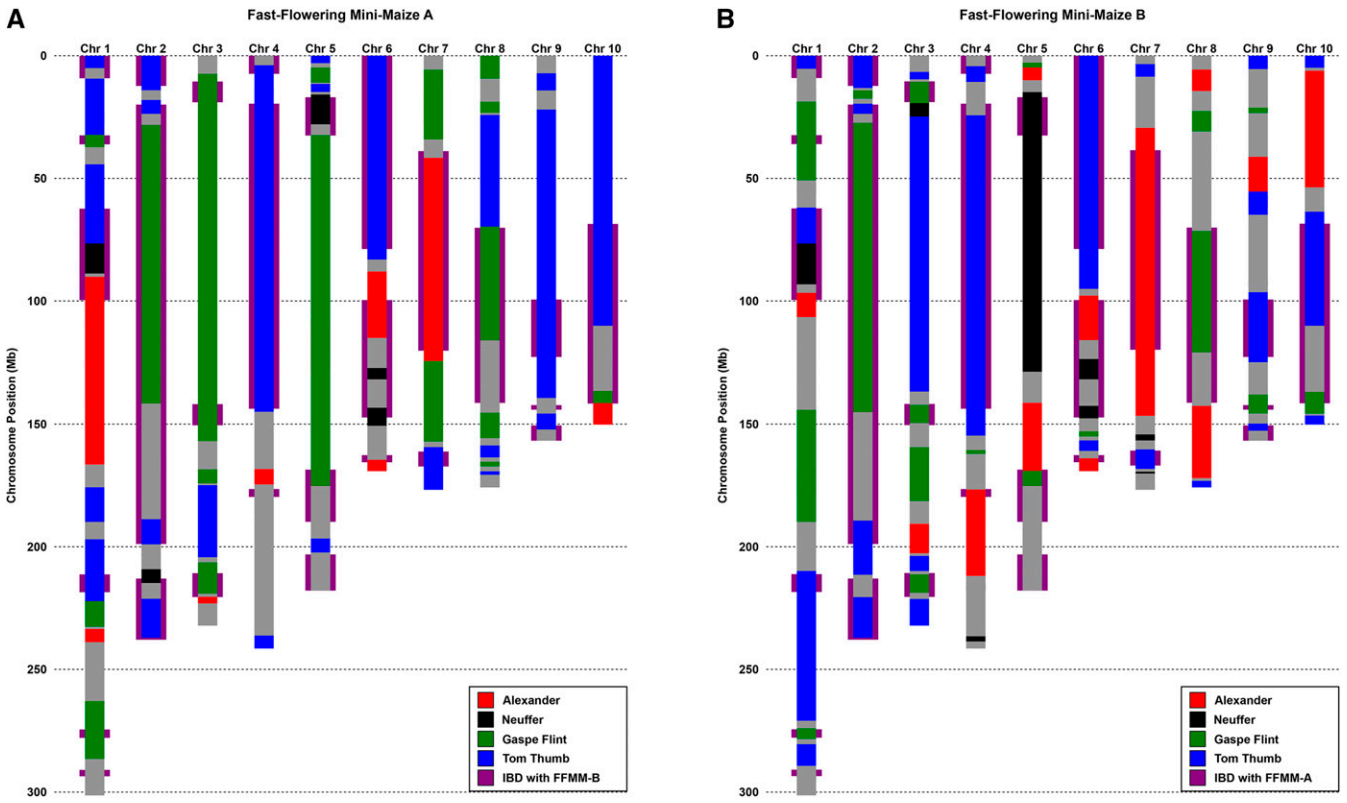

Figure 2 Inferred ancestry along the FFMM chromosomes. Genotyping-by-sequencing was performed on four individuals of each of the four founder populations, and haplotype similarity was used to infer probable ancestry across the FFMM-A (panel A) and B (panel B) genomes. Colored blocks indicate a region probably inherited from the corresponding parent population; regions of ambiguous ancestry are colored gray, and sections shared between the two FFMM lines are considered identical by descent (IBD) and are highlighted along the sides in purple. The full similarity traces across each chromosome are in Figure S1. Alexander, Alexander's Early Early Synthetic; Neuffer, Neuffer's ACR.

stature, pollen yield, and seed set. FFMM-A and -B were selected as having the best combination of these characteristics.

Whole-genome sequencing

FFMM-A whole-genome sequence was determined from a single greenhouse-grown plant. Tissue was collected by leaf punch ~1 month after planting and genomic DNA extracted with a DNeasy Plant Mini kit (QIAGEN, Valencia, CA). Illumina TruSeq DNA library preparation and sequencing were outsourced to the Genomics Facility at Cornell University; sequencing was performed on an Illumina HiSequation 2500 with paired-end, 151-bp reads, resulting in 412 million reads total (2 × 206 million). Raw sequencing data are available from the Sequence Read Archive, accession no. SRX834991, and whole-genome genotypes are included as sample "MM-1A" in Maize Hapmap3 (available from Panzea, http://cbsusrv04.tc.cornell.edu/users/panzea/filegateway.aspx?category=Genotypes).

Genomic ancestry determination

Four plants of each founder population plus two FFMM-B plants were grown to the seedling stage in a growth chamber (16 hr light/8 hr dark at a constant 25° and 30% relative humidity). Samples consisting of 5 cm^2 of leaf tissue were collected into 96-well plates; to increase the depth of coverage, each founder plant was sampled three times and each FFMM-B plant was sampled twice, for a total of 52 individual samples. These were sent to the Genomic Diversity Facility at Cornell University for genotyping by sequencing (GBS) (Elshire *et al.* 2011) on an Illumina HiSequation 2500, with *Ape*KI restriction enzyme used for complexity reduction. The resulting FASTQ files were processed to SNP calls using the GBS 2.7 production TOPM file from Panzea (available at http://www.panzea.org/lit/data_sets.html) and the ProductionSNPCallerPlugin in the TASSEL-GBS pipeline (Glaubitz *et al.* 2014) in TASSEL v5.1.0. The resulting genotype file is included in Supplemental Material, File S1.

The technical replicates from each founder plant were checked to confirm they clustered properly and were then merged into a single sample per plant using a custom Python script; all FFMM-B samples were similarly checked and merged into a single sample. The resulting SNPs were filtered to include only those that were polymorphic among these samples (208,501 SNPs total).

The corresponding FFMM-A genotypes were called by aligning the whole-genome resequencing reads to the maize AGPv2 genome using Bowtie2 (Langmead and Salzberg 2012) and calling SNPs with the Samtools mpileup command (Li *et al.* 2009). All calls that were supported by at least two reads and were at least 10 bp removed from an indel were chosen; those that overlapped with the GBS SNPs (above) were added to the GBS data set.

Local genomic ancestry was determined by calculating similarity among samples in a 1000-SNP sliding window (step size of 100 SNPs) along each chromosome. Regions were assigned to the most similar ancestor if the similarity score passed 55% (Figure 2). The computer-generated assignments were then manually adjusted based on visual inspection of the chromosome traces (see Figure S1).

All scripts used in these analyses are available in File S2.

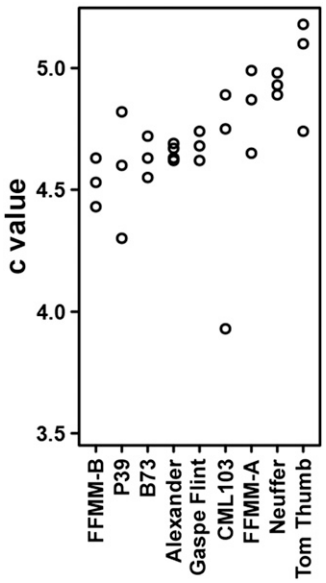

Figure 3 Genome-size estimation. The nuclear genome content (*c*-value) of both FFMM lines, samples from the four founder populations, and several inbred lines were determined by flow cytometry. Each point is from a separate plant, except for the Alexander and Tom Thumb samples, which had only two plants each (and thus some plants were sampled multiple times). Samples are arranged in order of increasing median *c*-value.

Flow cytometry

Three plants of each sample (see Figure 3) were grown to the seedling stage in a growth chamber (16 hr light/8 hr dark at a constant 25° and 30% relative humidity). Whole leaves were collected from each plant and sent to the Iowa State University Flow Cytometry Facility for genome size determination, with triploid trout nuclei (Biosure, Inc., Grass Valley, CA; product no. 1014) as the size marker.

