

QTL mapping of parthenocarpic fruit set in North American processing cucumber

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Abstract

Key message Through a novel phenotyping method, four QTLs were consistently associated with increased parthenocarpic fruit set in North American processing cucumber that accounted for over 75 % of observed phenotypic variation.

Abstract Parthenocarpy is a desirable trait with potential for increasing yield and quality in processing cucumber production. Although many successful parthenocarpic fresh market cucumber varieties have been developed, the genetic and molecular mechanisms behind parthenocarpic

expression in cucumber remain largely unknown. Since parthenocarpy is an important yield component, it is difficult to separate the true parthenocarpic character from other yield related traits. In the present study, we developed a novel phenotypic approach for parthenocarpic fruit set focusing on early fruit development. Two hundred and five F₃ families derived from a cross between the highly parthenocarpic line 2A and low parthenocarpic line Gy8 were phenotypically evaluated in three greenhouse experiments. Seven QTLs associated with parthenocarpic fruit set were detected. Among them, one each on chromosomes 5 and 7 (*parth5.1* and *parth7.1*) and two on chromosome 6 (*parth6.1* and *parth6.2*) were consistently identified in all experiments, but their relative contribution to the total phenotypic variation was dependent on plant growth stages. While each of the four QTLs had almost equal contribution to the expression of the trait at commercial harvest stage, *parth7.1* played an important role in early parthenocarpic fruit set. The results suggested that parthenocarpic fruit set can be accurately evaluated with as few as 20 nodes of growth. The QTLs identified in this study for parthenocarpic fruit set are a valuable resource for cucumber breeders interested in developing parthenocarpic cultivars and to researchers interested in the genetic and molecular mechanisms of parthenocarpic fruit set.

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Introduction

Fertilization and fruit development are critical to reproduction and dispersal of flowering plants. Upon successful pollination and fertilization, a number of physiological events occur that lead to the development of fruit and seed. In some instances, fruit set can occur independent of pollination and/or fertilization, which is referred to as

parthenocarpy. Parthenocarpy can be obligatory in which the plants always produce seedless fruits or facultative in which production of seedless fruits occurs only when pollination is prevented. Parthenocarpy is a desirable trait for the production of fruit and vegetable crops with undesirable large and/or hard seeds. In commercial production, parthenocarpic cultivars are especially beneficial when pollination is difficult or heavily impacted by environmental conditions.

The developmental switch that turns a gynoeceium into a growing fruit depends on the fertilization of ovules. In most angiosperms, the gynoeceium dies if not fertilized (O'Neill and Nadeau 1997). Following pollination and fertilization, various phytohormones play critical roles in the initiation of fruit and seed development (Gillaspy et al. 1993; Seymour et al. 2013). As such, parthenocarpic fruit production can be artificially induced by exogenous application of plant growth regulators, such as auxin, gibberellic acid, cytokinin, brassinosteroids, or their combinations (e.g., Robinson et al. 1971; Schwabe and Mills 1981; Gorguet et al. 2005; Fu et al. 2008; Vriezen et al. 2008; Pandolfini 2009; Pascual et al. 2009; Serrani et al. 2010). Parthenocarpic expression in crop plants can also be manipulated through transgenic expression of regulator genes in hormone signaling pathways. For example, parthenocarpic fruit set has been achieved by expression of the ovule-specific auxin-synthesizing *DefH9-iaaM* transgene construct in tomato, eggplant, strawberry, raspberry, and cucumber (Rotino et al. 1997; Ficcadenti et al. 1999; Donzella et al. 2000; Mezzetti et al. 2004; Yin et al. 2006). *TPRP-F1-rolB*, a second transgenic ovary-specific auxin-synthesizing construct has also been demonstrated to induce parthenocarpic expression in grape, tomato, and eggplant (Carmi et al. 2003; Rotino et al. 2005; Costantini et al. 2007).

Naturally occurring (genetic) parthenocarpy has been observed in many plants and parthenocarpic cultivars are common in citrus (*Citrus* spp.), grape (*Vitis* spp), cucurbit, and solanaceous crops. In several species, the mode of inheritance for parthenocarpic fruit set has been investigated and varies from a single gene to multiple quantitative trait loci (QTLs). For example, in tomato several parthenocarpic genes or QTLs have been identified (Mazzucato et al. 1998; Fos et al. 2000, 2003; Beraldi et al. 2004; Gorguet et al. 2005, 2008). Miyatake et al. (2012) identified two major-effect QTLs controlling parthenocarpic fruit set in eggplant (*Solanum melongena*). In summer squash (*Cucurbita pepo* L.), parthenocarpy was shown to be controlled by a single gene with incomplete dominance (de Menezes et al. 2005). In citrus, parthenocarpy is controlled by at least two dominant complementary genes (Vardi et al. 2008).

Parthenocarpic development of cucumber fruit has long been recognized as an important characteristic for

greenhouse production (Sturtevant 1890). Most fresh market cucumbers grown in protected environments today are parthenocarpic. Parthenocarpic pickling cultivars are also of major importance in Europe. In the US, the majority of processing cucumbers are grown in a once-over machine harvest production system (Tatlioglu 1992), and parthenocarpic cultivars have not been widely used. The yields of US processing cucumber have not substantially increased since the 1980's (Gusmini and Wehner 2008). A phenomenon known as first fruit inhibition, where the first fertilized fruit inhibits the growth of subsequent fruits is thought to be a major obstacle to yield improvement in cucumber with once-over machine harvest production systems (Denna 1973; de Ponti 1976; El-Shawaf and Baker 1981; Sun et al. 2006a). A potential solution is the use of gynoeceious (expression of only pistillate flowers) parthenocarpic cucumber varieties. The use of gynoecey in combination with parthenocarpy is necessary as cucumber exhibits facultative parthenocarpy, meaning seeded fruit set can occur in parthenocarpic varieties exposed to a pollen source. Gynoeceious varieties also benefit from increased numbers of pistillate flowers, and thus greater opportunities for fruit set. Parthenocarpic cucumber varieties offer several advantages over conventional seeded varieties. Parthenocarpic varieties are able to set fruits sequentially without suffering from first fruit inhibition (Denna 1973; Sun et al. 2006a). Parthenocarpic varieties do not require pollination, and are therefore less vulnerable to poor pollination conditions (abiotic and biotic) and the need for insect pollinators (Pike and Peterson 1969; de Ponti 1976; Varoquaux et al. 2000; Sun et al. 2006a). In addition, parthenocarpic varieties often have more uniformly shaped fruit desired by the processing industry (de Ponti 1976; Aalbersberg and van Wijchen 1987).

Parthenocarpic fruit set in cucumber has been manipulated both genetically and with the exogenous application of various synthetic phytohormones. The application of auxin, cytokinin, gibberellic acid, brassinosteroids, and auxin transport inhibitors all result in the induction of parthenocarpic fruit set in cucumber (e.g., Choudhury and Phatak 1959; Robinson et al. 1971; Beyer and Quebedeaux 1974; Elassar et al. 1974; Cantliffe and Phatak 1975; Kim et al. 1992a; Fu et al. 2008). However, this method has many drawbacks, including the need for continuous application of phytohormones throughout growth, increased input costs for growers, environmental impact concerns, and human dietary concerns related to consumption of phytohormones (Rotino et al. 1997).

