



Quantitative trait loci for bearing habit in a ‘Sparrow’ × ‘Schessler’ *Juglans nigra* mapping population

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Abstract

Eastern black walnut, *Juglans nigra* L., is an economically important tree species valued for its high-quality timber and edible nuts. A regional industry for the species’ nut and kernel products resides in Missouri, where over 9 million kg of hulled in-shell nuts are purchased in mast years. The crop is primarily based upon nuts harvested from wild trees, placing a ceiling on nut volume and quality (e.g. small nut size, dark pellicle color, and 10–14% kernel by weight). Orchards of named cultivars, like ‘Kwik Krop’ and ‘Sparrow’, supply up to 22,000 kg of nuts with a higher kernel percentage (> 26%) and improved quality. Such cultivars often represent chance wild or on-farm seedlings, clonally propagated since the late 1800’s by enthusiasts. Today, continued improvement of eastern black walnut as an orchard crop is limited by a long generation time, delayed expression of important traits, and space requirements – creating a strong need for marker-trait association studies that inform progeny selection. The first linkage map for *J. nigra* was recently created using the ‘Sparrow’ × ‘Schessler’ F₁ population and loci for phenology traits discovered. The objective of this study is to utilize these genetic resources to detect quantitative trait loci (QTL) and report associated DNA markers for the spur-bearing habit, which promotes precocity and high yield. Using single-year data from the 11-year-old population, we observe that segregation for the spur-bearing habit appears to be recessive and multigenic. Three QTLs ($p > 0.99$) were identified on linkage group (LG) 8, LG11, and LG16 that explain 7.2%, 8.7%, and 10% of trait variation, respectively. Regions between flanking DNA markers were 3.16 cM, 4.32 cM, and 9.69 cM, respectively. This study is the first to examine the genetic control of bearing habit and yield in eastern black walnut and informs breeders’ approach for their future genetic improvement.

Keywords *Juglans* · Eastern black walnut · EST-SSR · Heterodichogamy · QTL mapping · Spur bearing

Introduction

Eastern black walnut (*Juglans nigra* L.) is one of 21 species within the genus *Juglans*, residing in the section *Rhysocaryon* (Manning 1978). Eastern black walnut is distributed

throughout deciduous forests in eastern North America, occurring in scattered populations on favorable sites (Williams, 1990). It is a shade-intolerant, pioneering species with a long juvenile period, reaching reproductive maturity at 20–30 years (Schlesinger and Funk 1977). Before European colonization, eastern black walnut was important to indigenous peoples, for use as a staple crop and medicine, with evidence from historic village sites suggesting a level of cultivation and range expansion (Black et al. 2006; Wykoff 1991). The timber from mature eastern black walnut stands has been sought after since early colonial times, prized for its dark brown heartwood, workability, strength, and rot resistance (McKenna and Coggeshall 2018).

Compared to the related Persian walnut (*Juglans regia* L.), eastern black walnut is relatively new to cultivation as a nut crop and is still in the early stages of domestication. The Persian walnut is the most cultivated walnut species,

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with the California crop in 2021 valued at just over \$1 billion (USDA 2022). The success of *J. regia* as a global crop is due in part to its long history of human use and selection, having been actively cultivated by humans outside of its natural range for over 2,000 years (Salas-Salvadó et al. 2011). In contrast, some of the earliest clonally propagated eastern black walnut selections were made by landowners in the late 1800s (Zarger 1969). The value of kernels from Missouri's wild eastern black walnut crop, accounting for approximately 65% of the total United States supply, is estimated to be between \$ 2.6 - \$3.5 M (Hammons Products, personal comm.).

Despite its short period of domestication, over 700 named *Juglans nigra* cultivars exist; a testament to the species' significant ecological, economic, and cultural importance (Reid et al. 2004). These early selections mainly represent a strong focus on percent kernel, moving from 6 to 10% kernel in unimproved wild eastern black walnuts to above 30% in the best selections and cultivars (Reid 1990; Meier et al. 2022). Selection did not emphasize yield, bearing habit, disease resistance, or other important traits, meaning that many of these early cultivars are not viable options for commercial production (Reid et al. 2004). To improve the commercial viability of eastern black walnut, the University of Missouri Center for Agroforestry (UMCA) breeding program has used these historical selections since 2002 to carry forward systematic improvement schemes. Selections from the first generation represent promising improvements over traditional cultivars, with a few individuals combining desirable traits including spur-bearing habit, larger kernel size, and improved quality. A dozen UMCA selections have been transitioned to a preliminary release program for wide on-farm trialing by early adopters throughout Missouri (Webber et al. 2021).

Concerning the Persian walnut, highly productive cultivars exhibit a strong lateral bearing habit, observed when shoots emerge from the current season's new growth, forming lateral branches that terminate in pistillate blooms (Hendricks et al. 1998). Lateral bearing habit contrasts with terminal bearing, where new growth exhibits strong apical dominance, resulting in less branching and nuts borne on the tips of branches (Germain 1989). Lateral bearing is observed on a continuum, from fruitful trees displaying a small degree of branching to lateral fruitfulness. The character is of great commercial value to walnut industries worldwide due to its correlation with productivity and precocity (Amiri et al. 2010). Diversifying sources of lateral bearing and integrating them into breeding pipelines is a priority globally (Bernard et al. 2018). 'Payne' is the source of lateral bearing used in the Walnut Improvement Program at the University of California-Davis, and it is a parent or grandparent of all lateral bearing cultivars released by the program (Bernard et

al. 2018). 'Chandler', a grandchild of 'Payne', was released in 1979, with high yield and lateral bearing habit, late leafing, and light-colored kernels (Tulecke 1994). 'Chandler' is one of the world's most widely grown cultivars and represents over 70% of the Persian walnut trees planted in California (Bernard et al. 2018).