Heterosis and temperature effects on Mini-Maize traits

Heterosis and hybrid vigor are terms describing the observation that a hybrid produced by crossing two inbred parents is more vigorous than either parent. This effect is one of the foundations for modern maize agriculture and was responsible for a sharp rise in yield over openly pollinated varieties. Because FFMM-A and -B were derived from independent lineages from the original material and remained independent throughout selfing and selection, it is possible that they are genetically distinct enough that a cross between the two inbred lines will show hybrid vigor. A simple test for hybrid vigor was performed on the two lines as well as a test to determine whether a difference in temperature affected the flowering time and vigor of FFMM. A total of 90 plants were used in the heterosis trial, divided into two randomized blocks of 45 plants in each greenhouse. Because the inbred FFMM-A and FFMM-B lines showed delayed flowering in winter (when

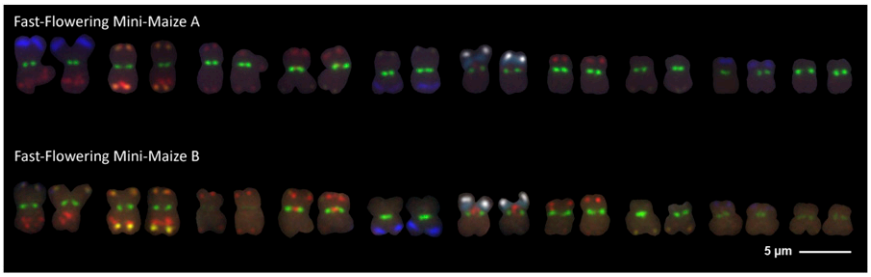

Figure 4 Karyotype of FFMM-A and FFMM-B. Karyotypes of FFMM-A and FFMM-B using the protocol described in Kato *et al.* (2004) with modifications of the probes used, as described in the materials and methods section under the subheading "Karyotypes." Red is Cent4, TAG, and subtelomere 1.1; green is CentC and subtelomere 4-12-1; blue is 180 bp Knob; white is TR-1 Knob; teal (green and blue) is NOR 173 ribosomal DNA; and yellow-orange (red and green) is 5S ribosomal DNA.

sunlight and temperature are both reduced), the two greenhouses were set to different temperature regimes to quantify this effect while also testing heterosis in two different conditions. The warmer greenhouse was set to an optimal temperature of 28° during the day and 25° at night; the cooler greenhouse was set to an optimal temperature of 25° for both day and night. These temperatures were optimal temperature settings for the automated greenhouse climate control system; the actual temperature in the greenhouse fluctuated above and below these settings throughout the experiment. Lighting in both greenhouses was set to a 16-hr day and 8-hr night schedule. The heterosis trial was performed in the Sears Greenhouse in Columbia, MO, from late October 2014 to early January 2015.

Each group of 45 plants contained 15 FFMM-A 12th generation self, 15 FFMM-B 12th generation self, and 15 hybrid FFMM-B 12th generation/FFMM-A 12th generation individuals. Test plots were arranged as a six-pot by seven-pot rectangle with the three remainder pots centered on one end and a 3- to 4-cm gap between each pot and its neighbors. By random assignment, one seed was planted in the center of each 21 × 21 cm round nursery pot containing Promix BX supplemented with 5.5 g of ferrous sulfate heptahydrate and 10.5 g of 20-20-20 fertilizer as a growth medium.

At 50 days after planting, the plants were photographed and the following traits were measured: height of plant from base to tip of tassel, ear leaf length from tip to stalk, ear leaf width at the widest point, and number of tassel branches with a branch containing at least four spikelets. Number of days to anther emergence was recorded as the number of days from seed planting to the first day anthers protruded from the tassel and visibly dispersed pollen. When ear shoot flag leaves appeared, they were trimmed daily so ~2 cm of ear flag leaf protruded. The number of days to silking was recorded as the time between planting and the first day that a silk with the flag leaves was cut. Ear length was measured from the base of the first kernels to the tip. For determination of the number of kernels and total kernel weight, the ears were shelled, and only kernels that appeared fully developed and viable were included. All ears were openly pollinated to assure a full seed set and were assisted with pollen shaken onto a piece of paper and applied to the silks.

Traits were analyzed using a two-way ANOVA with the two fixed factors being genotype (FFMM-A, FFMM-B, or hybrid) and temperature (warm or cool). Traits of ear leaf length, ear leaf width, height, ear length, kernel count, and total kernel weight met the two-way ANOVA model normality assumption. Any trait that did not meet the two-way ANOVA normality assumption was analyzed using the *K*-sample median test comparing the medians of six groups based on the cross product of the genotype greenhouse temperature. Number of days to anther emergence and silk emergence and number of tassel branches fell into this category. All statistical analyses were performed in IBM's SPSS Statistics software, version 22. Raw data used for these analyses is available in File S3.

Through the course of the experiment, one hybrid plant in the cooler greenhouse died and one FFMM-B plant in the warmer greenhouse displayed stunted growth; these plants

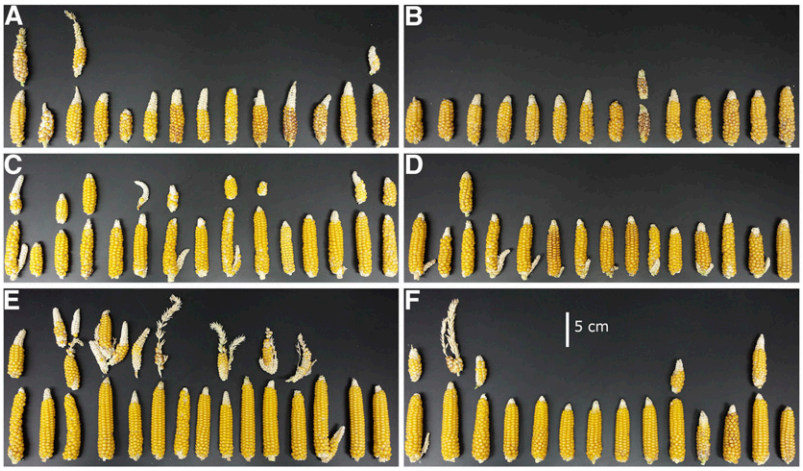

Figure 5 Ears produced in the heterosis trial. (A) FFMM-A from the warmer greenhouse, (B) FFMM-A from the cooler greenhouse, (C) FFMM-B from the warmer greenhouse, (D) FFMM-B from the cooler greenhouse, (E) hybrid from the warmer greenhouse, and (F) hybrid from the cooler greenhouse. The primary ear of each plant is arranged in a line along the bottom of each group; any additional ears from the same plant are aligned above the primary. Some additional ears were secondary ears while others were produced on tillers. All ears were openly pollinated with additional pollination performed by shaking pollen onto a sheet of paper and applying to the silks of the primary ear to ensure optimal seed set.

Table 1 Percentage genome contribution of parental lines

Parental line	FFMM-A (%)	FFMM-B (%)
Alexander's Early Early Synthetic	11.1	15.8
Neuffer's Early ACR	2.0	8.1
Gaspe Flint	29.2	16.2
Tom Thumb Popcorn	34.5	29.5
Unknown	23.3	30.5

Estimated genome contributions of each of the four parents of FFMM based on genotyping-by-sequencing data.

were excluded from analysis. One FFMM-A plant in each greenhouse failed to produce a viable primary ear; these plants were excluded from analysis of ear length and seed traits. The tip of the ear leaf of one hybrid plant in the warmer greenhouse was missing; this ear leaf length measurement was also excluded from analysis.

Standard care protocol

In the greenhouse, FFMM can be effectively grown with two plants per 21-cm diameter by 21-cm deep nursery pot. Seeds can be planted equidistant from each other and the sides of the pot, or close to the edge of the pot opposite each other. These pots can be arranged in a tightly packed configuration until the leaves begin to overlap, at which point the pots should be moved apart at least 10 cm and rotated to minimize leaf overlap. Care should be taken to avoid FFMM plants being shaded by taller maize plants. Growing FFMM on a table helps to alleviate shading by taller plants as well as being a more convenient height for pollination than growing on the floor.

Pollination of FFMM requires a slight alteration of standard maize pollination protocol. FFMM has flag leaves, which protrude from the ear shoot prior to silking. These flag leaves grow rapidly and must be trimmed every day or two until silks emerge far enough that they are trimmed with the flag leaves. Pollination can be performed the next day. Standard ear shoot bags are too long for FFMM and tend to fall off as the flag leaves extend. For this reason ear shoot bags should be shortened by cutting in half. Standard tassel bags are also too large for FFMM. Tassel bags should also be shortened to approximately half size before attachment to a FFMM plant. Additionally, when grown in the field, ear shoot bags have a tendency to blow off of FFMM. To avoid open pollination due to ear shoot bag loss, it is advisable to staple the otherwise discarded half of the cut ear shoot bag to the half covering the ear as a backing to encircle the stalk of the plant and hold the bag in place.

The main ear should be expected to emerge from the fourth or fifth node from the top. In the heterosis trial, the ear shoot emerged from the fourth node from the top in 14 of 30 FFMM-A plants, 12 of 30 FFMM-B plants, and 28 of 29 hybrid plants; the remainder emerged from the fifth node (File S3).

Kernel color markers

Kernel color markers *R1-scm2*, which conditions pigment in both the embryo and endosperm of kernels, and *y1*, which conditions white endosperm, were backcrossed from independent lines into FFMM-A, which has the original genotype *r1*; *A1*; *A2*; *C1*; *C2*; *Bz1*; *Bz2*; *Y1* (see Neuffer *et al.* 1997 for descriptions) for seven generations and then self-pollinated and selected for homozygosity.