The mode of genetic inheritance for parthenocarpic fruit set in cucumber remains unresolved although highly successful parthenocarpic greenhouse cultivars have been developed. Genetic studies have been largely inconsistent on the mode of inheritance for parthenocarpy in cucumber and have ranged from proposals of a single gene to complex

multigenic inheritance (for example, Pike and Peterson 1969; de Ponti and Garretson de Ponti and Garretsen 1976; El-Shawaf and Baker 1981; Kim et al. 1992b). Most recently, Sun et al. (2006b) reported four major QTLs associated with parthenocarpic expression and observed significant epistasis and large genotype \times environment interactions for this trait in processing cucumber. In another study with F_2 and $F_{2,3}$ populations derived from crosses between a gynoecious parthenocarpic and two monoecious non-parthenocarpic lines, Wu et al. (2015) detected seven QTLs for parthenocarpic fruit set with a major-effect QTL, *parth2-1* in chromosome 2.

The objective of this research was to determine a model of inheritance and to identify major QTLs associated with parthenocarpic fruit set so that the trait may be more effectively utilized by researchers and breeding programs to improve yield in processing cucumber. Since parthenocarpic is often considered a yield component, it is difficult to separate the true parthenocarpic character from other yield related traits. To address this, a new approach to phenotypic evaluation of parthenocarpic fruit set focused on early fruit initiation and development was implemented to better define true parthenocarpic expression. This approach sought to build upon previous studies that demonstrated that parthenocarpic fruit set is determined in the days before and immediately after anthesis (Gillaspy et al. 1993; Fos et al. 2000; Molesini et al. 2009; Pascual et al. 2009; Ruan et al. 2012). With a better definition of parthenocarpic fruit set, traditional QTL mapping approaches were employed to detect and construct optimal genetic models for parthenocarpic fruit set in cucumber.

Materials and methods

Plant materials

Two US processing cucumber inbred lines, 2A and Gy8, were employed in the present study. The highly parthenocarpic 2A consistently sets multiple parthenocarpic fruits in both open field and greenhouse environments, whereas Gy8 yields few to no fruit in the absence of pollination (Sun et al. 2006a) (Fig. 1). Both lines exhibit similar growth and fruit characteristics including stable gynoecious expression, indeterminate growth habit, multiple disease resistances, and blocky shaped fruits preferred by the processing industry with length to diameter ratios near 3.0 at commercial harvest. The QTL mapping population consisted of 205 $2A \times Gy8 F_2$ plants and their derived F_3 families.

Experimental design and phenotypic data collection

Three experiments, EXPT1, EXPT2, and EXPT3, were conducted to evaluate parthenocarpic fruit set.



Fig. 1 Representative photographs depicting typical parthenocarpic fruit numbers and fruit set locations in the highly parthenocarpic parent line 2A (left), low parthenocarpic parent line Gy8 (right), and their F_1 hybrid (center)

Each experiment was conducted across five individual $6.1 \text{ m} \times 6.1 \text{ m}$ greenhouses at the University of Wisconsin-Madison Walnut Street Research Greenhouse facility. EXPT1 (July–September 2011) was organized into a randomized complete block experimental design (RCBD) with one plant from a subset of 201 F_3 families in each greenhouse; four plants of 2A and Gy8 and 1 F_1 plant were included in each greenhouse as checks. To address potential plant overcrowding observed during EXPT1, the experimental design was amended in EXPT2 (September–November 2011) and EXPT3 (April–June 2012) in which the number of plants per F_3 family was reduced from 5 to 3. Since it was not possible to distribute one plant from each F_3 family into each block of the RCBD, an augmented randomized complete block (ARCB) experimental design was implemented with three plants from each of the 205 F_3 families randomly distributed across the five greenhouses and the check varieties represented in complete blocks in agreement with EXPT1 (Federer and Raghavarao 1975; Federer and Crossa 2012). The number of F_3 families was increased to 205 to provide balanced rows of plants in each greenhouse. The goal of this experimental design change was to recover inter-experiment and inter-genotype information for use in finding the appropriate model for the phenotypic data prior to QTL mapping.

All experiments were conducted under controlled environmental conditions with standardized plant treatment and maintenance. Plants were grown under a 14 h photoperiod and 29–31 °C day/24–26 °C night temperatures. The plants were provided with supplemental artificial high pressure sodium lighting when natural light levels dropped below 650 μ E. All plants were vertically bound to bamboo poles. To account for potential differences in lateral branching and to avoid detrimental crown setting of fruit, the first five nodes of each plant were cleared of all vegetation, including flowers. In addition, all lateral branches from the rest of the plant were continuously removed throughout growth at regular intervals. Plants were limited to one pistillate flower per plant node to ensure that each plant developed an equal number of pistillate flowers and opportunity for fruit set.

Parthenocarpic fruit set was measured as the number of ovaries initiating parthenocarpic growth on each plant. A formal minimum fruit size requirement was not used and ovaries displaying any signs of growth and expansion were considered to have initiated parthenocarpic growth. Ovaries that initiated parthenocarpic growth, but later ceased at any point during development were counted as successfully initiated parthenocarpic fruit (see examples in supplemental Fig. S1). In some instances, ovaries were observed to abort development prior to anthesis. No differentiation was made in this study between ovaries aborting development prior to or following anthesis. Data collection on plant nodes 6–30 was conducted when ~95 % of plants had reached 35 plant nodes in maturity (approximately 60 days after germination). In addition, the plant node location of each ovary exhibiting parthenocarpic growth was recorded. All parthenocarpic fruits were cut to inspect for seeds resulting from rogue pollination; however, seed-bearing fruits were not observed during this examination.

Statistical analyses of phenotypic data

Statistical analyses of phenotypic data were performed using the statistical software R (Version 3.2.3, <http://www.r-project.org/>). A Spearman rank correlation of F_3 family means was performed between pairs of experiments as a measure of suitability for pooling observed data from the three experiments (EXPT1-3). The ability to pool data would be beneficial in alleviating the severity of sampling errors resulting from sampling 3–5 individuals from each F_3 family in each experiment. To determine if greenhouse environments were similar between experiments, two sample t tests were performed between pairs of experiments from experiment means calculated with data from all individuals. With the mean value of EXPT1 found to be statistically different from EXPT2 and EXPT3 with t tests, an ANOVA was performed with the check varieties (2A, Gy8, and their F_1) grown in each experiment.

Three different analyses were performed to model phenotypic data based on the performance of check varieties in each experiment. The first analysis adjusted the phenotype of plants in each experiment based upon the performance of the check varieties in their respective experiment by the following equation:

Model 1: Adjustment =

- Grand mean of check varieties in all experiments
- Combined mean of check varieties in each experiment.

The second analysis utilized a linear mixed model to estimate best linear unbiased estimators (BLUES) for each F_3 family, where Genotype referred to both the check and F_3 family genotypes and both were treated as fixed effects, while Experiment was treated as a random effect. The model is:

Model 2 : Parthenocarpic fruit initiated
= Mean + Genotype + Experiment.