Eastern black walnut displays a different pattern of non-terminal bearing, which is less like the lateral bearing of Persian walnut, and more like that of spur-type apple – short, compressed internodes terminating in pistillate blooms (Fig. 1). These compact branches arising from larger limbs drastically increase the number of fruiting sites throughout the canopy (Reid et al. 2004). While lateral and spur-type bearing are not directly analogous, their effects on fruiting sites and yield are functionally similar. As in Persian walnut, spur-bearing in eastern black walnut appears to occur on a continuum, with terminal bearing also present. A deeper understanding of spur-bearing and genetic architecture of yield in eastern black walnut would benefit breeding scheme design and aid marker-assisted selection (MAS) development, as generation time is long and land use requirements are high.

Quantitative Trait Loci (QTL) analysis recently became possible in *J. nigra* with the completion of the first linkage map for the species, using a bi-parental family of 'Sparrow' × 'Schessler' (Chatwin et al. 2023). The linkage map consists of 62 EST-SSR markers and 356 SNP markers. The EST-SSRs are common to both *J. nigra* and *J. regia*, allowing comparisons of QTL positions on maps of the two species. Chatwin et al. (2023) reported QTLs for heterodichogamy flowering and phenological characters, mapping a single locus for protandry to linkage group (LG) 11 and drawing comparisons to QTL discovered in *J. regia*. As the population enters its mature bearing years, there is a great opportunity to expand QTL analysis to other economically important traits.

The study seeks to evaluate segregation for the spur-bearing habitat in the 'Sparrow' × 'Schessler' mapping population and conduct QTL analysis, comparing results to discoveries made in *J. regia* and assessing DNA markers for potential use in MAS.

Materials and methods

Parent cultivars

Both parent cultivars used to construct the mapping population are wild selections. The maternal tree, 'Sparrow' was named in 1935 after selection from the wild in Lomax, Illinois. Evaluations at the University of Missouri Horticulture and Agroforestry Research Center (HARC) in New

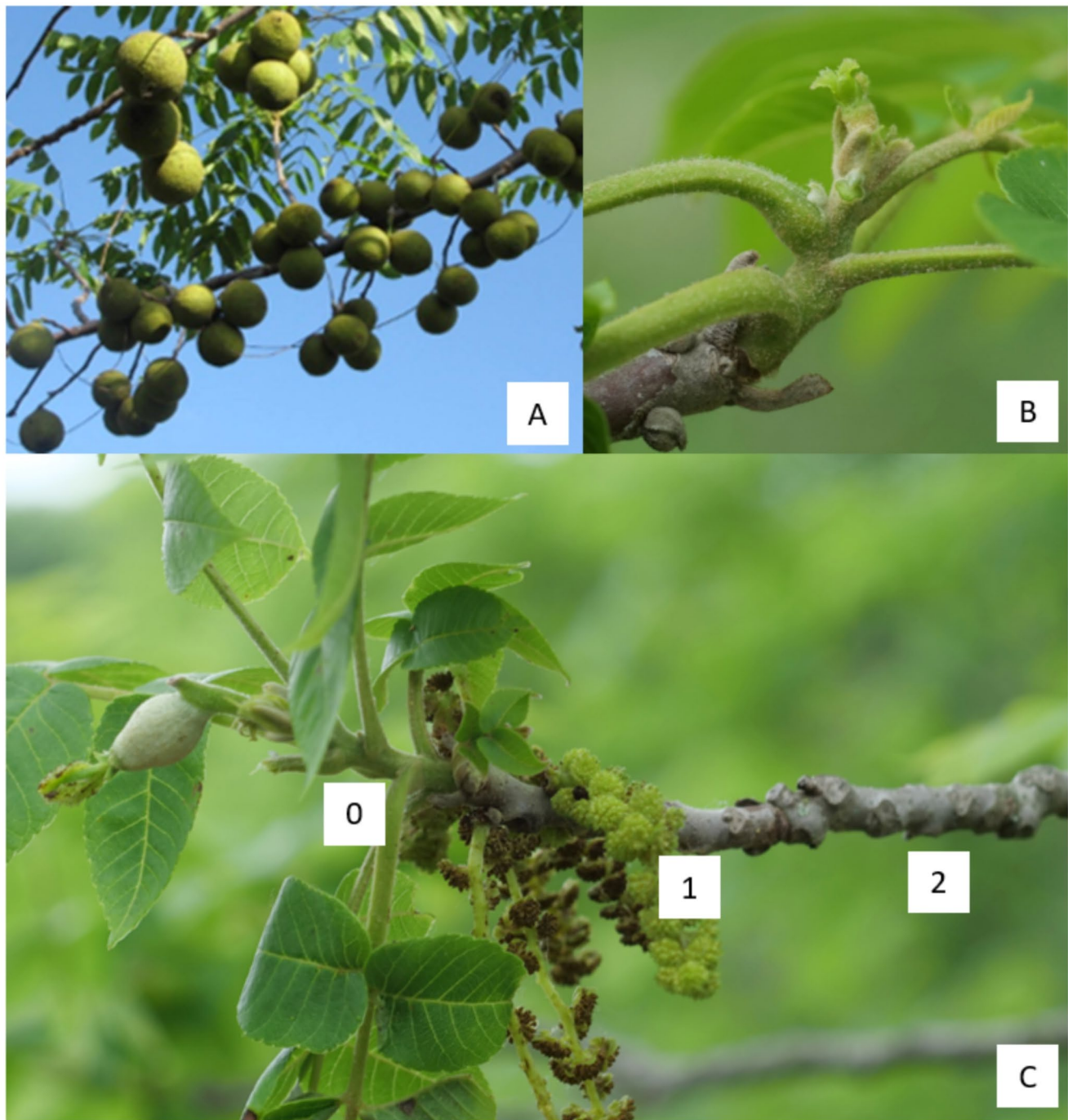


Fig. 1 (A) Productive eastern black walnut tree showing a high degree of spur-bearing. Nuts are produced on short spurs that develop on main branches. (B) Fruiting spurs terminate in pistillate blooms after grow-

ing only a few leaves. (C) A fruiting spur with the current season's growth (0), last year's wood (1), and two-year old wood (2). Short, compressed internodes are typical characteristics of fruiting spurs