Karyotypes

A karyotype (Figure 4) was prepared as a reference for chromosome studies of FFMM using a procedure described previously (Kato *et al.* 2004) with the following modifications. NOR 173 was labeled with green and blue to produce a teal color; Cent4 was labeled with red and the sequence used was altered from the original (Kato *et al.* 2004) to remove Knob homology; a green 5′ end-labeled oligo was used to label CentC. Other probes used included 5S ribosomal RNA (2-3-3) labeled both red and green, TAG (1-26-2) red, TR-1 (M77) far-red (pseudocolored white in figures), 4-12-1 green, 1.1 red, and Knob as blue. Excluding the CentC oligo, all green probes were labeled with fluorescein dUTP (2′-deoxyuridine 5′-triphosphate), red with Texas Red dCTP (2′-deoxycytidine 5′-triphosphate), blue with coumarin dUTP, and far-red with Cy5 dUTP. All probes except the CentC oligo were nick translated as described (Kato *et al.* 2004).

Table 2 Comparison of vigor in a relatively warm greenhouse

Trait	Hybrid warmer greenhouse	FFMM-A warmer greenhouse	FFMM-B warmer greenhouse
Plant height	105.9 ± 7.8 cm	81.3 ± 9.4 cm***	95.1 ± 8.9 cm***
Ear leaf length	77.4 ± 3.2 cm	64.2 ± 3.2 cm***	67.5 ± 4.0 cm***
Ear leaf width	40.8 ± 3.4 mm	31.7 ± 2.3 mm***	40.1 ± 4.0 mm
Number of kernels on primary ear	180.5 ± 32.3 kernels	80.4 ± 34.5 kernels***	117.8 ± 23.4 kernels***
Total dry seed weight of primary ear	16.2 g ± 3.5 g	7.8 ± 3.2 g***	9.8 ± 2.6 g***
Primary ear length	10.6 ± 0.9 cm	7.5 ± 1.3 cm***	8.5 ± 0.9 cm***
Number of tassel branches[a]	4 branches	3 branches	9 branches[b]
Days from planting to anther emergence[a]	33 days	34 days***	35 days***
Days from planting to silking[a]	31 days	34 days***	36 days***

Comparison of both FFMM-A and FFMM-B parents to an F_1 hybrid from a cross of the two lines. All significance levels are denoted after the value for each parent. Significance levels are only assigned if the value of the trait in the parental line is considered less vigorous than the hybrid. The first six trait values are given as a mean ± SD, while the last three trait values are the median value. *** represents a significance value of 0.001.

[a] Median value is given in table; a *K*-sample median test was used to analyze significance of interaction instead of two-way ANOVA.

[b] Value significantly larger than hybrid.

Table 3 Comparison of vigor in a relatively cool greenhouse

Trait	Hybrid cooler greenhouse	FFMM-A cooler greenhouse	FFMM-B cooler greenhouse
Total plant height	92.1 ± 6.2 cm	72.0 ± 4.5 cm***	83.1 ± 5.9 cm**
Ear leaf length	71.7 ± 4.6 cm	60.2 ± 3.6 cm***	68.0 ± 2.6 cm*
Ear leaf width	39.2 ± 5.3 mm	30.6 ± 1.8 mm***	43.1 ± 4.1 mm[a]
Number of kernels on primary ear	157.6 ± 27.4 kernels	94.6 ± 25.6 kernels***	108.2 ± 27.8 kernels***
Total dry seed weight of primary ear	16.6 ± 3.8 g	9.1 ± 2.5 g***	10.3 ± 2.5 g***
Primary ear length	8.8 ± 0.9 cm	6.7 ± 0.8 cm***	7.8 ± 0.7 cm*
Number of tassel branches[b]	4 branches	3 branches	8 branches[a]
Days from planting to anther emergence[b]	37 days	38 days	39 days**
Days from planting to silking[b]	35.5 days	38 days	41 days**

Comparison of both FFMM-A and FFMM-B parents to an F_1 hybrid from a cross of the two lines. All significance levels are denoted after the value for each parent. Significance levels are only assigned if the value of the trait in the parental line is considered less vigorous than the hybrid. The first six trait values are given as a mean ± SD, while the last three trait values are the median value. *, **, and *** represent significance values of 0.05, 0.01, and 0.001, respectively.

[a] Value significantly greater than hybrid.

[b] Median value is given; a *K*-sample median test was used to analyze significance of interaction instead of two-way ANOVA.

Data availability

FFMM is available from the Maize Genetics Cooperation Stock Center through www.MaizeGDB.org: FFMM-A, Co-op ID: TX40J http://www.maizegdb.org/data_center/stock?id=9024759; FFMM-B, Co-op ID: TX40K http://www.maizegdb.org/data_center/stock?id=9025545; FFMM-A *R1-scm2*, Co-op ID: TX40JA http://www.maizegdb.org/data_center/stock?id=9031115. FFMM-A *y1*, Co-op ID: TX40JB http://maizegdb.org/data_center/stock?id=9032716. File S1 is a genotype file based on SNP calls. File S2 contains the scripts used to determine FFMM ancestry. File S3 is an Excel spreadsheet that contains measurements from the heterosis and greenhouse temperature experiment.

Results and Discussion

Development of FFMM

The two lines of FFMM are the descendants of a cross of an F_1 hybrid of Neuffer's Early ACR (from M. G. Neuffer, University of Missouri) by Alexander's Early Early Synthetic (from Maize Genetics Cooperation Stock Center, University of Illinois), and an F_2 plant of Tom Thumb Popcorn (Johnny's Selected Seeds) (Bass *et al.* 2001) by Gaspe Flint (PI 214279 from North Central Regional Plant Introduction Station, Ames, IA) that had been selected for fast flowering. The four parental lines were known to be fast flowering, and each subsequent generation was selected for fast-flowering progeny. Gaspe Flint in addition contains a variant of *vgt1* (Salvi *et al.* 2002, 2007), which bypasses the juvenile growth phase of maize. Selected individuals were selfed for 11 generations with selection to create two independent inbred lines. Lines A and B were selected based on flowering time, pollen yield, seed set, and an ear shoot that protrudes from the leaf axil before silking to prevent contamination by open pollination. FFMM outperforms its progenitor lines in terms of flowering time. Of the progenitor lines, Gaspe Flint is the most similar for onset of anthesis, but suffers from poor fertility under many conditions and a less optimal morphology.

Characteristics of FFMM

The FFMM lines can complete a generation within 60 days of planting. Flowering time varies to slightly under or over 1 month (depending on seasonal conditions), but harvest can consistently be completed at 60 days. Thus, five generations per year can be easily achieved. It is conceivable that with optimization of growth conditions, six generations could be completed within a year. An average of 87.5 kernels are produced on the primary ear of FFMM-A and

Table 4 Comparison of FFMM hybrid traits in a warmer *vs.* cooler greenhouse

Trait	Hybrid warmer greenhouse	Hybrid cooler greenhouse
Plant height	105.9 ± 7.8 cm***	92.1 ± 6.2 cm
Ear leaf length	77.4 ± 3.2 cm***	71.7 ± 4.6 cm
Ear leaf width	40.8 ± 3.4 mm	39.2 ± 5.3 mm
Number of kernels on primary ear	180.5 ± 32.3 kernels*	157.6 ± 27.4 kernels
Total dry seed weight of primary ear	16.2 g ± 3.5 g	16.6 ± 3.8 g
Primary ear length	10.6 ± 0.9 cm***	8.8 ± 0.9 cm
Number of tassel branches[a]	4 branches	4 branches
Days from planting to anther emergence[a]	33 days***	37 days
Days from planting to silking[a]	31 days***	35.5 days

Comparison of the vigor of traits in an F_1 hybrid of FFMM-A and -B in two different relative temperature conditions, representing a subset of a larger group of two-way ANOVA and *K*-sample median tests. The significance values are assigned to the more vigorous treatment if there was a significant difference between the two. The first six trait values given are a mean ± SD, while the last three are median values. * and *** represent significance values of 0.05 and 0.001, respectively.

[a] Median value is given; a *K*-sample median test was used to analyze significance of interaction instead of two-way ANOVA.

Table 5 Comparison of FFMM-A traits in a warmer *vs.* cooler greenhouse

Trait	FFMM-A warmer greenhouse	FFMM-A cooler greenhouse
Total plant height	81.3 ± 9.4 cm***	72.0 ± 4.5 cm
Ear leaf length	64.2 ± 3.2 cm**	60.2 ± 3.6 cm
Ear leaf width	31.7 ± 2.3 mm	30.6 ± 1.8 mm
Number of kernels on primary ear	80.4 ± 34.5 kernels	94.6 ± 25.6 kernels
Total dry seed weight of primary ear	7.8 ± 3.2 g	9.1 ± 2.5 g
Primary ear length	7.5 ± 1.3 cm*	6.7 ± 0.8 cm
Number of tassel branches[a]	3 branches	3 branches
Days from planting to anther emergence[a]	34 days***	38 days
Days from planting to silking[a]	34 days***	38 days

Comparison of the vigor of traits of FFMM-A in two different relative temperature conditions, representing a subset of a larger group of two-way ANOVA and *K*-sample median tests. The significance values are assigned to the more vigorous treatment if there was a significant difference between the two. The first six trait values given are a mean ± SD, while the last three are median values. *, **, and *** represent significance values of 0.05, 0.01, and 0.001, respectively.
[a] Median value is given; a *K*-sample median test was used to analyze significance of interaction instead of two-way ANOVA.