The third analysis employed a linear mixed model to predict best linear unbiased predictions (BLUPs) for each F_3 family. For this analysis, check varieties were treated as fixed and F_3 families were treated as random effects. Two new variables, Check Entry and Test Family, were created as described by Wolfinger et al. (1997). Check Entry is a categorical variable with four levels that retained the individual identities for each of the checks but pooled all F_3 families into one measurement. This variable was used to model the means for each check variety and a common population mean for F_3 families. Test Family is a categorical variable with two levels that distinguishes genotypes into either check or F_3 family categories (Wolfinger et al. 1997). In Model 3, Check Entry was treated as a fixed effect, while Experiment and the Genotype \times Test Family interaction were treated as random effects. The model is:

Model 3 : Parthenocarpic fruit initiated
= Mean + Check entry + Experiment
+ Genotype \times Test family

Genotyping and linkage map construction

Cucumber SSR markers reported by Ren et al. (2009), Cavanaugh et al. (2010), and Yang et al. (2012) were screened for polymorphisms between 2A and Gy8. To identify additional molecular markers, 2A and Gy8 were sequenced with Illumina HiSeq 2000 (Illumina Inc., San Diego, CA) using the 2 \times 100 bp paired ends module with a mean coverage of 10 \times . The resulting short reads were mapped against the Gy14 draft genome assembly following Yang et al. (2012). Indel identification and SNP-calling were performed by the SAM tools software. Primer design was

Table 1 Summary statistics for the number of parthenocarpic fruit set per plant in EXPT1-3

Datasets	# Plants	Mean \pm SD	95 % Mean CI ^a	Range	2A	Gy8	2A \times Gy8 F ₁
EXPT1	1018	3.23 \pm 2.6	3.10–3.36	0–15	3.8	3.1	2.8
EXPT2	653	3.74 \pm 1.7	3.57–3.90	0–10	6.5	3.2	2.8
EXPT3	658	3.76 \pm 1.7	3.60–3.92	0–11	6.0	3.0	3.2
Pooled	2329	3.53 \pm 2.1	3.44–3.62	0–15	5.5	3.1	2.9

^a 95 % Mean confidence interval

performed with Primer3web (<http://primer3.ut.ee/>) (Rozen and Skaletsky 2000). For SNP genotyping, the SNPs were converted to dCAPS markers for CAPS assay.

Protocols for DNA isolation, PCR amplification, and gel electrophoresis followed Li et al. (2011). Linkage analysis was conducted using JoinMap 3.0 software. Linkage groups were identified using a minimum LOD threshold of 4.0. Linkage order and genetic distances were calculated with the regression mapping algorithm and the Kosambi mapping function. A ripple function was performed after the addition of each marker locus to construct an optimized marker order.

QTL analyses

QTL analyses were performed with data obtained via Models 1-3 and the pooled observed data from EXPT1-3 and then compared to determine the dataset that provided the best estimate of F₃ family phenotypes. Due to the observed differences in the timing of parthenocarpic fruit set associated with plant overcrowding in EXPT1, as well as the amended experimental design that was implemented in EXPT2 and EXPT3, two datasets, EXPT1 data and combined data from EXPT2 and EXPT3 were analyzed separately. To further explore the dynamics of genetic control of parthenocarpic fruit set, QTL analyses were performed with datasets consisting of data from only the first 10 (NODE10) and first 20 (NODE20) plant nodes of the combined phenotypic data collected for EXPT2 and EXPT3. Data from EXPT1 was not included in these analyses due to the noted delay in fruit set related to overcrowding.

QTL analyses were conducted in R/qtl (Version 1.30.4) (Broman et al. 2003). For each dataset, QTLs were first detected with Interval Mapping (IM), then with Composite Interval Mapping (CIM) and Multiple Interval Mapping (MIM) to refine QTL positions. Genome wide LOD significance thresholds at $\alpha = 0.05$ were estimated by 1000 permutations. A 1.5-LOD support interval was calculated for each QTL and was defined by flanking right and left molecular markers. QTLs were denominated with the prefix *parth* followed the number of the chromosome in which it is located and then followed by a decimal indicating the order in which it appears on the chromosome. As an example, *parth6.1* and *parth6.2* denote the first and second

parthenocarpic fruit set QTLs identified on chromosome 6, respectively.

Results

Phenotypic data analysis

The mean number of parthenocarpic fruit set per plant for each experiment, as well as pooled data from all three experiments are presented in Table 1; the frequency distributions for each experiment and EXPT1-3 pooled data are presented Fig. 2. In all three experiments, 2A consistently yielded more parthenocarpic fruit per plant than Gy8, whereas the F₁ more closely resembled Gy8 in both the timing and number of parthenocarpic fruit set. Significant transgressive variation for parthenocarpic fruit set was observed in each experiment (Table 1; Fig. 2). While the frequency distribution for the number of parthenocarpic fruit initiated per plant in EXPT1 skewed toward fewer fruits, the distributions for EXPT2, EXPT3, and the pooled data from EXPT1-3 followed a continuous normal distribution (Fig. 2). Two sample *t* tests did not reveal significant differences between EXPT2 and EXPT3 ($t_{1309} = 0.25$, $P = 0.80$). Conversely, the mean number of parthenocarpic fruits initiated per F₃ plant in both EXPT2 and EXPT3 varied significantly from that of EXPT1 (EXPT1/EXPT2: $t_{1669} = 4.47$, $P = 8.43E-6$; EXPT1/EXPT3: $t_{1674} = 4.69$, $P = 2.91E-6$). Thus, an ANOVA was performed with only the check varieties, which detected a significant difference between the experiments but not between individual blocks (greenhouses) in any of the experiments (supplemental Table S1). ANOVA also detected significant experiment \times genotype interactions indicating that the check varieties performed differently in each experiment (Table S1). Data from all three experiments was pooled and then adjusted according to Models 1–3 to account for the differences between experiments. In addition, a Spearman rank correlation of F₃ family means was performed between each of the experiments in pairs. The order of rank for the F₃ families in all of the Spearman rank correlation pairs was significant and positively correlated ($r_s = 0.48-0.54$, $P < 0.01$) and supported the use of a pooled dataset from EXPT1-3 for comparison to modeled data in QTL mapping.