Franklin, Missouri, show consistent productivity, with an alternate bearing index (a measure of tendency to produce alternating low and high yields, ranging from 0 to 1) of 0.5–0.7 and yield efficiency of 10.86 nuts per cm² of trunk area (Coggeshall, unpublished). In recent breeding and selection trials, ‘Sparrow’ has been used as a source or cultivar standard for a high degree of spur-bearing. The paternal cultivar

‘Schessler’ was originally a tree selected in the wild in Genoa, Ohio, in 1937. Based on evaluations between 2002–2009, the alternate bearing habit is greater in ‘Schessler’ with an index of 0.7–0.8, and lower yield efficiency at 8.03 nuts per cm² of trunk area.

Mapping population, genotyping, and linkage map development

Mapping population development is described in full by Chatwin et al. (2023), where open-pollinated nuts were collected from a ramet of the cultivar Sparrow. Three thousand and nine seedlings were grown from these nuts, after which paternal exclusion analysis was performed with 11 gSSRs from *J. nigra* (Coggeshall 2010). Of these, ‘Schessler’ was the parent of 329 them. The identified ‘Sparrow’ × ‘Schessler’ seedlings were grown in pots for a year until field planting in 2010. The trees were planted on 6-meter centers at the University of Missouri Horticulture and Agroforestry Research Farm in New Franklin, Missouri (39°00′50″ N lat., 92°46′12″ W long). As of 2021, 318 of the 329 trees remain alive.

Tissue sample collection, and genotyping were performed and described in depth by Chatwin et al. (2023). In 2016, twig tissue from 277 trees in the mapping population was collected for double-digest reduced representation sequencing (ddRADseq) and stored in a -80 °C freezer. Tissue samples were ground in liquid nitrogen using a SPEX 6850 Freezer mill (Metuchen, NJ). DNA was extracted using Norgen Biotech Plant/Fungi DNA extraction kits (Norgen Biotek Corp®, Thorold, ON), and DNA quality was confirmed by electrophoresis on agarose gels and using a ThermoScientific NanoDrop spectrophotometer (ThermoFisher Scientific, Waltham, MA). An additional leaf sample was collected in the spring of 2017 for EST-SSR marker development. EST-SSR markers were developed via a bait-capture approach (Chatwin et al. 2023). EST-SSR markers were mined for *J. nigra* and *J. regia* using the Hardwood Genomics database (<https://www.hardwoodgenomics.org/>) and MISA using the MicroSatellite identification tool (<http://pgrc.ipk-gatersleben.de/misa/>). Markers were filtered and removed if they did not match at least 80 bp from *Juglans* species in the NCBI database BLAST search algorithm or if the sequence contained more than one SSR. Bait design was conducted by MYcroarray®, now Arbor Biosciences™, and a bait-capture approach selected high-quality EST-SSR markers suitable for genetic mapping in both *J. regia* and *J. nigra*, to enable comparative mapping of these species. The parent cultivars ‘Sparrow’ and ‘Schessler,’ and 160 seedlings with the most complete ddRADseq data were used for target capture. Samples were prepared using the NEBNext® Ultra™ II FS DNA Library Prep Kit for Illumina with the NEBNext® Multiplex Oligo Set (Dual-index) adapters (New England BioLabs® Inc., Ipswich, MA), and validated with the Qubit™ dsDNA H.S. Assay Kit (ThermoFisher Scientific, Waltham, MA) and Bioanalyzer DNA 7500 kit (Agilent Technologies, Santa Clara, CA).

Linkage map construction

Chatwin et al. (2023) reported the *J. nigra* ‘Sparrow’ × ‘Schessler’ linkage map used in this study (Table S1) and the procedures used in its development. The map was constructed in JoinMap 4.1 (Van Ooijen 2013) using 248 individuals from the population and informative markers in pseudo-testcross configuration (Table S2). Markers from the female parent segregating 1:1 were in <lm x ll> format, markers from the male parent segregating 1:1 were in <nn x np> format, and markers segregating 1:2:1 were in <hk x hk> format, while SSR markers were in <hk x hk> or <ab x cd> or <ef x eg> format. The completed consensus map contains 62 EST-SSR markers and 356 SNP markers across the species’ 16 LGs. The total map length is 1646 cM, with an average distance of 4.2 cM between markers, and 19 gaps greater than 10 cM. There are an average of 26 markers per LG. The least dense LG is LG5, with 14 SNPs and 2 EST-SSRs. The EST-SSR markers mapped to the *J. regia* ‘Chandler’ V2.0 genome reveal a high degree of collinearity with some structural changes on chromosomes 3 and 7 (Chatwin et al. 2023). With the included EST-SSR markers, comparative analysis will be possible between *J. nigra* and other species in the *Juglandaceae*.

Spur-bearing and yield phenotyping

The population produced its first substantial crop in 2021, where the segregation pattern of bearing habit could be observed. Before harvest, the total number of nuts borne on spurs was counted for each tree in the orchard, while excluding nuts borne on terminal branches. Spur-borne nuts (SbN) were defined as nuts borne on short branches from larger limbs (Fig. 1). The total count of SbN per tree was used to quantify the degree of spur-bearing for each individual in the population.

Nuts were harvested from each tree as they ripened by gently shaking trees to measure the total yield. The harvest date was determined by checking the hull’s resistance to finger pressure. Nuts with hulls that dimpled from soft pressure were considered ready to harvest. To maintain kernel quality, nuts were hulled within five days of harvest date using a mechanical huller. After hulling, a pressure washer was used to remove remaining hull fragments that could influence total in-shell mass or lead to spoilage. Cleaned nuts were hung in high-density polyethylene mesh bags on racks for approximately four weeks at room temperature. Box fans and spaces between bags ensured adequate airflow. In-shell mass (kg) was recorded using a digital scale.