112.8 on the primary ear of FFMM-B based on the winter heterosis trial. Many plants produced secondary ears (Figure 5).

Genomic ancestry of Mini-Maize

To determine which genomic regions were inherited from each founder population, we performed GBS on four plants of each founder population and on two FFMM-B plants. The resulting genotypes were merged with those from whole-genome data on FFMM-A and similarity between samples calculated in sliding windows across the genome (see Figure S1). The inferred ancestry blocks for both FFMM-A and FFMM-B are shown in Figure 2, and the total percentages from each founder are in Table 1. Not all genomic regions could be confidently assigned an identity, probably because the specific haplotype was not captured in any of the plants sampled.

Roughly 41% of the genome is shared between FFMM-A and -B. Some of these shared regions probably contribute to the short, fast-flowering phenotype, but with only two samples in the comparison it is not possible to differentiate those regions from ones that are shared due to chance. The flanking sequences of the *vgt1* variant from Gaspe Flint (Salvi *et al.* 2002, 2007) are present, indicating its inclusion in the final selection.

Flow cytometry

It has been proposed that genome size correlates with various life-history traits in plants, including flowering time (Bennett 1972). To test if Mini-Maize had also selected for changes in genome size, we performed flow cytometry analysis of nuclear DNA content on both FFMM lines, the four founder populations, and several reference inbred lines (Figure 3). Because FFMM-B has the smallest apparent genome size among these samples and FFMM-A is among the largest, we conclude that there was not coordinated selection on genome size in these two lines.

Heterosis and greenhouse temperature

Five of nine tested traits showed evidence of hybrid vigor in FFMM (Table 2 and Table 3). Length of primary ear leaf, total plant height, primary ear length, kernel count of primary ear, and total seed weight of the primary ear were all significantly greater in the hybrid than either parent (see Figure S2, Figure S3, Figure S4, Figure S5, and Figure S6). In addition, the time to anther emergence and silk emergence were significantly shorter in the hybrid when compared to the two parents in the warmer greenhouse conditions (see Figure S7 and Figure S8). In the cooler greenhouse conditions the flowering times of the hybrid and FFMM-A were not significantly different.

Table 6 Comparison of FFMM-B traits in a warmer *vs.* cooler greenhouse

Trait	FFMM-B warmer greenhouse	FFMM-B cooler greenhouse
Total plant height	95.1 ± 8.9 cm***	83.1 ± 5.9 cm
Ear leaf length	67.5 ± 4.0 cm	68.0 ± 2.6 cm
Ear leaf width	40.1 ± 4.0 mm	43.1 ± 4.1 mm*
Number of kernels on primary ear	117.8 ± 23.4 kernels	108.2 ± 27.8 kernels
Total dry seed weight of primary ear	9.8 ± 2.6 g	10.3 ± 2.5 g
Primary ear length	8.5 ± 0.9 cm*	7.8 ± 0.7 cm
Number of tassel branches[a]	9 branches	8 branches
Days from planting to anther emergence[a]	35 days***	39 days
Days from planting to silking[a]	36 days***	41 days

Comparison of the vigor of traits of FFMM-B in two different relative temperature conditions, representing a subset of a larger group of two-way ANOVA and *K*-sample median tests. The significance values are assigned to the more vigorous treatment if there was a significant difference between the two. The first six trait values given are a mean ± SD, while the last three are median values. * and *** represent significance values of 0.05 and 0.001, respectively.
[a] Median value is given; a *K*-sample median test was used to analyze significance of interaction.

The number of tassel branches did not show hybrid vigor; FFMM-B has significantly more tassel branches than FFMM-A or the hybrid (see Figure S9).

FFMM showed significantly faster flowering in the warmer greenhouse compared to the cooler greenhouse (Table 4, Table 5, and Table 6). This result suggests that it may be possible to optimize growing conditions to further reduce the generation time of the FFMM lines. A few other traits were significantly of a greater magnitude in the warmer greenhouse, including total plant height, ear length, ear leaf length in the hybrid and FFMM-A, and the kernel count of hybrid ears. The only trait observed to be significantly greater in the cooler greenhouse was ear leaf width in FFMM-B plants (see Figure S10 and Table 3).

Conclusions

Fast-Flowering Mini-Maize has many qualities that make it both an excellent research model and an interesting teaching tool. FFMM provides the benefits of a short generation time and economical use of growing space to an already well-established model organism. Additionally, a genome sequence aligned to the B73 reference genome makes FFMM-A ready to use for genomic applications. FFMM is also a potentially interesting hands-on teaching material that can be used to demonstrate basic genetic principles such as dominant/recessive alleles, segregation, and independent assortment using the introgressed color markers.

Acknowledgments

Statistical analysis of the heterosis and greenhouse temperature data was advised by Ray Bacon and Rebecca Ewing of the University of Missouri Social Science Statistics Center through the Statistical Software Support Outreach program. Research for this project was supported by the National Science Foundation Plant Genome Project (nos. 0820619 and 1238014), the U.S. Department of Agriculture–Agricultural Research Service, a Graduate Assistance in Areas of National Need fellowship (Department of Education grant no. P200A120101), and the University of Georgia.

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Communicating editor: S. Poethig

GENETICS

Supporting Information
www.genetics.org/lookup/suppl/doi:10.1534/genetics.116.191726/-/DC1

Fast-Flowering Mini-Maize: Seed to Seed in 60 Days

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DOI: 10.1534/genetics.116.191726

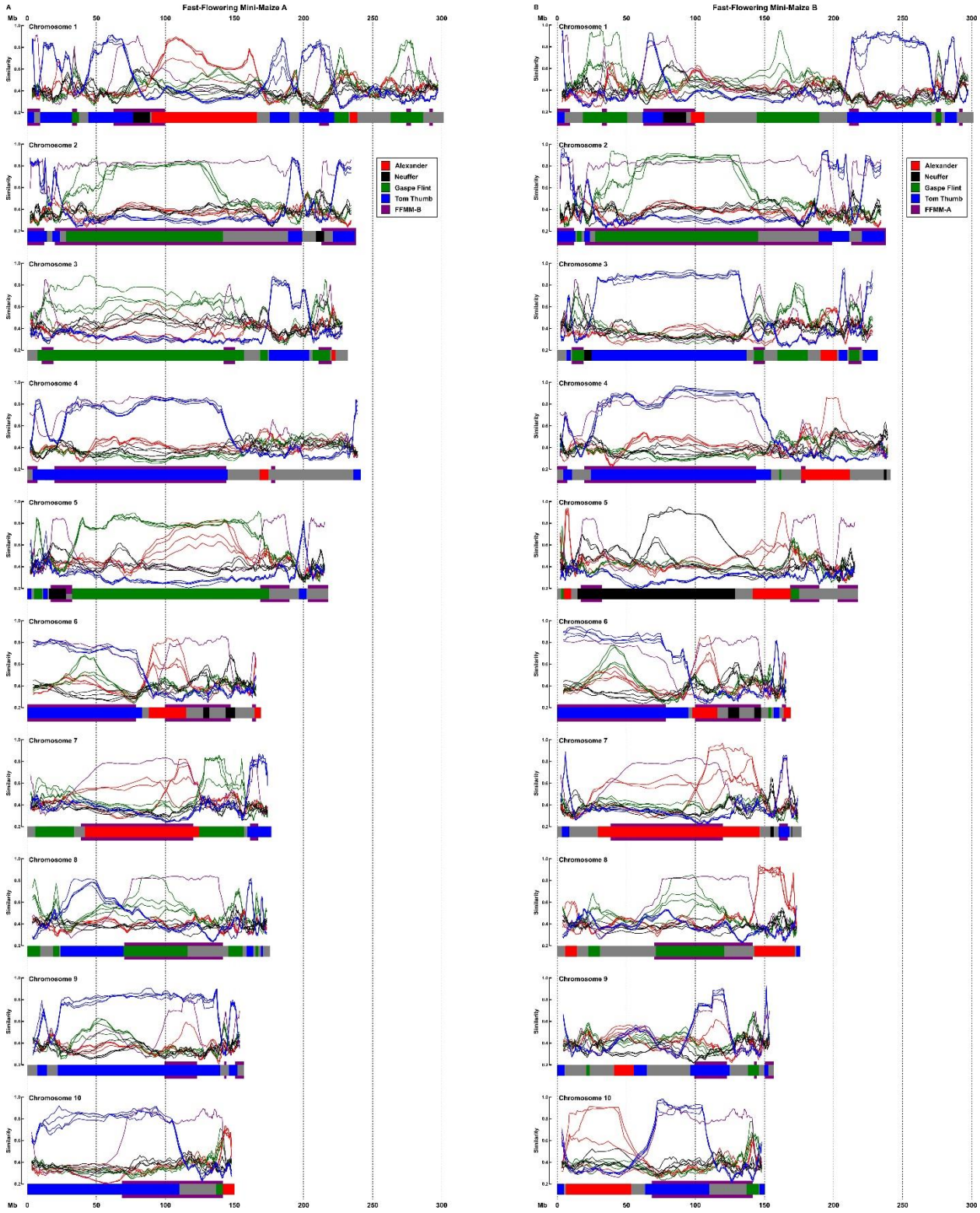
A
Fast-Flowering Mini-Maize A
B
Fast-Flowering Mini-Maize B
Mb
Similarity
Chromosome 1
Chromosome 2
Chromosome 3
Chromosome 4
Chromosome 5
Chromosome 6
Chromosome 7
Chromosome 8
Chromosome 9
Chromosome 10
Alexander
Neuffer
Gaspe Flint
Tom Thumb
FFMM-B
FFMM-A

