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# Genetic Variation Among 54 Eastern Black Walnut Cultivars for Phenological and Morphological Traits

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Abstract. **Eastern black walnut (*Juglans nigra*) is a large tree endemic to the eastern United States and is highly sought after for its timber products and uniquely flavored nuts. The University of Missouri Center for Agroforestry in New Franklin, MO, USA, hosts an eastern black walnut cultivar repository. This collection supports an ongoing breeding program to improve economic performance for nut production (kernel weight, nut quality, precocity, and yield). In 1996, 54 cultivars were grafted and planted in a series of experimental orchards for evaluation. From 2001 until 2015, trees were evaluated for 12 phenological and eight nut quality/yield traits. Economically relevant traits including kernel weight, kernel percentage, and precocity (total nuts produced age 6 to 10 years) ranged from 1.1 to 8.6 g, 5.3% to 39.3%, and 16 to 1522 total nuts per tree, respectively. Kernel percentage was positively correlated with kernel weight ($r = 0.51$) and precocity ($r = 0.38$). Precocity was negatively correlated with the first ($r = -0.39$) and last ($r = -0.30$) female bloom. Principal component analysis and biplot analyses revealed high levels of variation among the cultivars. The first two components explain 43.4% of the total variation. Nut dimensions (nut length and nut thickness), nut and kernel weight, and kernel percentage are the largest drivers of variation in the collection. Eigenvectors for precocity and kernel percentage load together and are orthogonal to kernel weight, suggesting these three important traits can be improved simultaneously. Also, nut length loads with kernel weight, providing a candidate indirect selection parameter to increase kernel weight. These data inform strategies for crossing scheme design, expectations for multitrait genetic gain, complementary hybridization, and identifying unique recombinants.**

Eastern black walnut (*Juglans nigra*) is one of 21 species within the genus *Juglans* and a member of the subsection *Rhysocaryon* (Manning, 1978). The species is an economically important hardwood prevalent across North America and prized for its edible nuts and rich heartwood timber (Miller and Chambers, 2013; Reid et al., 2009; Walker et al., 2002). Persian walnut (*Juglans regia*) is the basis of the worldwide industry and has a long history of domestication and organized genetic improvement (Vahdati et al., 2019). Although the value of the eastern black walnut shell and kernel market is smaller in comparison, the species supports an important regional industry for rural communities in the midwestern United States, where considerable opportunity remains for genetic improvement and growth.

Most commercially harvested eastern black walnut nuts are of wild origin. As a result, this supply is highly variable in yield and quality. Missouri's crop ranges from 5 to 7 million kilograms (≈65% of the total supply in the United States) and is valued at \$2.6 to \$3.5 million annually. However, masting causes alternate bearing and steep declines in production in the off years (Hammons Products, unpublished). Nuts from wild trees also garner a low price (\$0.35/kg) due to their low kernel percentage (6% to 14%) (Coggeshall, 2002) and highly variable quality, with many kernels having a dark color and acrid floral/fermented aroma and flavor (Warmund et al., 2009a). However, recently there has been an increased demand for nuts harvested from grafted cultivars exhibiting improved nut quality traits and spur-bearing habit (Thomas and Prindle, 2016).

In contrast to wild sources, nuts derived from improved selections exhibit >30% kernel and other preferred attributes, resulting in wholesale purchases of hulled, wet in-shell nuts ≈\$1.65/kg by Hammons Products (Stockton, MO, USA). It is estimated that current annual production levels derived from wild sources could be produced on 800 ha of improved cultivar orchards (Hammons Products, unpublished). Consistent production of nuts with desirable traits makes improved cultivars attractive to both commercial growers and consumers. Shifting regional production from wild trees to orchards would allow industry growth by increasing supply. Persian walnut improvement is a helpful comparison in analyzing genetic variation in eastern black walnut because *J. nigra* and *J. regia* are phylogenetically related species (Bernard et al., 2018).

Since 1996, the University of Missouri Center for Agroforestry (UMCA, New Franklin, MO, USA) has curated and maintained a collection of 54 eastern black walnut cultivars representing much of the native range, prioritizing high kernel percentage (Coggeshall and Woeste, 2010). Cultivars from this collection (with kernel percentages in the upper 20s) were used to initiate the first organized

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eastern black walnut improvement program for nut/kernel traits beginning in 2001 (Coggeshall, 2002). The goals of this eastern black walnut improvement program include: 1) germplasm acquisition and evaluation, 2) development of pedigreed seedling populations through controlled pollinations, and 3) establishing field trials that focus on germplasm performance for a series of key traits across multiple environments. Evaluation of phenological and nut quality characteristics in this collection from 2001 to 2015 revealed high levels of variation for a diverse range of traits (Coggeshall, 2011).