Statistical analysis and QTL detection

The number of SbN and total yield was transformed with a square root transformation before further analysis. Pearson's correlation coefficients were estimated using `ggpairs()` function of the `GGally` package in Rstudio for SbN and total yield in kg (R Core Team 2022; RStudio Team 2022). Phenotypic data was imported into MapQTL 6 software for analysis (Van Ooijen 2009). SbN and total yield datasets were analyzed separately. Analysis was performed with the interval mapping function, using the default parameters. A 1,000-iteration permutation test was used to determine the significant logarithmic odds ratio (LOD) threshold of potential QTLs. Significant loci were detected using the chromosome-level significance threshold of $P < 0.01$.

Results

Phenotypic data analysis

In 2021, 219 of the 318 'Sparrow' × 'Schessler' trees bore nuts. Genotype data was available for 158 bearing trees and used for QTL analysis. Sixty-six of the genotyped trees bore no nuts. These trees were retained in the QTL analysis due to the established correlation between precocity and lateral bearing in *J. regia* (Germain 1989). A histogram of raw SbN values for the 318 seedlings is presented in a histogram (Fig. 2a) and shows a non-normal distribution wherein most trees produced between 0 and 58 SbN. Square root transformed data presented as a histogram (Fig. 2b) resembles a segregation pattern for multiple recessive loci. The data distribution (square root transformed) for individuals retained in QTL analysis (Fig. 2c) resembles the population. As expected, there was a high correlation between SbN (square root transformed) and the total yield ($R^2 = 0.971$, $p > 0.01$). The single most productive tree produced 10.4 kg of cleaned, in-shell nuts, while a large majority of offspring (72%) produced 1 kg or less. The frequency distribution

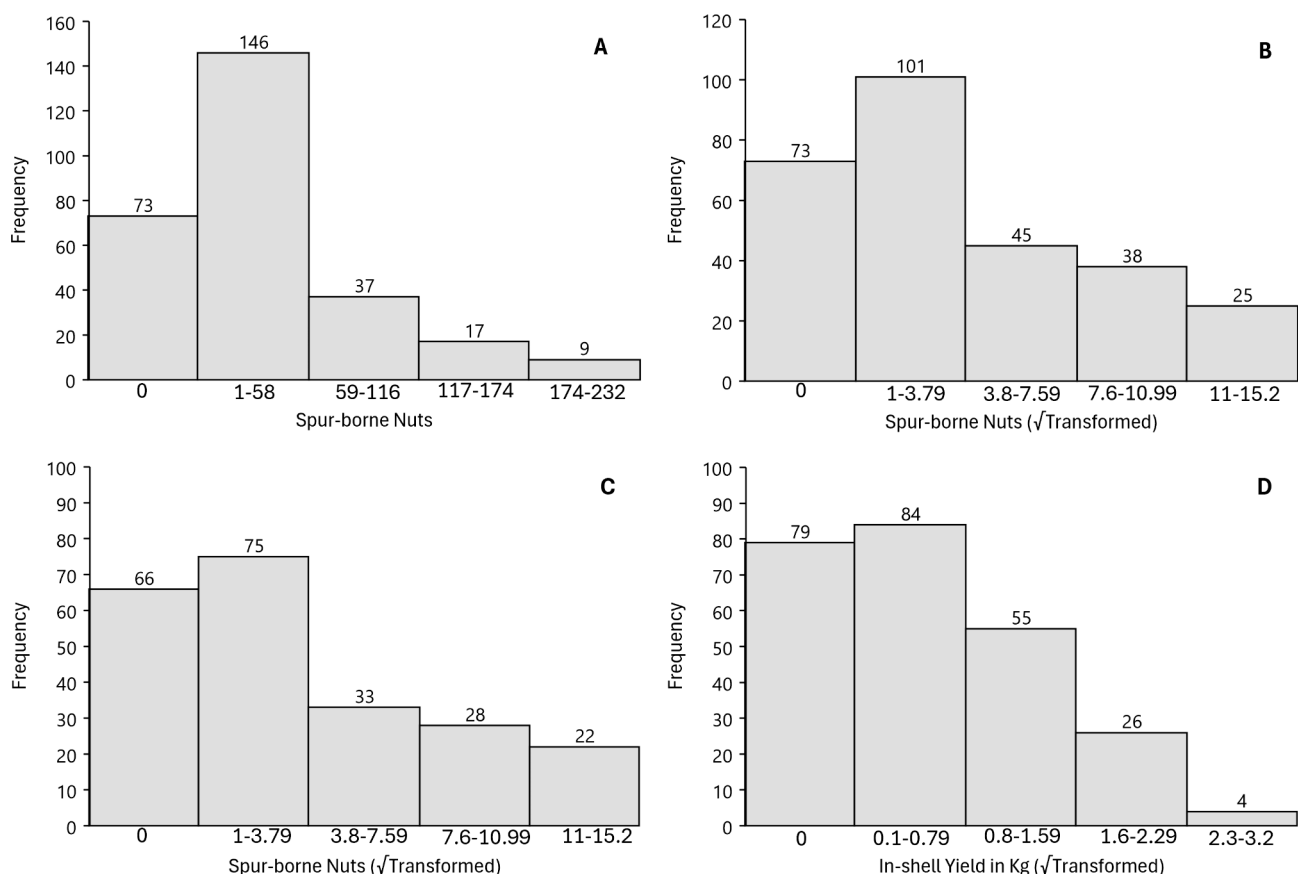
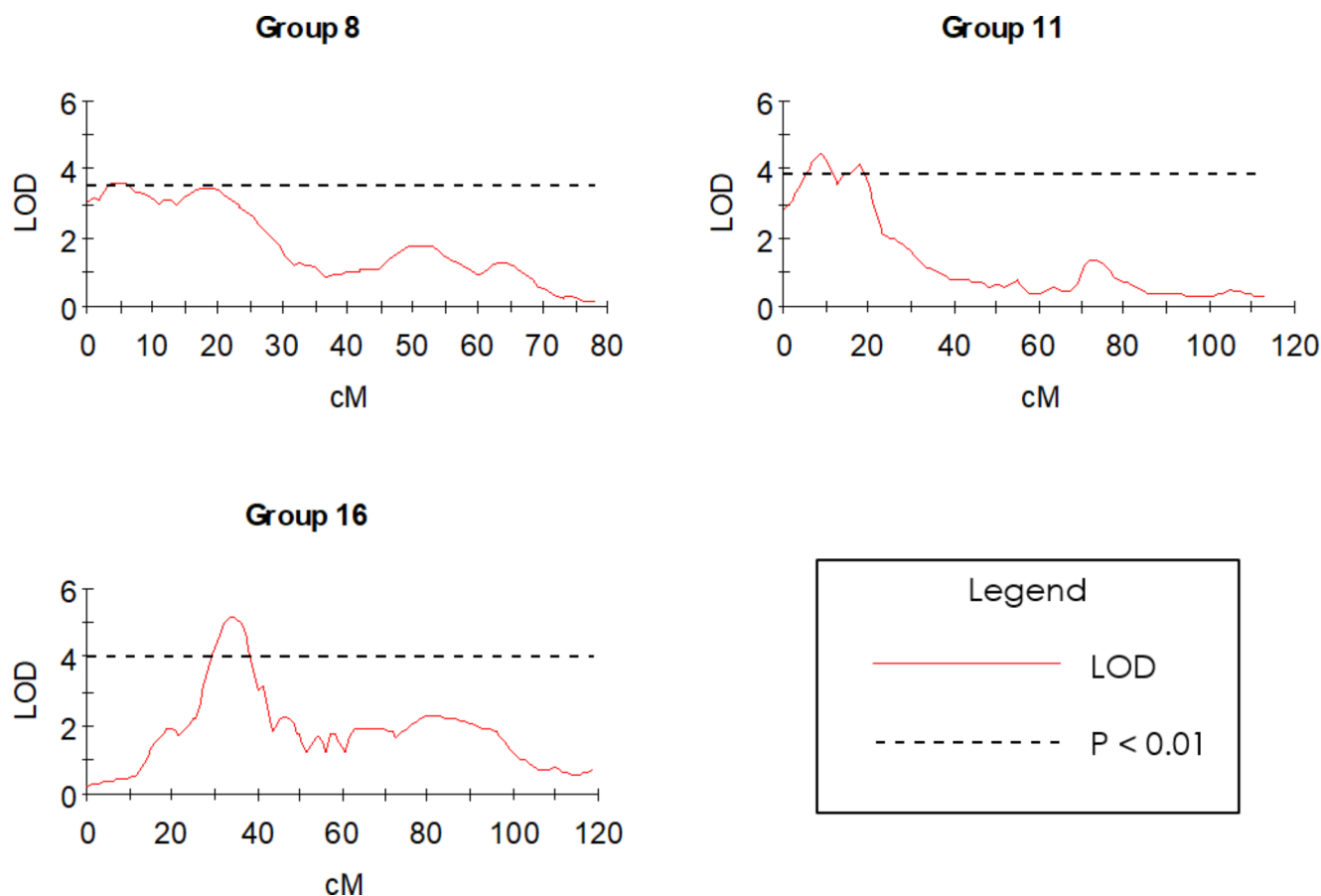