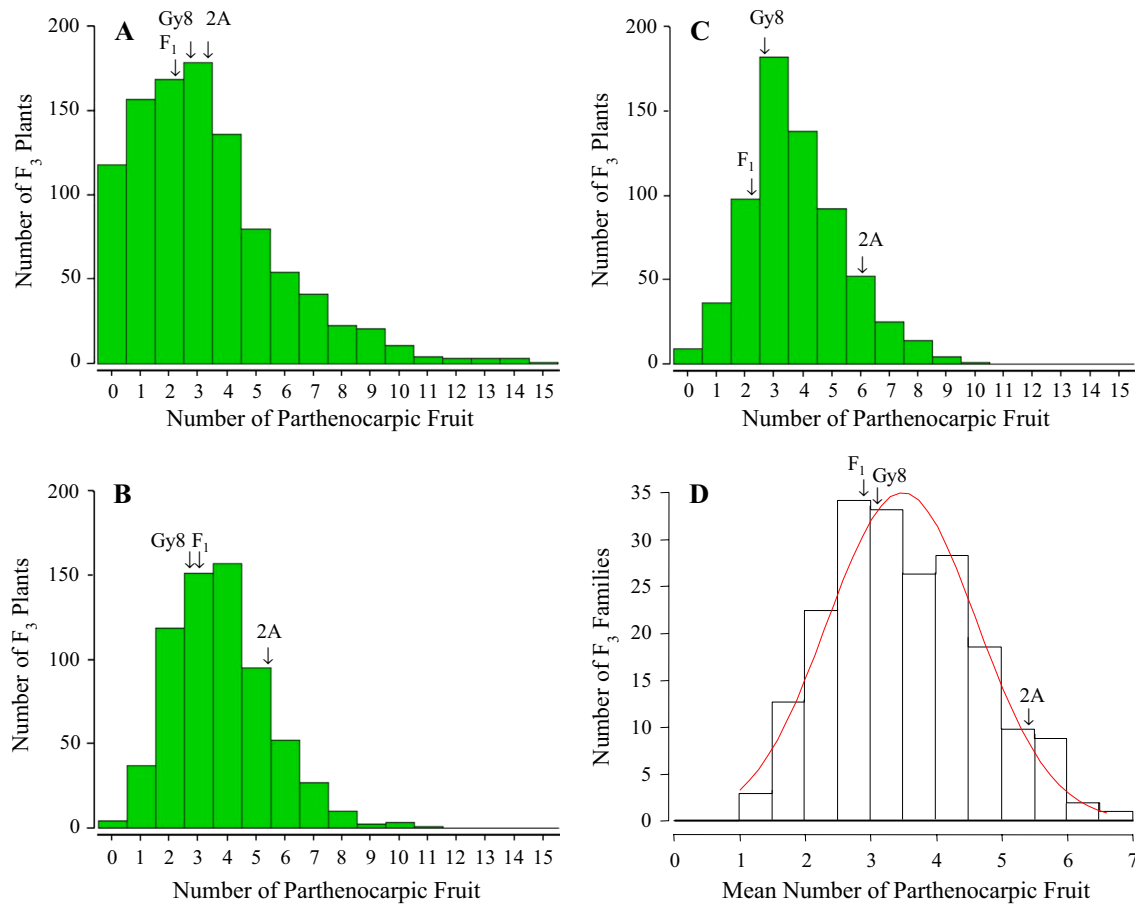


Fig. 2 Frequency distributions for the total number of parthenocarpic fruit initiated per plant in EXPT1 (a), EXPT2 (b), EXPT3 (c), and pooled F₃ family grand means from EXPT1-3 (d). For each experi-

ment the mean values of the *parental* lines and 2A × Gy8 F₁ hybrid are designated with *arrows*

Distribution of parthenocarpic fruits along main stem

In all experiments, the plant node where parthenocarpic fruit set occurred was recorded and the frequency distributions of parthenocarpic fruits along the main stem (nodes 6–30) in these experiments are shown in Fig. 3. A difference was observed in the peak location for parthenocarpic fruit set between EXPT1 (plant node 19) and EXPT2/EXPT3 (both at plant node 12). This difference could be attributed to plant overcrowding in EXPT1 which led to the abortion of some ovaries located on lower plant nodes, and thus created a delay in the onset of parthenocarpic fruits.

In EXPT2 and EXPT3, a bimodal distribution of parthenocarpic fruits was observed (Fig. 3). The ability of an individual plant to support a second flush of fruits may be a possible confounding factor for accurate measurement of parthenocarpic fruit set. In US commercial processing cucumber once-over machine harvest production, fruit harvest typically

occurs when plants reach ~20 plant nodes. In consideration of this, the NODE20 dataset was created to be inclusive of data from only plant nodes 6–20 in EXPT2 and EXPT3 (EXPT1 data were excluded due to the noted delay in fruit set). The frequency distributions for the number of parthenocarpic fruits occurring on plant nodes 6–20 in EXPT2 and EXPT3 followed a continuous normal distribution (Fig. S2).

The highly parthenocarpic parent 2A was consistently observed to initiate parthenocarpic fruit development on plant nodes 6–10 which was rarely observed in the low parthenocarpic parent Gy8 (Fig. 1). We formed a hypothesis that early parthenocarpic fruit set in the first ten nodes may be indicative of overall parthenocarpic expression. The NODE10 dataset was compiled from data collected for plant nodes 6–10 in EXPT2 and EXPT3 to explore this hypothesis. This dataset resembled a logarithmic distribution with Gy8 and 2A averaging 0.2 and 2.6 parthenocarpic fruits per plant, respectively (Fig. S2).

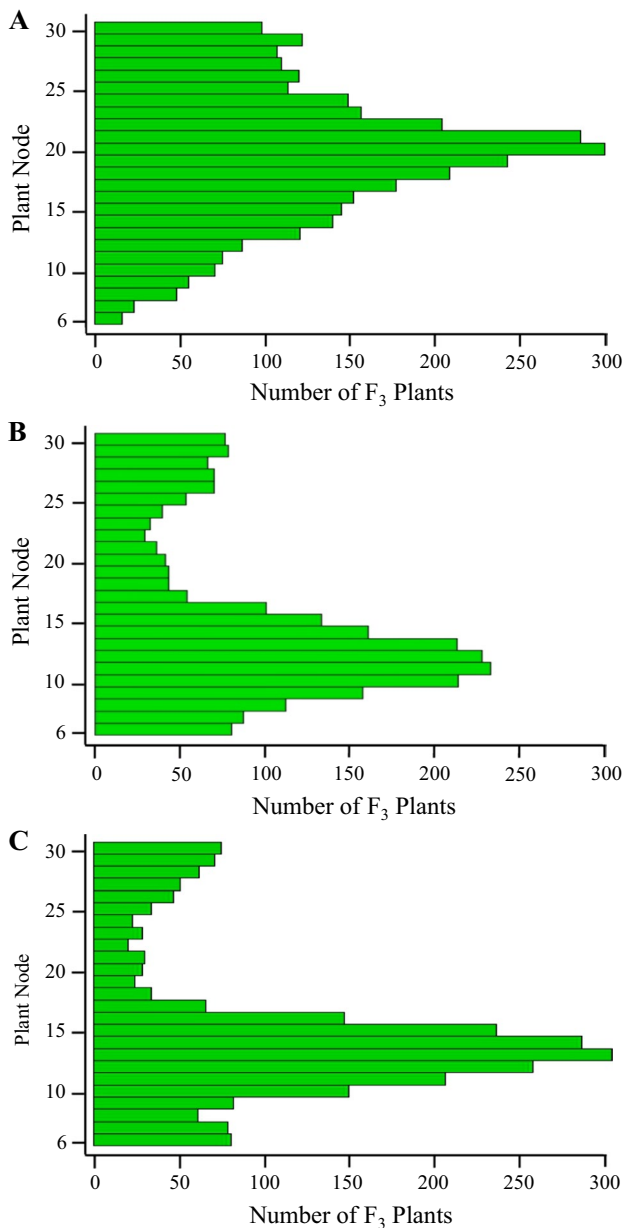


Fig. 3 Frequency distributions for parthenocarpic fruit set location along the main stem of all plants in EXPT1 (a), EXPT2 (b), and EXPT3 (c). Numbers on the Y axis represent node positions. All plants were cleared of vegetation and flowers on nodes 1 through 5 as a part of the experimental design

Linkage map construction

Of the 3532 SSR markers screened, only 235 (6.7 %) were polymorphic between 2A and Gy8 and 185 were mapped. Seven additional markers including five indels and two dCAPS markers were developed from resequencing data to fill large gaps on the SSR-based map. The resulting genetic map had 192 marker loci in seven linkage groups which spanned 571.7 cM and physically covered 164.3 Mbp of

the 193.2 Mbp (85 %) Gy14 draft genome assembly. Main features of this map are summarized in supplemental Table S2, and more details including individual marker information of the map are provided in supplemental Table S3.