Figure S1 – Full ancestry assignments across chromosomes. The probable ancestry of each portion of the FFMM-A and B chromosomes was determined by comparing their similarity to samples from each of the four founder populations. The traces show calculated similarity in 1000-SNP sliding windows (step size of 100 SNPs) across each chromosome, with each line representing a different individual plant. The inferred ancestry is shown as blocks below each trace; gray regions denote areas where the ancestry is uncertain, and regions shared between the two FFMM lines are highlighted in purple.

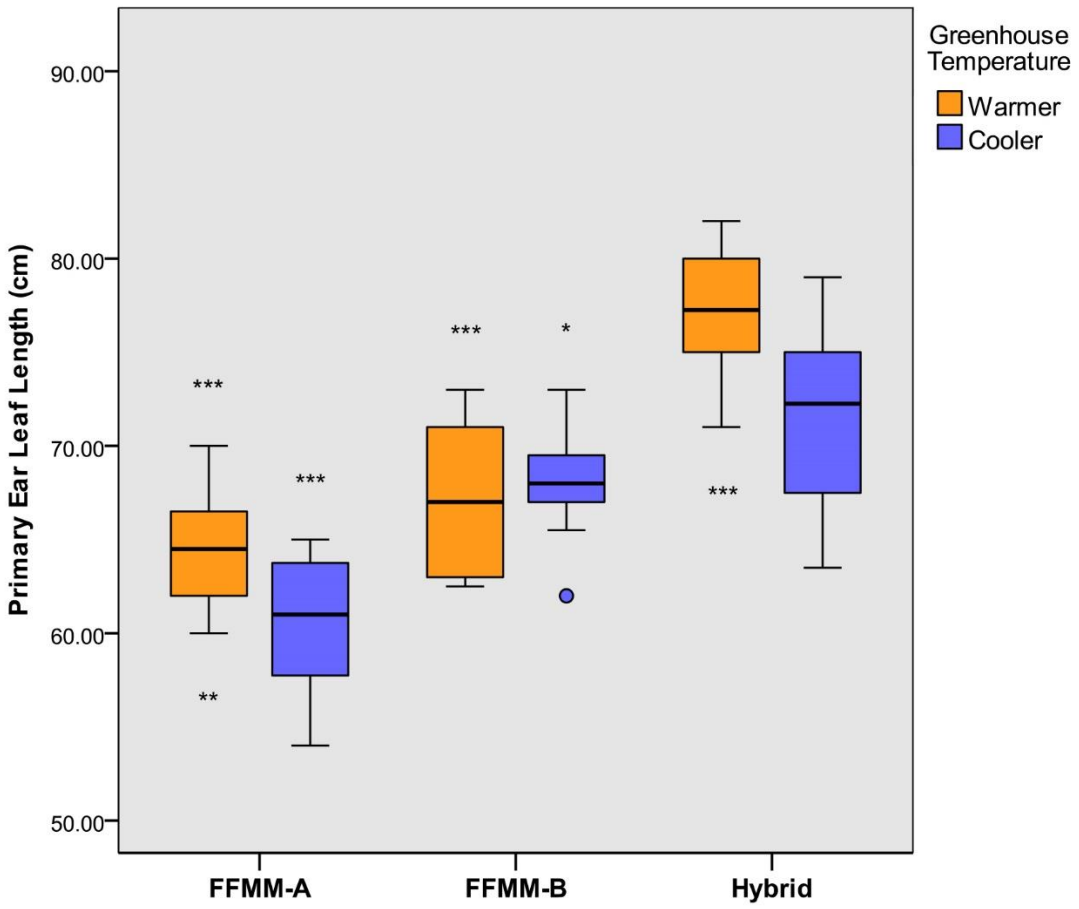

Figure S2 – Primary Ear Leaf Length – Means, Two-Way ANOVA

A box-plot representing the data used for a two-way ANOVA comparing the length of the leaf at the primary ear node. Asterisks above FFMM-A or FFMM-B boxes denote significance compared to the hybrid in the same conditions. Asterisks below a box represent significance compared to siblings in the other temperature condition, with the asterisk under the superior group.

*, **, and *** represent significance levels of .05, .01, and .001, respectively.

Points marked as a filled in circle represent outliers and fall between 1.5 times and 3.0 times the interquartile range.

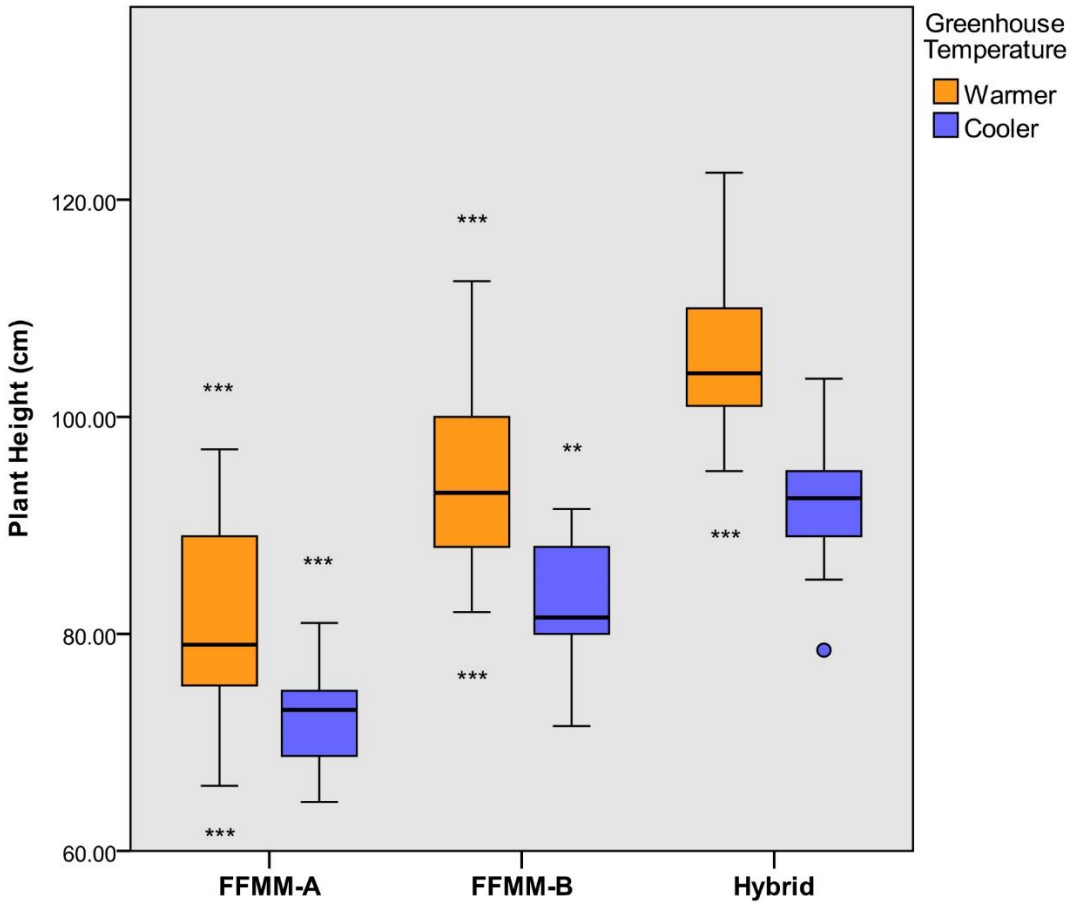

Figure S3 - Plant Height – Means, Two-Way ANOVA

A box-plot representing the data used for a two-way ANOVA comparing the final plant height after completion of flowering from base of plant to tip of tassel. Asterisks above FFMM-A or FFMM-B boxes denote significance compared to the hybrid in the same conditions. Asterisks below a box represent significance compared to siblings in the other temperature condition, with the asterisk under the superior group.