Fig. 2 Histograms show the spur-borne nuts distribution and in-shell yield in the F_1 'Sparrow' × 'Schessler' population. All individuals (A), all individuals transformed (B), genotyped individuals included

in QTL analysis (C), and In-shell Yield for all individuals transformed (D). Bins are divided by percentile

Table 1 Summary of QTLs identified in the F_1 full-sibling ‘Sparrow’ $\times$ ‘Schessler’ population for yield and degree of spur-bearing

QTL Target	LG	LOD	LOD Threshold ($P < 0.01$)	% Variance Explained	Position (cM)	Flanking Marker Span (cM)
Yield	8	4.27	3.6	7.7%	4.81	3.16
Yield	16	4.34	3.9	7.8%	34.42	9.69
Spur-bearing	8	3.62	3.5	7.2%	3.81	3.16
Spur-bearing	11	4.43	3.9	8.7%	9.24	4.32
Spur-bearing	16	5.13	4.0	10.0%	34.42	9.69

**Fig. 3** LOD profiles for spur-bearing on linkage groups 8, 11, and 16 using the interval mapping function in MapQTL6. The $P < 0.01$ significance level was determined for each linkage group with a permuta-

tion test, represented by the dashed line. LOD values exceeding the threshold were detected, showing significant QTLs on linkage groups 8, 11, and 16

histogram of total yield in kg (Fig. 2d) follows a similar pattern to the histogram displaying SbN count (Fig. 2a-c). Data is made available in MapQTL format in Table S3.

QTL analysis

The LOD significance thresholds ($P < 0.01$) were determined for each LG using a 1,000-iteration permutation test, where LOD scores exceeding the threshold indicate significant QTLs. Based on the number of SbN, QTLs were detected on LGs 8, 11, and 16, accounting for 7.2%, 8.7%, and 10% of the variation, respectively (Table 1; Fig. 3). The QTL peak on LG8 is positioned at 3.80 cM, with one SNP marker surpassing the significant LOD threshold, 20299_63,

an estimated 1.16 cM upstream. On LG11, the QTL spans 12.18 cM, with the peak at 9.24 cM. Two SNPs and two EST-SSRs are within this QTL, with the nearest two flanking markers of the QTL spanning a region of 4.32 cM. The peak of the QTL on LG16 is at 34.41 cM and contains the only significant SNP marker 45269_86 above the significance LOD threshold. The analysis based on total yield (square root transformed) detected the same significant QTLs on LGs 8 and 16, accounting for 7.7 and 7.8% of the variation (Table 1). However, the QTL detected on LG11 was below the $P < 0.01$ significance threshold (Fig. 4). Linkage map visualization shows the relative position of each of the identified QTL regions (Fig. 5).