A few large intervals without marker coverage are present on this genetic map, particularly on chromosomes 1 and 5 (Table S3). These large intervals were primarily due to the extremely low polymorphism levels observed between 2A and Gy8 which were found to be genetically very similar. The marker-deficient regions were likely under selection for processing cucumber profiles during the development of the two lines. However, these regions were unlikely to affect detection of QTLs for parthenocarpic fruit set in the present study (see below for discussion).

Identification of QTLs associated with parthenocarpic fruit set

QTL analyses were performed with three QTLs detection methods (IM, CIM and MIM) and datasets constructed from data adjusted by Models 1-3, pooled data from EXPT 1-3, EXPT1 data, and data from EXPT2-3 combined. Details of QTLs detected with all methods and different datasets are presented in supplemental Table S4. All three QTLs detection methods returned highly consistent results, with the MIM approach providing the best fitting QTL models. The results from QTL analyses performed with MIM are summarized in Table 2. Going forward, all following discussions will be based on the results obtained via MIM. With highly consistent map positions detected with each dataset, seven QTLs could be identified which were designated as *parth2.1* (chromosome 2 at 0.0–7.2 cM), *parth4.1* (chromosome 4 at 73.8–87.7 cM), *parth5.1* (chromosome 5 at 26.4–54.7 cM), *parth6.1* (chromosome 6 at 3.3–16.2 cM), *parth6.2* (chromosome 6 at 80.0–90.8 cM), *parth6.3* (chromosome 6 at 43.8–63.7 cM), and *parth7.1* (chromosome 7 at 0–26.3 cM). All analyses indicated complementation between the parental lines for parthenocarpic fruit set. Favorable alleles for increased parthenocarpic fruit set were contributed by the highly parthenocarpic parental line 2A at *parth5.1*, *parth6.1*, and *parth7.1*. Favorable alleles for increased parthenocarpic fruit set were contributed by the low parthenocarpic parental line Gy8 at *parth2.1*, *parth4.1*, *parth6.2*, and *parth6.3*.

QTL analyses of adjusted data from Models 1–3 and the pooled data from EXPT1-3 all returned nearly identical results (Table 2, Table S4; Fig. S3). In light of this, the pooled dataset from EXPT1-3 was deemed to be the most suitable for use in further QTL analyses. QTL analyses of the pooled EXPT1-3 data identified the presence of seven QTLs accounting for 75.5 % of the observed phenotypic variation (R^2) for parthenocarpic fruit set. Two QTLs on chromosome 6, *parth6.1* ($R^2 = 12.2$ %) and *parth6.2*

Table 2 QTLs for parthenocarpic fruit set detected with multiple interval mapping (MIM) for all datasets used in the present study

QTL	Chr	Peak location	LOD Score	% Var (R^2) ^a	Additive effects ^c	Dominance effects ^c	1.5-LOD Interval		Dataset Detecting the QTL ^b
							Left (cM)	Right (cM)	
MODEL			62.7	75.5					E1-3
<i>parth2.1</i>	Chr2	0.0	9.1	5.6	-0.37	-0.11	0.0	7.2	M1, M2, M3, E1-3, E1
<i>parth4.1</i>	Chr4	83.2	8.0	4.8	-0.34	-0.09	73.8	87.7	M1, M2, M3, E1-3, E1
<i>parth5.1</i>	Chr5	32.3	12.9	8.2	0.49	0.16	26.4	52.6	M1, M2, M3, E1-3, E1, E2-3
<i>parth6.1</i>	Chr6	9.7	17.9	12.2	0.61	-0.06	3.3	16.2	M1, M2, M3, E1-3, E1, E2-3
<i>parth6.3</i>	Chr6	53.0	11.9	7.5	-0.56	-0.01	51.2	63.7	M1, M2, M3, E1-3, E1
<i>parth6.2</i>	Chr6	83.0	19.9	13.8	-0.71	0.11	80.0	90.8	M1, M2, M3, E1-3, E1, E2-3
<i>parth7.1</i>	Chr7	21.8	8.6	5.2	0.37	0.03	0.0	26.3	M1, M2, M3, E1-3, E2-3

The datasets include Model 1 (M1), Model 2 (M2), Model 3 (M3), EXPT1-3 pooled (E1-3), EXPT1 (E1), and EXPT2-3 combined (E2-3)

^a Percentage of phenotypic variation (heritability due to the QTL) explained by the full model and each individual QTL

^b M1 = Model 1, M2 = Model 2, M3 = Model 3, E1-3 = EXPT 1-3 pooled data, E1 = EXPT1 data, E2-3 = EXPT2-3 combined data

^c Additive and dominance effects are in reference to the parthenocarpic parental line 2A

($R^2 = 13.8\%$), together accounted for approximately 26 % of the observed phenotypic variation with the remaining QTLs each accounting for less than 10 % of the observed phenotypic variation.

The consequences of plant overcrowding and the experimental design change between EXPT1 and EXPT2-3 were explored with a comparison of QTLs detected with the EXPT1 and the combined EXPT2-3 datasets (Table 2; Fig. 4a). The EXPT1 dataset detected six QTLs accounting for 69.0 % of the observed phenotypic variation as well as an epistatic interaction between *parth6.1* and *parth6.3* ($R^2 = 2.9\%$), but failed to detect *parth7.1* (Table 2). On the other hand, QTL analyses of the combined data from EXPT2-3 identified four QTLs, *parth5.1*, *parth6.1*, *parth6.2*, and *parth7.1*, accounting for 54 % of the observed phenotypic variation, while failing to detect *parth2.1*, *parth4.1*, and *parth6.3* or any epistatic interactions between QTLs (Table 2). The relative contributions of some QTLs detected with the two datasets were also different. The four QTLs detected with EXPT2-3 data had nearly equal contributions to the observed phenotypic variation ($R^2 \approx 11\text{--}15\%$). However, some of these same QTLs in EXPT1 were observed with significantly different contributions to the total phenotypic variation. In particular, the effect of the *parth5.1* QTL was diminished in EXPT1 ($R^2 = 4.2\%$ in EXPT1 versus 13.5 % in the EXPT2-3

dataset) and *parth7.1* was undetectable. In QTL analyses of the EXPT2-3 dataset, only *parth6.2* showed favorable alleles for increased parthenocarpic fruit set as being contributed from Gy8, which aligned better with the expectation that favorable alleles would be contributed by 2A.