*, **, and *** represent significance levels of .05, .01, and .001, respectively.

Points marked as a filled in circle represent outliers and fall between 1.5 times and 3.0 times the interquartile range.

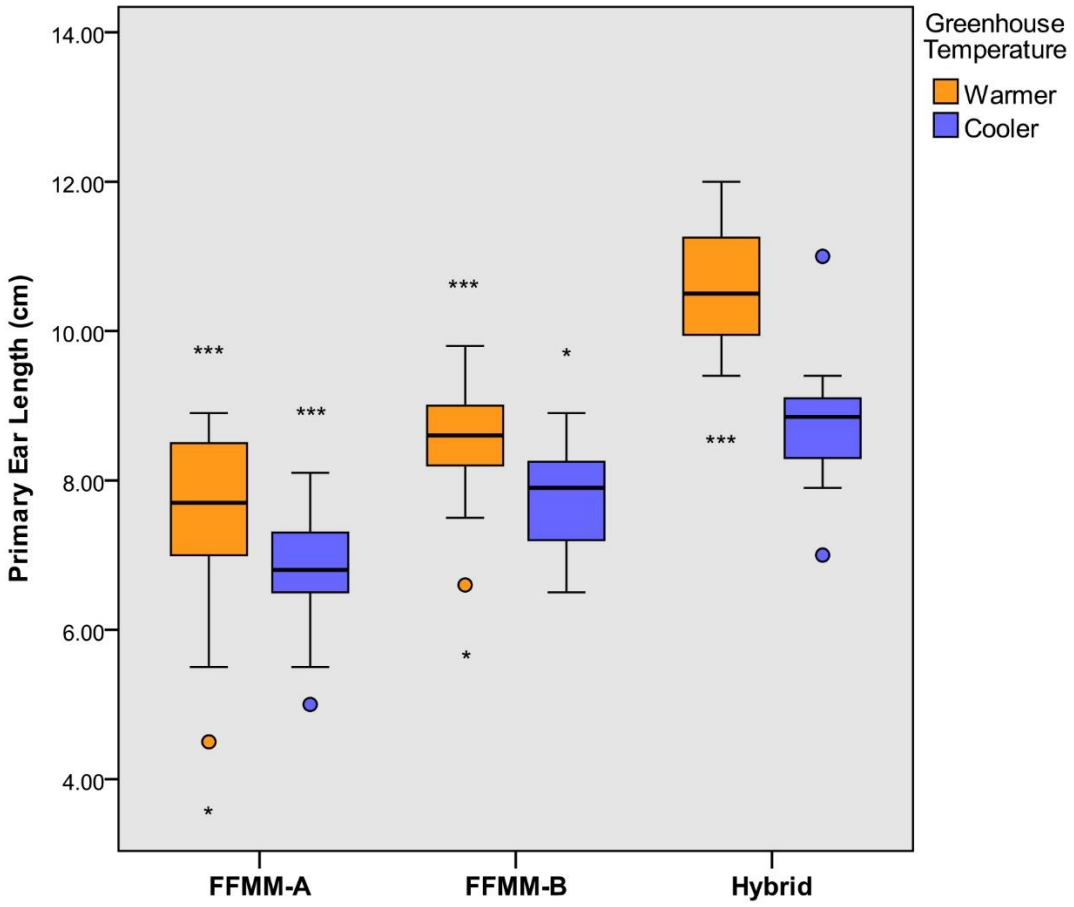

Figure S4 - Primary Ear Length – Means, Two-Way ANOVA

A box-plot representing the data used for a two-way ANOVA comparing the primary ear length from base of kernels to tip of cob. Asterisks above FFMM-A or FFMM-B boxes denote significance compared to the hybrid in the same conditions. Asterisks below a box represent significance compared to siblings in the other temperature condition, with the asterisk under the superior group.

*, **, and *** represent significance levels of .05, .01, and .001, respectively.

Points marked as a filled in circle represent outliers and fall between 1.5 times and 3.0 times the interquartile range.

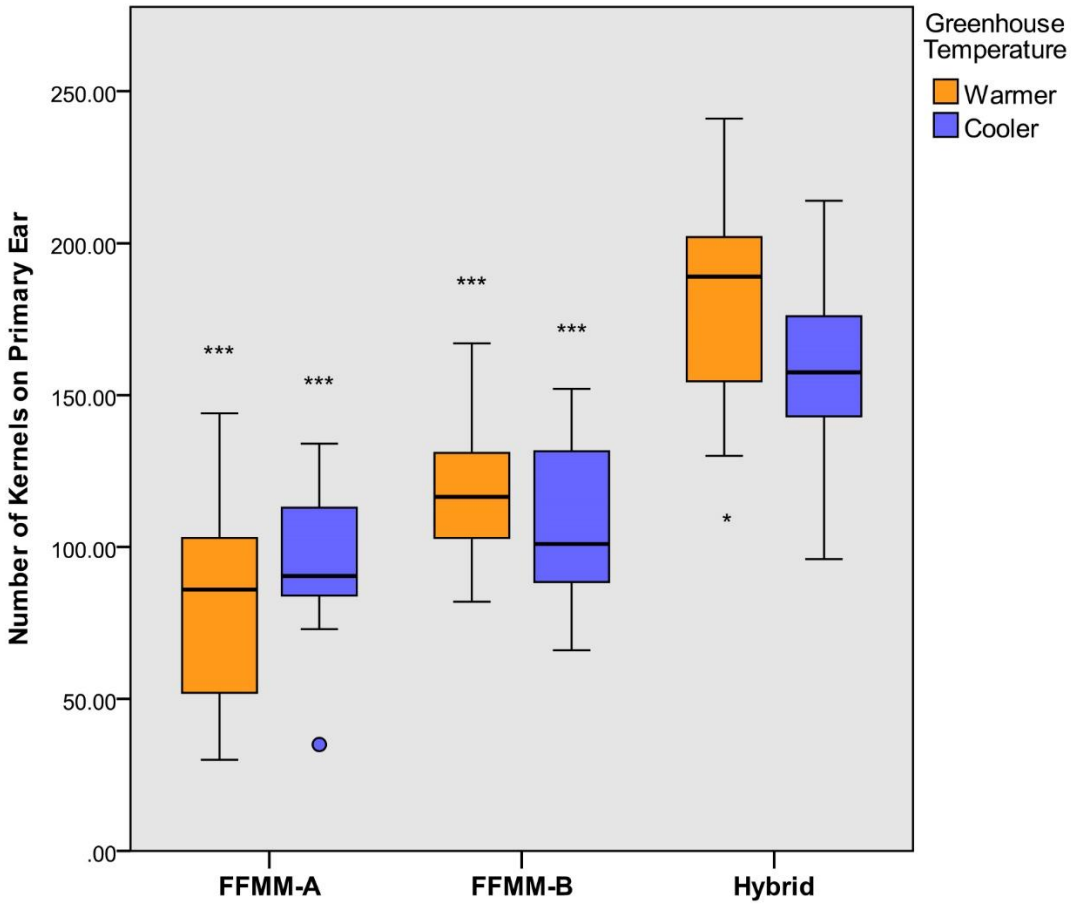

Figure S5 – Primary Ear Kernel Count- Means, Two-Way ANOVA

A box-plot representing the data used for a two-way ANOVA comparing the number of apparently viable kernels based on presence of an embryo and dense endosperm. Asterisks above FFMM-A or FFMM-B boxes denote significance compared to the hybrid in the same conditions. Asterisks below a box represent significance compared to siblings in the other temperature condition, with the asterisk under the superior group.

*, **, and *** represent significance levels of .05, .01, and .001, respectively.

Points marked as a filled in circle represent outliers and fall between 1.5 times and 3.0 times the interquartile range.