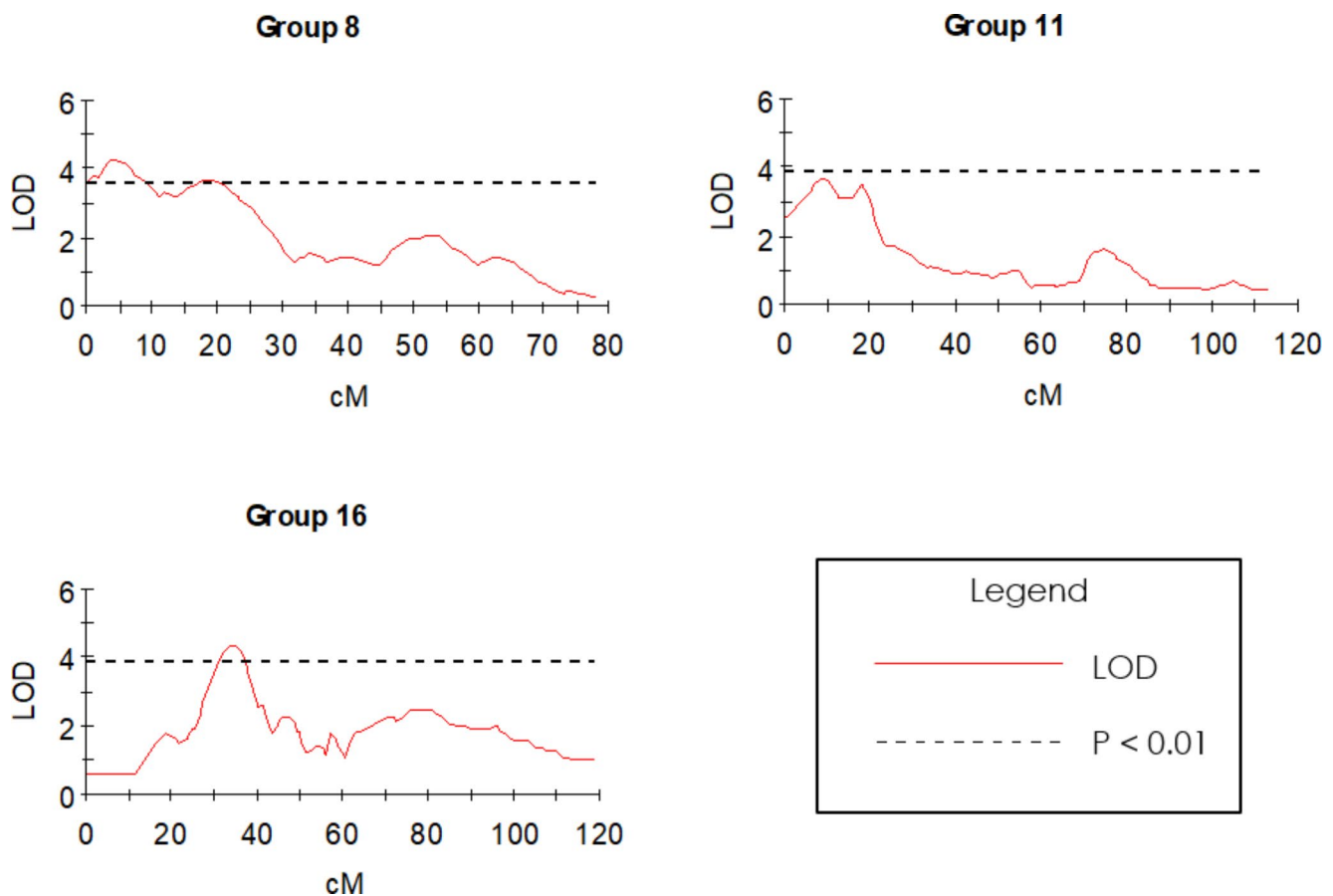


Fig. 4 LOD profiles for in-shell yield on linkage groups 8, 11, and 16 using the interval mapping function in MapQTL6. The $P < 0.01$ significance level was determined for each linkage group with a permutation test, represented by the dashed line. LOD values exceeding the

threshold were detected, showing significant QTLs on linkage groups 8 and 16. The LOD score based on yield data was higher on LG8, but fell short of the threshold on LG11

Discussion

This study was the first to explore the genetic architecture of bearing habit and in-shell yield for eastern black walnut. This early evidence suggests that multiple loci may be responsible for spur-bearing habit in this species; QTLs were detected on LG8, 11, and 16 in the ‘Sparrow’ $\times$ ‘Schessler’ F_1 population, using an interval mapping approach. The segregation pattern for SbN suggests multiple loci of recessive control. These loci correspond with QTLs for total in-shell yield, although the QTL peak on LG11 is just below the significance threshold of $P < 0.01$. These findings are useful to breeders, offering context for trait inheritance and genetic control, as well as the selection intensity required to improve spur-bearing during complementary hybridization crossing schemes. Further, these data reinforce that selection for spur bearing habit is a valid approach for improving nut yield, as seen through shared QTLs and a high correlation between traits ($R^2 = 0.97$, $P > 0.01$) using transformed SbN.