QTLs associated with early parthenocarpic fruit set

To determine if the observed differences between the parental lines for the timing of parthenocarpic fruit set would reveal unique QTLs, analyses were performed with the NODE20 and NODE10 datasets and compared with data from all 30 plant nodes in EXPT2-3. The QTLs detected with the NODE10, NODE20, and EXPT2-3 (NODE30) datasets are presented in Table 3 and illustrated in Fig. 4B. Analyses of the NODE20 dataset indicated the presence of five QTLs, *parth4.1*, *parth5.1*, *parth6.1*, *parth6.2*, and *parth7.1*, accounting for 65 % the observed phenotypic variation for parthenocarpic fruit set (Table 3). These QTLs were consistent with those identified for the EXPT2-3 dataset except for the inclusion of *parth4.1* ($R^2 = 4\%$). While *parth5.1*, *parth6.1*, and *parth7.1* had similar contributions to observed phenotypic variation ($R^2 = 10\text{--}15\%$) between NODE20 and EXPT2-3, in NODE20, *parth6.2* had a significantly larger contribution ($R^2 = 23.7\%$) which was nearly twice the effect of other QTLs (Table 3).

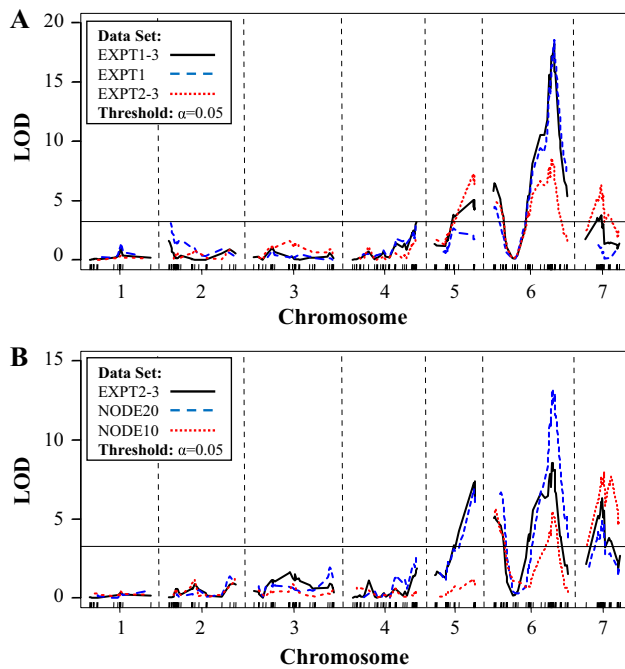


Fig. 4 Genome-wide LOD profiles for parthenocarpic fruit set QTLs detected with different datasets. **a** QTLs detected with EXPT1 data (dashed line), pooled data from EXPT1-3 (solid line), as well as EXPT2-3 combined (dotted line). **b** QTLs detected with EXPT2-3 (solid line), NODE20 (dashed line), and NODE10 (dotted line). All three datasets were pooled data from EXPT2 and EXPT3 only. Note that the profiles were from interval mapping in R/qtl in which *parth2.1* and *parth6.3* are below the LOD threshold for significance

Three QTLs, *parth6.1*, *parth6.2*, and *parth7.1*, were identified with the NODE10 dataset and accounted for 40.5 % of the observed phenotypic variation. The three QTLs were consistent with those identified with NODE20 and EXPT2-3 datasets. The *parth7.1* QTL had the strongest effect among the three ($R^2 = 17.4\%$) which was elevated in comparison to the effects observed for this QTL with the NODE20 and EXPT2-3 datasets (Table 3; Fig. 4b). The appearance of a potential second QTL linked to *parth7.1* in the NODE10 dataset was determined to be an artifact of the linkage map marker order deviating from the expected physical map order in this location (Fig. 4b; supplemental Table S3). The marker order in this QTL region could not be resolved with the data available in this study. NODE10 failed to detect *parth5.1* which was present in all other QTL analyses. In the NODE10 dataset, only *parth6.2* showed favorable alleles for increased parthenocarpic fruit set as being contributed by Gy8.

Discussion

Phenotypic evaluation of parthenocarpic fruit set in cucumber

One goal of this study was to limit yield as a confounding factor in the measurement of parthenocarpic fruit set. This is often difficult to achieve since parthenocarp can

Table 3 Development stage dependent parthenocarpic fruit set QTLs detected with the MIM method

Datasets ^a	QTL	Chr	QTL peak (cM)	LOD score	R^2 (% Var ^b)	QTL Effects		1.5 LOD interval	
						Additive ^c	Dominance ^c	Left (cM)	Right (cM)
EXPT2-3 (NODE30)	MODEL			34.69	54.12				
	<i>parth5.1</i>	5	54.7	11.4	13.5	0.52	0.15	32.3	54.7
	<i>parth6.1</i>	6	0.0	9.7	11.1	0.49	-0.09	0.0	13.3
	<i>parth6.2</i>	6	80.0	13.1	15.7	-0.60	0.02	76.2	83.0
	<i>parth7.1</i>	7	21.5	9.8	11.3	0.51	0.01	19.1	33.2
NODE20	MODEL			46.65	64.93				
	<i>parth4.1</i>	4	86.9	5.0	4.2	-0.26	-0.08	84.7	87.7
	<i>parth5.1</i>	5	52.9	12.1	10.9	0.41	0.13	32.3	54.7
	<i>parth6.1</i>	6	0.0	14.8	13.8	0.49	-0.07	0.0	3.3
	<i>parth6.2</i>	6	80.0	23.0	23.8	-0.66	-0.08	77.4	83.0
	<i>parth7.1</i>	7	24.1	9.6	8.5	0.40	0.04	23.0	42.0
NODE10	MODEL			23.13	40.52				
	<i>parth6.1</i>	6	3.3	8.3	12.2	0.26	-0.18	0.0	9.3
	<i>parth6.2</i>	6	80.0	8.7	12.8	-0.29	0.10	63.7	83.0
	<i>parth7.1</i>	7	32.1	11.4	17.4	0.33	0.02	19.1	42.0

^a All three datasets were from the combined data of EXPT2 and EXPT3

^b Percentage of phenotypic variation (heritability due to the QTL) explained by the full model and each individual QTL

^c Additive and dominance effects are in reference to the parthenocarpic parental line 2A

be viewed as a yield component; in fact, in many cases, parthenocarpy is indirectly selected for while breeding for increased yield (Robinson and Reiners 1999). By scoring parthenocarpic fruit set as a binary trait at a predetermined number of ovaries, we limited variation attributable to plant physiological source/sink relationships and thus yield. The decision to allow parthenocarpic fruit to develop undisturbed until the conclusion of each experiment may still be considered a confounding factor as large parthenocarpic fruits were observed, but by scoring ovaries as parthenocarpic after any signs of development, we likely captured a more accurate measurement of parthenocarpic expression.

The phenotypic evaluation of parthenocarpic fruit set used in the present study is comparable to the methods used by de Ponti (1976) in cucumber, as well as Kikuchi et al. (2008) and Miyatake et al. (2012) in eggplant. A simple parthenocarpic percentage statistic (number of parthenocarpic fruits divided by the number of pistillate flowers) was proposed by de Ponti (1976) as the most effective way to evaluate parthenocarpy in cucumber. The use of parthenocarpic percentage may be useful for the evaluation of parthenocarpic expression when working with lines differing in the number of pistillate flowers, fruit size, and yield capacity. For simplification, a parthenocarpic percentage statistic was not formally used in the present study as many of these factors were already accounted for in the selection of parent lines with similar morphological and agronomic characteristics and by limiting plants to one pistillate flower per node.