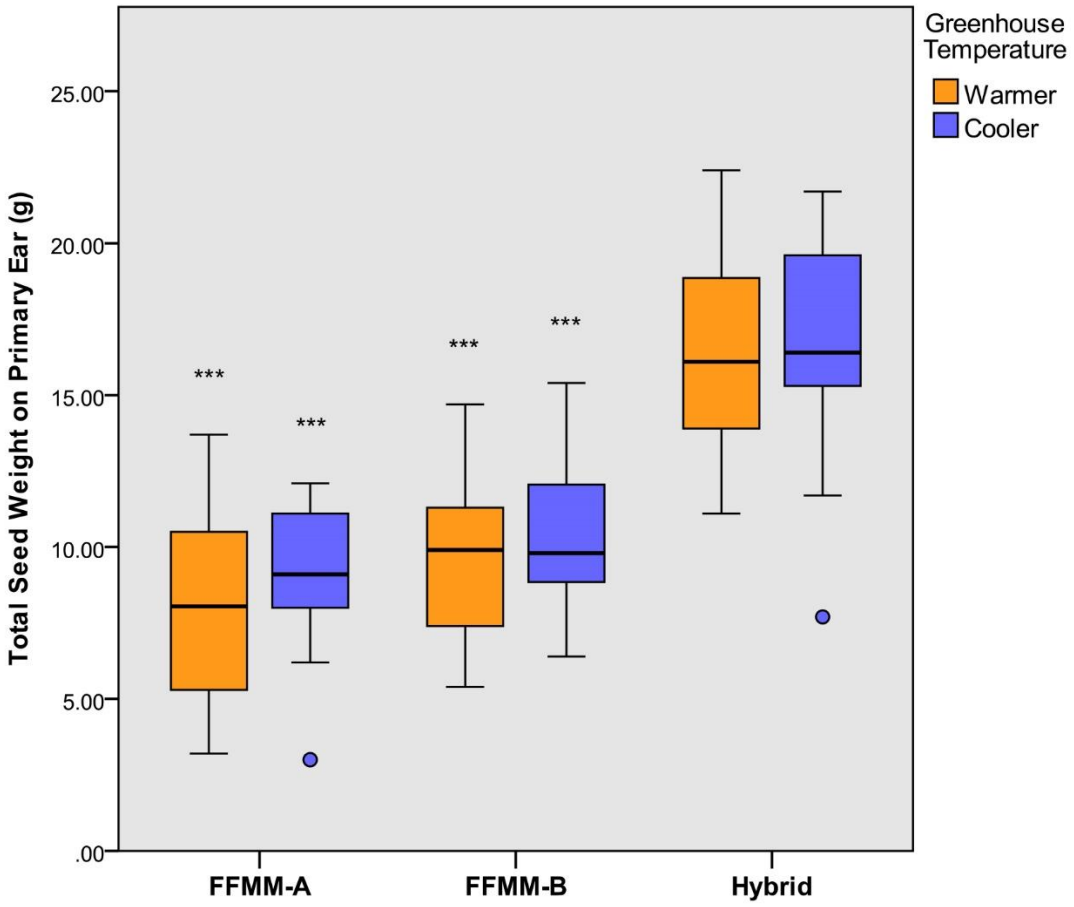

Figure S6 - Total Dry Seed Weight on Primary Ear- Means, Two-Way ANOVA

A box-plot representing the data used for a two-way ANOVA comparing the total dry seed weight (after removal from the cob) on the primary ear. Asterisks above FFMM-A or FFMM-B boxes denote significance compared to the hybrid in the same conditions. Asterisks below a box represent significance compared to siblings in the other temperature condition, with the asterisk under the superior group.

*, **, and *** represent significance levels of .05, .01, and .001, respectively.

Points marked as a filled in circle represent outliers and fall between 1.5 times and 3.0 times the interquartile range.

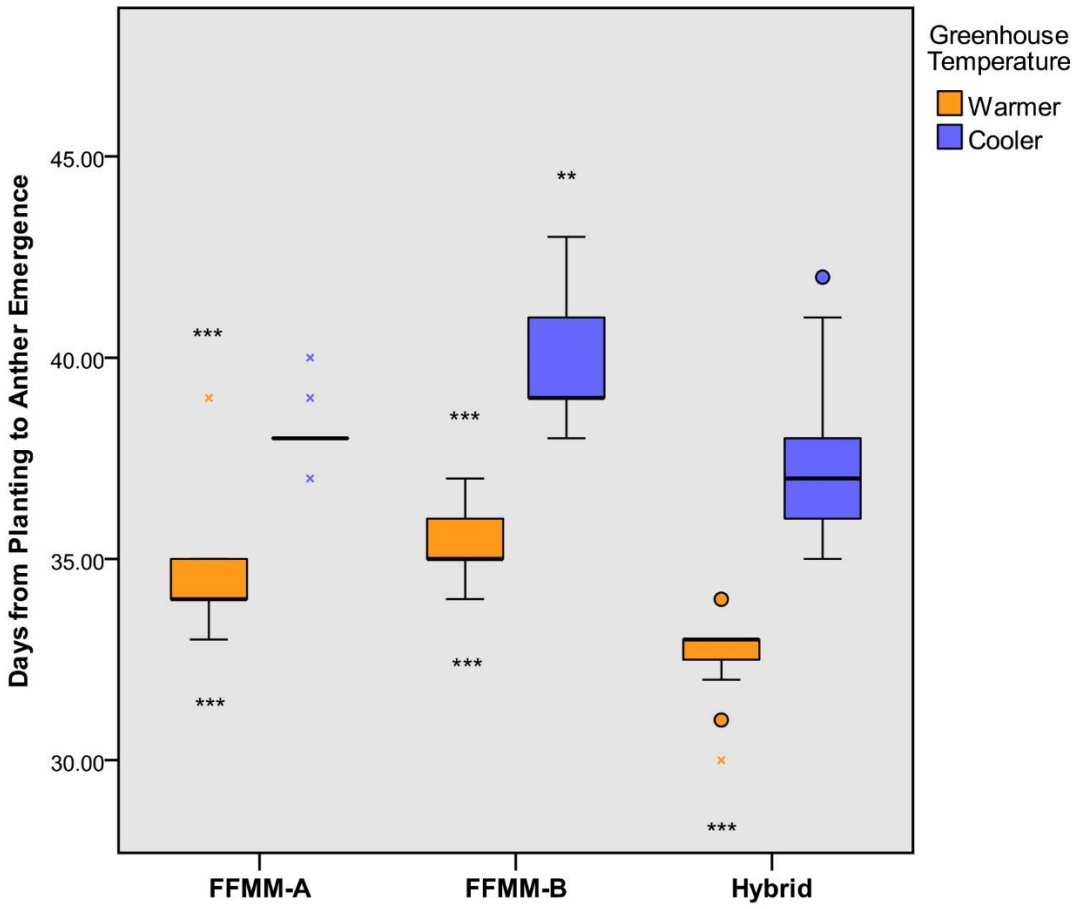

Figure S7 - Days to Anther Emergence - Median Test

A box-plot representing the data used for a median value comparison of the number of days between planting and anther emergence. Asterisks above FFMM-A or FFMM-B boxes denote significance compared to the hybrid in the same conditions. Asterisks below a box represent significance compared to siblings in the other temperature condition, with the asterisk under the superior group.

*, **, and *** represent significance levels of .05, .01, and .001, respectively.

Points marked as a filled in circle represent outliers and fall between 1.5 times and 3.0 times the interquartile range.

Points marked as an x represent “outlier points” that are more than 3.0 times the interquartile range.

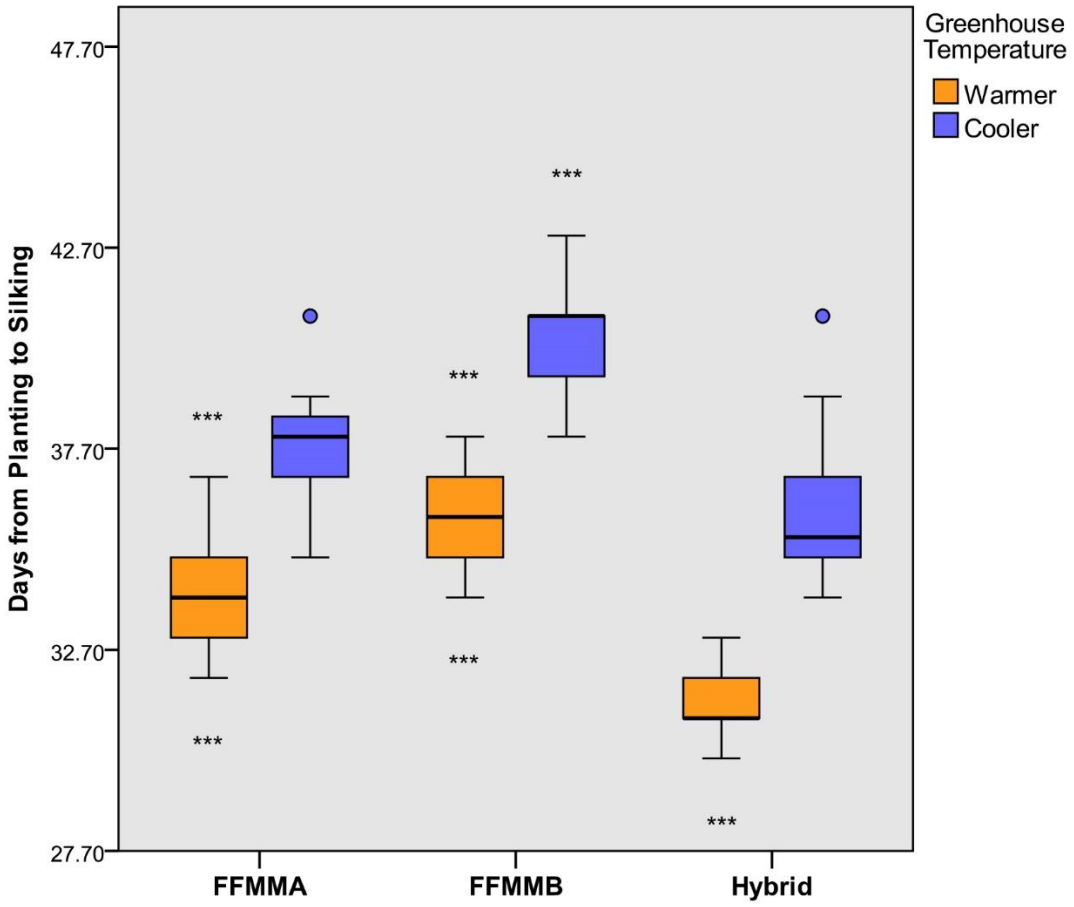

Figure S8 - Days to Silking - Median Test

A box-plot representing the data used for a median value comparison of the number of days between planting and silking. Asterisks above FFMM-A or FFMM-B boxes denote significance compared to the hybrid in the same conditions. Asterisks below a box represent significance compared to siblings in the other temperature condition, with the asterisk under the superior group.