Despite much lower marker density and resolution (i.e., recombination frequency), the results reported here have similarities to marker-trait association studies of lateral bearing in *J. regia*. Dvorak et al. (2015) first identified the major lateral bearing locus in *J. regia* (Dvorak et al. 2015). Utilizing an F_2 progeny of ‘Chandler’, two markers were discovered flanking the associated genomic region on LG11. This QTL resides in a similar region to that of the *J. nigra* LG11 QTL discovered in this study, which co-locates with the heterodichogamy QTLs in *J. nigra* and *J. regia* near EST-SSR markers estJN1025 and estJN1060 (Chatwin et al. 2023). Incorporating lateral bearing sources from diverse origins is a priority of walnut breeding programs worldwide (Bernard et al. 2018). Consequently, subsequent marker-trait association studies have sought to expand the understanding of the genetic basis of lateral bearing in more diverse germplasm and with greater mapping resolution to refine candidate genes of interest. Spur bearing is present to varying degrees in over a dozen cultivars in the UMCA repository, including Cranz, Davidson, Kwik Krop, Sparks 127, and Sparrow, to name a few.

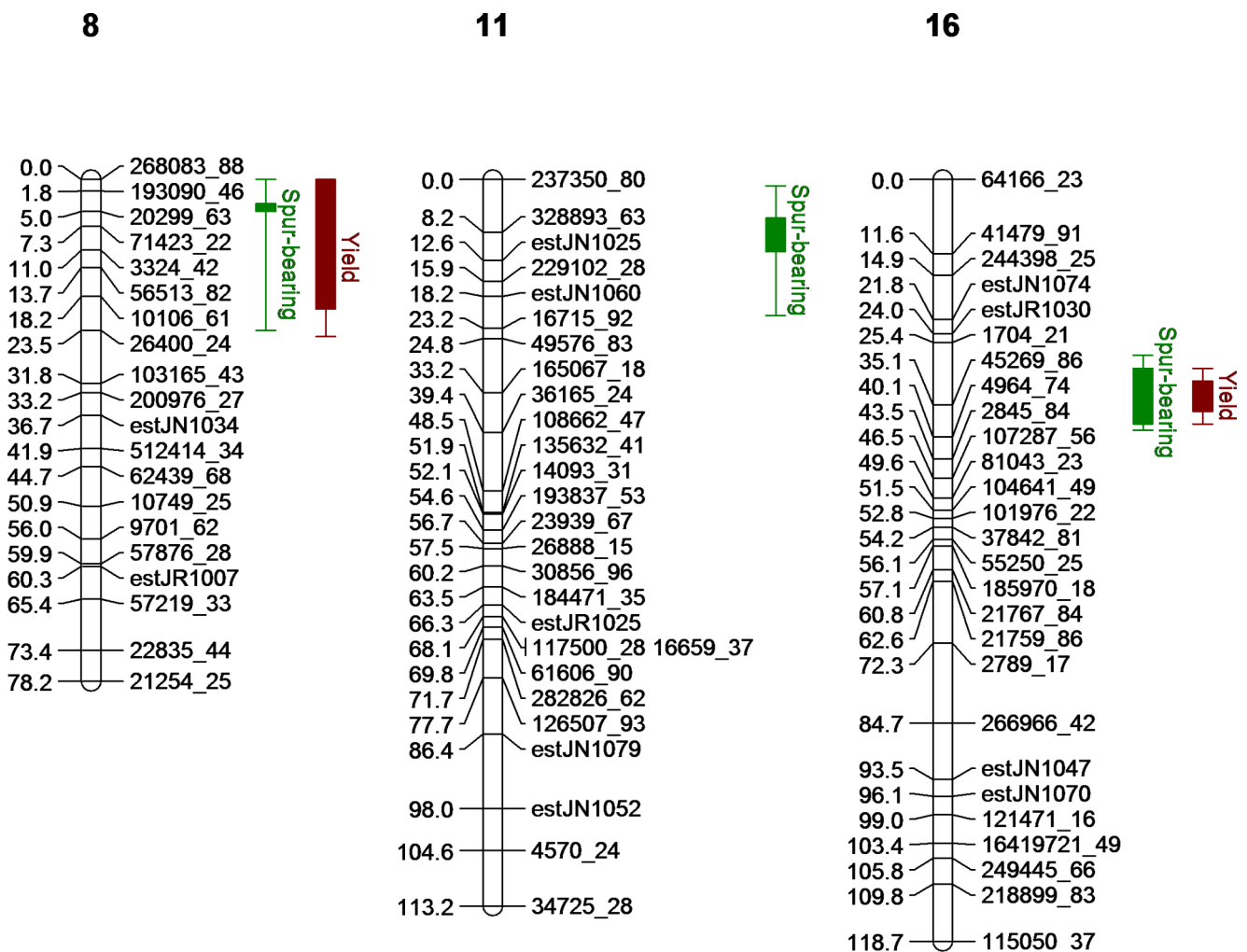


Fig. 5 Genetic linkage map of linkage groups 8, 11, and 16 showing QTLs associated with a high degree of spur-bearing and in-shell yield. Each marker shown on the right of linkage groups is labeled with its

position in centimorgans on the left. Bars indicate the markers located within each QTL region at the $P < 0.05$ level and $P < 0.01$ level

Marrano et al. (2019) explored marker-trait associations for lateral bearing, yield, and various other characteristics using a dual approach of QTL mapping and Genome-Wide Association Study (GWAS). QTL mapping utilized 1,880 SNPs segregating in a ‘Chandler’ × ‘Idaho’ population of 312 trees. GWAS benefited from >250,000 SNPs and a population of 896 offspring representing the diversity within the University of California at Davis Walnut Improvement Program pedigree. Using QTL analysis, a major QTL was identified in the same region on LG11 of ‘Chandler’ as discovered in Dvorak et al. (2015). Interestingly, this LG11 QTL explained only 38.05% of the variation in nut yield but 69.67% of the lateral fruitfulness variation. Estimates of variation explained by total yield in our study were comparable to SbN (Table 1) and much lower than Marrano et al. (2019). This difference may be a function of experimental power, as Marrano et al. (2019) used over four times as many markers and a population one generation more advanced.