Physiological constraints on expression of parthenocarpic fruit set in cucumber

During EXPT1, some plants were observed to be losing foliage and failed to set parthenocarpic fruits on the earliest plant nodes. The plants appeared to be suffering from overcrowding; presumed to be due to the high density planting used in EXPT1. Although fruit set was hampered on earlier plant nodes, fruit set was still occurring at later plant nodes and overall fruit set appeared at near expected levels. Further evidence for overcrowding came from EXPT2 and EXPT3, in which the number of plants in each greenhouse was reduced by 40 %, and symptoms of plant overcrowding were not observed. To account for these differences, Models 1-3 were used to adjust the pooled data for the measured differences between experiments. Although each model returned different mean values for each F_3 family, QTLs identified with datasets from all three models were nearly identical and were also consistent with QTL mapping results obtained with the pooled data from EXPT1-3 (Table S4; Fig. S3). These findings indicated that the differences in experimental conditions that were observed during EXPT1 did not have an impact on overall QTL detection,

and the results from EXPT1 still provided valuable information on QTLs underlying parthenocarpic fruit set expression in this population.

In EXPT2 and EXPT3, a bimodal distribution of fruit initiation was observed during the first 30 nodes of plant growth (Fig. 3). This was anticipated to be due to source/sink relationships and reflects that once plants have begun fruit set, plants would continue to set fruit until they were unable to support any additional fruits with available assimilates (de Stigter 1969; Lloyd 1980; Stephenson 1981; Lee and Bazzaz 1982a, b; Schapendonk and Brouwer 1984; Stephenson et al. 1988). Following this, there was a quiescent period and some flowers were aborted. Once active fruits reached a certain level of maturity, fruit set resumed and another flush of fruits was initiated. Fruit distribution in EXPT1 was expected to follow this same phenomenon, but due to the delayed fruit set, a bimodal distribution was not observed during the first 30 nodes of plant growth (Fig. 3).

Genotyping the 2A × Gy8 population for QTL mapping

To develop the linkage map, we screened 3532 SSRs and identified only 235 (6.7 %) polymorphisms between 2A and Gy8. This low level of polymorphism between the two lines was also observed by Sun et al. (2006b) working on the same cross with AFLP and RAPD markers. This observation is likely attributable to common ancestry between the two parental lines. Although a direct common ancestor could not be identified, both parental lines have pedigrees that include numerous lines developed thru the cucumber research breeding program at the University of Wisconsin-Madison. Both parental lines also have close relationships with the public gynoecious inbred line series (e.g., the Gy gynoecious series), with 2A descending from Gy7 and Gy8 itself descending from Gy14. Further, breeding efforts to incorporate traits relating to fruit quality, disease resistances, and favorable processing characteristics into elite processing cucumber lines by a small number of public breeding programs may have also led to the incorporation of common genomic regions.

A comparison of the linkage map presented here with the marker locations in the Gy14 cucumber draft genome assembly (Yang et al. 2012) revealed 11 large intervals without marker coverage that each physically span more than 3 Mbp (Table S3). Fruit size (length and diameter) and fruit epidermal features are important attributes differentiating different market classes. Many genes or QTLs underlying these traits have been mapped and a review of the literature (e.g., Yuan et al. 2007, 2008; Miao et al. 2011; Wei et al. 2014; Yang et al. 2014a, b, c; Bo et al. 2014; Weng et al. 2015) revealed that a number of genes or QTLs associated with fruit size, sex expression, fruit epidermal

features, as well as various disease resistances mapped to these large, monomorphic intervals on the 2A × Gy8 linkage map (data not shown). It is conceivable that the regions harboring genes for processing cucumber identity or disease resistances were under strong selection during line development, which resulted in the high level of homogeneity and low polymorphism in these regions. Since 2A and Gy8 have contrasting levels of expression for parthenocarpic fruit set, major QTLs for parthenocarpic fruit set are unlikely to be located in these monomorphic regions.

QTLs for parthenocarpic fruit set

Our approach focusing on early fruit initiation and development for accurate phenotypic evaluation of parthenocarpic fruit set was effective and accounted for a large amount of the observed phenotypic variation (75.5 %) (Table 2). Further, analyses of individual datasets obtained from EXPT1 alone, EXPT2-3 combined, and the first 10 and 20 nodes of plant growth consistently indicated the presence of four QTLs (*parth5.1*, *parth6.1*, *parth6.2*, and *parth7.1*) with moderate to large effect (approximately 10–20 %) on the expression of parthenocarpic fruit set (Tables 2, 3). Sun et al. (2006b) conducted QTL mapping of parthenocarpic fruit set using an F₃ population derived from a different 2A × Gy8 cross, which was genotyped with RAPD and SCAR markers. In the previous study, plants were grown in isolated open field plots and parthenocarpic potential was measured as the number of fruit exceeding 2.8 cm in diameter during a single harvest performed when 15 % of fruit were at least 5 cm in diameter (Sun et al. 2006b). They identified four major genomic regions associated with parthenocarpic expression. We added SSR markers to the linkage map developed by Sun et al. (2006b) for alignment of the genetic maps from the two studies, and we found that the three QTLs in Sun et al. (2006b) on chromosome 6 were at similar positions as in the present study. However, none of the other QTLs identified by either study could be validated by both studies. The Sun et al. study suggested that some QTL associated with parthenocarpic expression in their study corresponded with QTL identified by Fazio et al. (2003) for fruit number per plant at first harvest (yield). To investigate this observation, an additional comparison was made between the QTL identified by this study and QTL associated with fruit yield by Fazio et al. (2003). A strong association between the QTL for the two traits was not observed. This highlights a key difference between the Sun et al. (2006b) study and the present study and why each study identified slightly different QTLs. The approach to phenotypic evaluation undertaken by the present study demonstrates an improved and effective evaluation of parthenocarpic expression in cucumber while limiting interference from confounding traits related to environment and yield.

In three seasons, Wu et al. (2015) phenotyped the percentage of parthenocarpic fruits in F₂ and F_{2,3} populations derived from crosses between a gynoeocious parthenocarpic line and two monoecious non-parthenocarpic lines. They identified 1, 2, 2, 1, and 1 parthenocarpic QTLs (total 7) on chromosomes 1, 2, 3, 5 and 7, respectively. Among these QTLs, only the major-effect QTL, *parth2-1* on Chr2 ($R^2 = 10.2\text{--}17.4\%$) had largely consistent location with *parth2.1* detected in the present study. The discrepancies may reflect the different lines, the environmental conditions or phenotyping methods employed in these studies.