*, **, and *** represent significance levels of .05, .01, and .001, respectively.

Points marked as a filled in circle represent outliers and fall between 1.5 times and 3.0 times the interquartile range.

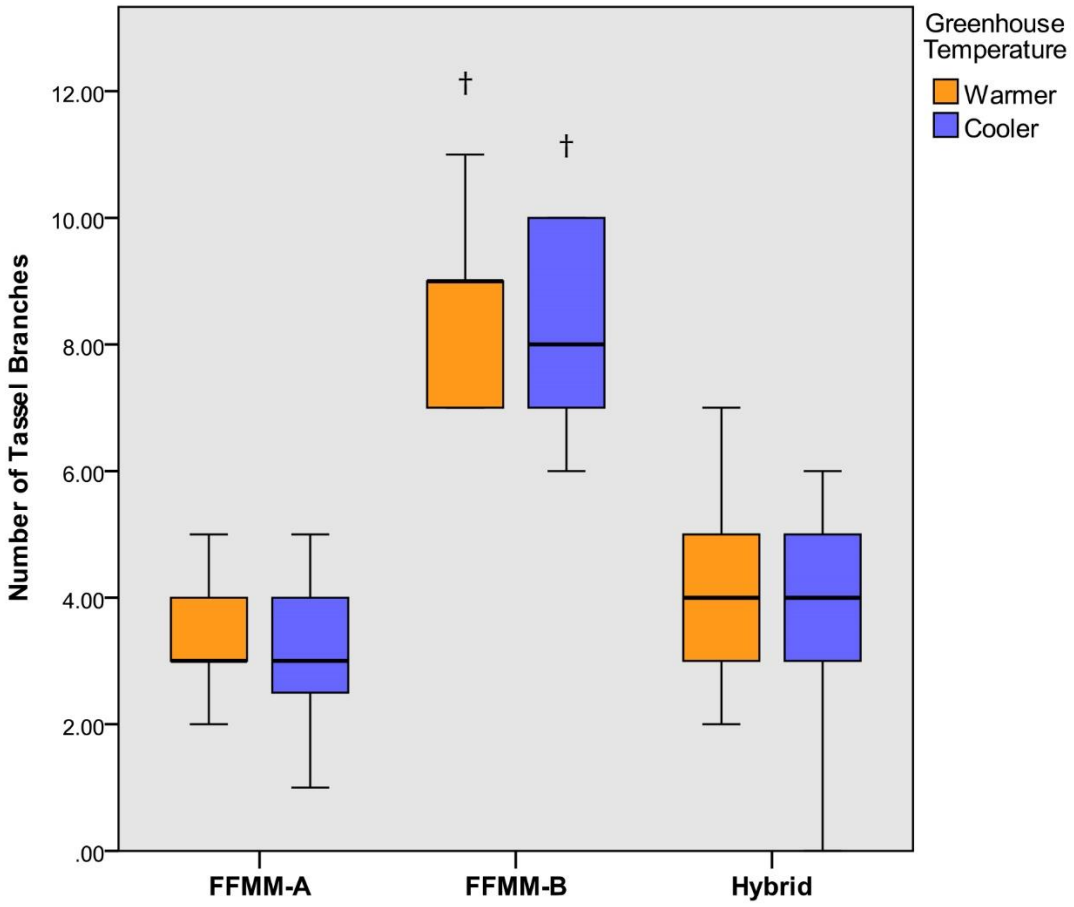

Figure S9 - Number of Tassel Branches - Median Test

A box-plot representing the data used for a median value comparison of the number of tassel branches not including the main spike. Asterisks above FFMM-A or FFMM-B boxes denote significance compared to the hybrid in the same conditions. Asterisks below a box represent significance compared to siblings in the other temperature condition, with the asterisk under the superior group.

*, **, and *** represent significance levels of .05, .01, and .001, respectively.

† denotes that the parental line is significantly superior to the hybrid.

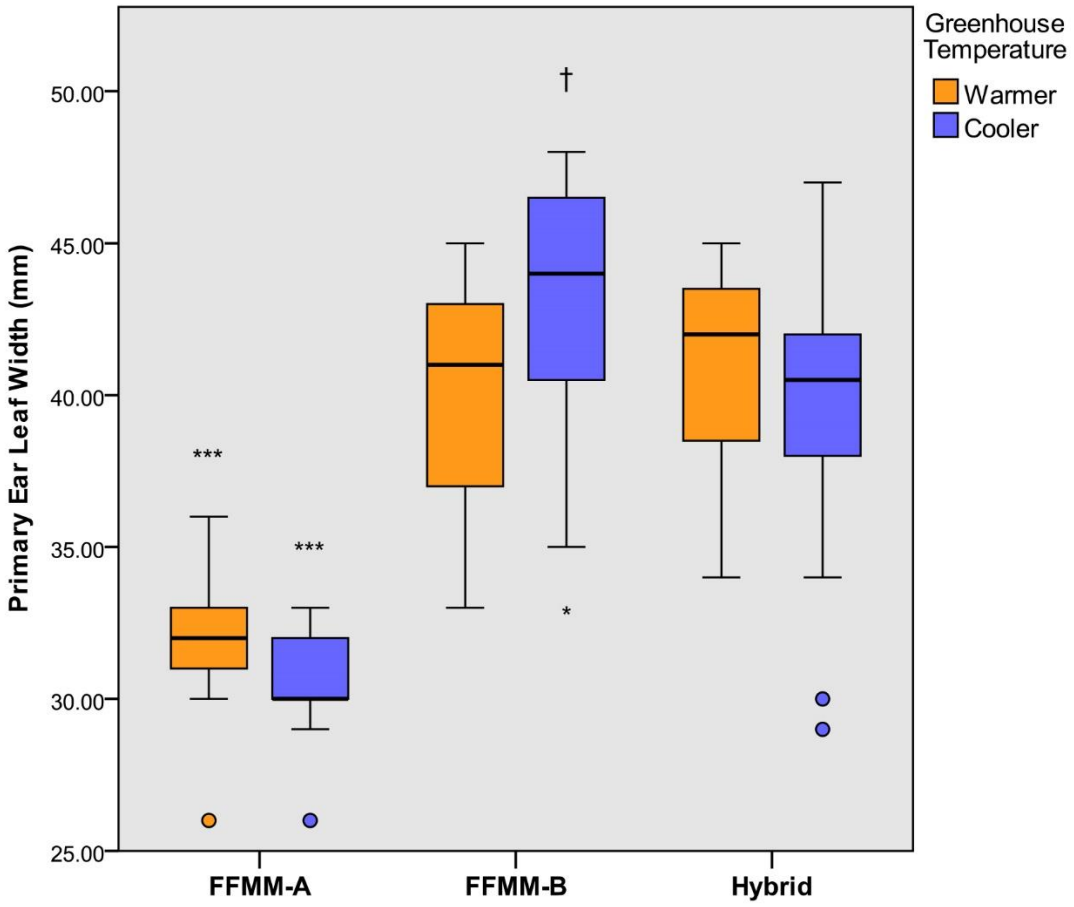

Figure S10 – Primary Ear Leaf Width – Means, Two-Way ANOVA

A box-plot representing the data used for a two-way ANOVA comparing the widest point of the leaf at the primary ear node. Asterisks above FFMM-A or FFMM-B boxes denote significance compared to the hybrid in the same conditions. Asterisks below a box represent significance compared to siblings in the other temperature condition, with the asterisk under the superior group.

*, **, and *** represent significance levels of .05, .01, and .001, respectively.

Points marked as a filled in circle represent outliers and fall between 1.5 times and 3.0 times the interquartile range.

† denotes that the parental line is significantly superior to the hybrid.

File S1: GBS genotypes of Fast-Flowering Minimaize B and the four founder populations. (.zip, 14 MB)

Available for download as a .zip file at:

http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.116.191726 /-/DC1/FileS1.zip

File S2: Bioinformatic scripts to determine ancestry of different chromosome segments. (.zip, 0 B)

Available for download as a .zip file at:

http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.116.191726 /-/DC1/FileS2.zip

File S3: Trait measurements from the heterosis trial. (.xlsx, 13 KB)

Available for download as a .xlsx file at:

http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.116.191726/-/DC1/FileS3.xlsx