That said, it is also worth highlighting that after applying simple interval mapping, like in our study, Marrano et al. (2019) performed Multiple QTL Mapping, selecting cofactor markers based on multiple regression and backward elimination (Arends et al. 2014; Jansen 1993). This method tends to increase estimates of variation explained and LOD.

Multiple QTL Mapping also discovered additional minor QTL for yield on LG1 and lateral bearing on LG6 (7.51% of variation explained) (Marrano et al. 2019). The suggestion of multiple loci follows the model of genetic control proposed by our results in *J. nigra*. Nevertheless, it’s reasonable to expect some variation in QTL results between the species, as the biological processes of the bifurcation patterns are different, and segregation patterns also vary. While we report a low rate of spur bearing recovery based on direct counting of SbN, studies in *J. regia* report a bimodal-like distribution (i.e. two peaks reflecting major gene segregation) by visually estimating the percentage of flowering

lateral buds on a 0–9 scale (Marrano et al. 2019). Different phenotyping approaches likely also introduce variation but are likely limited to the degree of explained variation. Due to the large size of the trees used in our study, it was not possible to accurately implement the Marrano et al. (2019) phenotyping approach of quantifying flowering shoots.

Using GWAS, lateral bearing yielded the largest number of significant marker-trait associations of the five traits studied in Marrano et al. (2019), with 18 SNPs detected across six chromosomes, 1, 4, 8, 9, 11, and 13. The analysis validates the major QTL on LG11, previously discovered by QTL mapping, where seven significant SNPs were detected over a 25 Mb region and explained around 90% of trait variation. Marrano et al. (2019) reported 91 candidate genes for lateral bearing in the most significant portion of this region, many of which have known roles in flowering and shoot meristem growth (Marrano et al. 2019). Bernard et al. (2020) validated this major QTL on chromosome 11, performing GWAS in French germplasm held at the Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement. The most associated SNP, AX-171,191,765, at this chromosome 11 locus caused lateral fruiting by an allelic substitution but had an uncharacterized functional annotation.

The results presented in this study provide a preliminary assessment of the genetic control of spur-bearing in eastern black walnut, informing breeding and further investigation. The limitations of this study include one replication per genotype (typical in many studies of this type), a single source of spur-bearing, and number of seedlings and number of markers. QTL mapping is inherently limited in its mapping resolution and effect size estimation due to the number of recombination events (Collard et al. 2005; Xu 2003). Nevertheless, using 62 EST-SSRs, 356 SNPs, and 158 'Sparrow' × 'Schessler' individuals, three QTLs were discovered for spur-bearing habit on LG8, LG11, and LG16. QTL results for total yield mirror those of spur-bearing, as reported in *J. regia* (Marrano et al. 2019). Marrano et al. (2019) note that the correlation between spur-bearing and yield may mask QTL specific to each trait. The QTL discovered on LG11 in *J. nigra* resides in a similar region as the major loci for lateral bearing discovered on chromosome 11 in *J. regia*, strengthening support for the involvement of this QTL for spur bearing in *J. nigra*. While the loci detected on LG8 and LG16 represent possible additional QTL, further investigation with greater power and resolution is needed to validate these regions and determine their effect size.

Further refinement of QTL regions and the development of flanking markers to assist progeny selection requires genotyping and adding markers to the linkage map. To support this need, *J. nigra* has been incorporated into a 70k multispecies SNP array with chestnut, hazelnut, and grape (Bianco et al. 2023). Five thousand markers on the array have been allotted to *J. nigra*, which will increase the LG coverage over 10x on

average. Additionally, consideration should be given to developing populations with greater recombination. Heavy spur-bearing selections in the 'Sparrow' × 'Schessler' F1 population should be used to create F2 families, crossing between other selections and individuals lacking spur-bearing. Other opportunities for high-resolution mapping will arise as UMCA breeding program pedigrees expand and GWAS can be employed, but such an approach will take 6 or more years for population development and maturation. Further, while the EST-SSR markers used in this study offer an invaluable tool for comparing QTL positions between *J. regia* and *J. nigra*, a chromosome-scale reference genome of 'Sparrow' would offer great insight on synteny between the species as well as within QTL regions of interest.

Conclusion

By utilizing a single year of data from a 'Sparrow' × 'Schessler' F1 population and an interval mapping approach, we were able to detect quantitative trait loci and report associated DNA markers for both total in-shell yield and the spur-bearing habit, which is correlated with precocity and high yield. For the first time, we observed three QTL on LG8, LG11, and LG16 that explain 7.2%, 8.7%, and 10% of trait variation, respectively. While these regions are associated with significant variation in yield and bearing habit, the spacing of flanking markers limits the application of current markers for selection. Future plans to increase marker density, continue phenotypic data collection, and sequence the population will aim towards increasing QTL marker accuracy. This preliminary study informs breeding approaches for the future genetic improvement of eastern black walnut.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11295-024-01664-8>.

Author contributions All authors contributed to the study conception and design, while the mapping population itself was developed by M. Coggeshall in advance of this study. Material preparation, data collection and analysis were performed by B. Jablonski, R. Revord, and J. Romero-Severson. The first draft of the manuscript was written by B. Jablonski and R. Revord. All authors read and approved the final manuscript.

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Data availability The genetic map, genotype, and phenotype data are available in MapQTL format as supplemental data.

Declarations

Competing interests The authors declare no competing interests.

Data archiving statement Raw sequence data for both RRS and EST-SSR bait capture is being submitted to NCBI's Short Read Archive (SRA). Accession numbers will be provided once available. Our genetic maps will be submitted to the TreeGenes database, and an accession number will be provided on acceptance.

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