In this study, the favorable alleles for increased parthenocarpic fruit set at each of the four QTLs were attributed to the highly parthenocarpic parental line 2A with the exception of *parth6.2*, where the favorable allele was contributed by the low parthenocarpic line Gy8. The remaining three minor-effect QTLs (*parth2.1*, *parth4.1*, and *parth6.3*), which were not consistently found in all analyses, all had favorable alleles being contributed by Gy8. Due to the noted experimental differences related to plant overcrowding observed in EXPT1, more confidence may be placed in the combined data from EXPT2-3. It is plausible that the *parth2.1* and *parth4.1* QTLs detected in the EXPT1 dataset were related to parthenocarpic fruit set and/or yield in high stress environments and should be considered cautiously. At best, these two QTLs could only be considered minor-effect QTLs as they each only accounted for ~5 % of the observed phenotypic variation in the pooled EXPT1-3 dataset (Table 2). Similarly, the high stress environment and subsequent delayed fruit set may potentially explain the absence of *parth7.1* in the analysis of data from EXPT1. Regardless, future research on parthenocarpic fruit development in cucumber should focus on the four moderate to large effect consensus QTLs identified in the present study.

The presence of *parth6.3* as a third QTL on chromosome 6 linked to *parth6.2* was not detectable in the analyses of combined data from EXPT2-3. Comparison of the LOD curves obtained through interval mapping with datasets from EXPT1 alone and EXPT2-3 combined showed large broad QTL peaks centered at *parth6.2* (Fig. 4). In addition, there was a slight uptick in LOD scores around 53.0 cM (*parth6.3*) in both datasets, although the change in LOD score was less than 1.0 in data collected from EXPT2-3 (Fig. 4). These observations indicated that an additional QTL linked to *parth6.2* may be present. The analyses of the EXPT1 dataset, where the presence of the linked QTLs were detectable, indicated that the potentially linked QTLs were in coupling phase and this may explain the large LOD scores attributed to *parth6.2* in the other datasets (Table S4). The epistatic interaction detected in EXPT1 between *parth6.1* and *parth6.3* may also partially explain the detection of the *parth6.3* QTL with this dataset (Table S4). The presence of *parth6.3* as a QTL linked to *parth6.2*

on chromosome 6 remains unclear and will require further marker saturation in this genomic region and potentially require validation with a larger population.

The contribution of favorable alleles for increased parthenocarpic fruit set at *parth6.2* by the low parthenocarpic parent Gy8 is an interesting example of genetic complementation (Tables 2, 3, Table S4). Nearly, all F₃ families with the highest observed expression of parthenocarpic fruit set were homozygous for the Gy8 allele at *parth6.2*. However, only 11 F₃ families achieved higher mean measurements of parthenocarpic fruit set than the parental line 2A across all experiments. One possible reason why so few families were found to exceed 2A may be related to the decision to collect phenotypic data at a single time when plants had reached 35 nodes (30 scorable plant nodes) in growth. It is possible that 2A and F₃ families with a high potential for parthenocarpic fruit set were never observed at their full potential due to plant capacity for fruit load as a confounding trait. In this scenario, the ideal genotype for increased parthenocarpic fruit set may be one that includes the Gy8 allele at *parth6.2*, but a noticeable increase in parthenocarpic expression was not observed over 2A because the fruit load capacity of an individual plant had already been realized. This would imply the presence of at least some remaining residual interference from a yield related trait. What remains to be answered is if the favorable allele possessed by Gy8 is favorable in all gene combinations. The question of why 2A is capable of high parthenocarpic potential while lacking the favorable allele at *parth6.2* suggests that the idealized genotype for high parthenocarpic fruit is complex and dependent upon the genotypes at multiple QTLs.

Optimal timing for phenotypic evaluation of parthenocarpic fruit set potential

The QTL mapping results from the NODE20 dataset were largely the same as those from the data collected for the first 30 plant nodes in EXPT2-3, although the contributions of each QTL had changed slightly (Table 3). This implies that future studies may be able to phenotypically evaluate parthenocarpic fruit set with as few as 20 nodes of plant growth. This observation complements the fact that processing cucumber lines are typically commercially harvested at approximately 20 nodes of plant growth. In addition, this observation satisfies any concern related to the ability of an individual plant to set a second flush of fruit as a confounding factor in this study, as the second flush of fruits had not yet commenced at 20 nodes of growth in EXPT2-3 (Fig. 3).

There was a significant increase in the effect of *parth6.2* in the NODE20 dataset versus the complete EXPT2-3 (NODE30) dataset (Table 3) and the reason is unclear. It

may be a reflection of the importance of the locus to parthenocarpic fruit set in the first 20 nodes of growth. More likely, as discussed earlier, it may be related to the possibility of a linked QTL in coupling phase in this region (*parth6.3*). However, if two linked QTLs do exist in this region they were not inseparable in the NODE20 dataset by all QTL detection methods employed.

The increased effect of *parth7.1* in the data collected from the first ten nodes of plant growth (NODE10 dataset) (Table 3; Fig. 4) suggests that it is important in early parthenocarpic fruit set. This may explain the inability to detect this QTL with the EXPT1 dataset (Table S4) since early parthenocarpic fruit set was disrupted by stress due to plant overcrowding. Overall, phenotypic selection for parthenocarpic fruit set is possible with as few as ten nodes of plant growth. However, in doing this there is some risk of omitting QTLs that may be important to fully maximizing parthenocarpic potential, such as *parth5.1*. We suggest that phenotypic selection should be done with 20 nodes of plant growth as active fruit set of the first flush of fruits is often continuing at node 10 and beyond in the highly parthenocarpic parental line 2A (Fig. 3). Limiting phenotypic evaluation to ten nodes of plant growth (only five scorable plant nodes) may be too restrictive and plants should be allowed to finish set of the first flush of fruits to maximize observed expression.

This study takes a new approach to phenotypic evaluation of parthenocarpic fruit set in cucumber. By focusing on fruit initiation and early fruit development, a major step has been taken in separating the true parthenocarpic character from other yield related traits that have confounded past studies. This study may still be confounded by the differing capacities of individual plants to support heavy fruit loads. Future studies may wish to address this by instituting a continuous harvest of fruit as soon as fruits can be conclusively declared to have either succeeded or failed to initiate parthenocarpic development. However, following this approach may in itself be complicated by plant stresses related to wounding and changes in fruit dominance if fruits are being continuously removed from the plant (Gruber and Bangerth 1990). Future studies may also wish to differentiate between ovaries aborting development prior to and following anthesis. Although unexplored here, the abortion of ovaries prior to anthesis are likely to be the result of physiological processes related to source/sink relationships and are not an indication of an overall non-parthenocarpic character. These points exemplify the complexity in accurately assessing parthenocarpic potential. Though an idealized protocol may not be obtainable, future studies should continue to focus on the events during early fruit development as the key to parthenocarpic fruit set. The QTLs identified for parthenocarpic fruit set by this study are a valuable resource for cucumber

breeders interested in developing parthenocarpic cultivars and to researchers interested in the genetic and molecular mechanisms of parthenocarpic fruit set. Future efforts will be needed in fine mapping of the QTL regions identified here to identify the underlying candidate genes and to elucidate a mechanism for parthenocarpic expression in cucumber.

Author contribution statement CL performed the research, analyzed the data, and wrote a draft of the manuscript. SP and HZ participated in marker analysis. YW and MJH designed the experiment, participated in data analysis, and preparation of the manuscript. All authors reviewed and approved this submission.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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