Ideally, eastern black walnut cultivars used for commercial nut production should exhibit characteristics such as high kernel weight, high kernel percentage, light-colored kernel, and easy extraction, with little emphasis on the upright growth habit ideal for veneer log production (Fowells, 1965). Horticultural and phenological characteristics such as spur-bearing habit, regular annual production, disease resistance, late budbreak date, and early harvest date also profoundly impact yield potential and success. Eastern black walnuts are long-lived trees that generally begin producing around age 6 years for grafted trees and age 10 years or more for seedlings (Rink et al., 2017). Precocity refers to the timespan required for a tree to begin flowering. The most precocious eastern black walnut selections in the UMCA collection began to flower and fruit 5 years after grafting. In Persian walnut, precocity is correlated with lateral bearing, yield, and early leafing/fruiting (Germain, 1990; Solar et al., 2001), with wide-spreading tree crowns being most productive (Amiri et al., 2010). Similar to lateral bearing in Persian walnut, spur-bearing in eastern black walnut greatly increases productivity and is also associated with precocity (Reid et al., 2009). Spur-bearing habit refers to a crown architecture with multiple, short, compact branches that are at least 2 years old that arise along primary limbs and annually produce a fruiting cluster (Reid et al., 2009). Alternate bearing refers to heavy crop loads that impact the succeeding year's crop by reducing available nutrients resulting in decreased flower initiation or retention and low yields. Therefore, year-to-year yield stability is equally relevant to high production within a given year. Diseases such as anthracnose caused by *Gnomonia leptostyla* and thousand cankers disease caused by *Geosmithia morbida* pose potential costs for commercial growers (Blood et al., 2018).

Field and laboratory evaluations of important characteristics have helped advance the eastern black walnut improvement program by defining which cultivars in the repository would be best suited for production in Missouri. DNA fingerprinting was used in tandem with phenotypic evaluations to verify cultivar identities (Coggeshall and Woeste, 2010). These genotypic data were also used to determine the top-level hierarchical genetic structure of the collection and pair candidate breeding parents from across different genetic groups (Coggeshall, 2011; Warmund et al., 2009a, 2009b; Zhao et al., 2017). Additional insight could be derived from multivariate data analysis from past field evaluations, as described previously, from 2001 to 2015 (Coggeshall, 2011).

Persian walnut breeders have used similar germplasm characterization and multivariate analysis to organize selections from wild populations for breeding (Bernard et al., 2018; Cosmulescu and Stefanescu, 2018; Ghasemi et al., 2012). Characterization studies have included desirable nut traits, improved yields, cold tolerance, late flowering, and tolerance to walnut blight caused by *Xanthomonas arboricola* pv. *juglandis* and anthracnose (Aslantaş, 2006; Bayazit, 2012; Bükücü et al., 2020; Cosmulescu and Stefanescu, 2018; Martinez-Garcia et al., 2017; Miletić et al., 2003; Zeneli et al., 2005). Strong relationships have been found between nut weight and nut length, nut weight and nut width, and kernel weight and nut width (Arzani et al., 2008; Bujdoso and Cseke, 2021; Mahmoodi et al., 2019). Multivariate analysis of phenological and nut quality traits can reveal how variation is distributed across multiple traits, inform the strategic use of potential breeding parents, and point to outliers (Arzani et al., 2008). Principal component analysis of Persian walnut germplasm in Iran and Turkey demonstrated distinct variation patterns that governed nut/kernel weight and kernel percentage, late leafing, and early leaf drop (Arzani et al., 2008; Bükücü et al., 2020). In some cases, evaluations have led to the immediate identification of promising selections for release as new cultivars or as parents for continued genetic development (Arzani et al., 2008; Ghasemi et al., 2012; Shah et al., 2021). Such studies require a robust phenotypic dataset to determine relationships between traits and their genetic effects (Bernard et al., 2018).

The present study uses a historical phenological and nut quality characteristics dataset to evaluate genetic diversity in 54 cultivars in the UMCA eastern black walnut germplasm repository. Here, we report values for the traits and correlations among them. These data identified the most promising cultivars for propagation, orchard establishment, and use as parents for genetic improvement.

## Material and Methods

**Plant material.** In 1996, 54 eastern black walnut cultivars were each grafted using two to four ramets per cultivar at the University of Missouri Horticulture and Agroforestry Research Center (HARC), in New Franklin, MO, USA (lat. 39°01'N, long. 92°74'W). Open-pollinated seedlings from 'Thomas' were used as rootstocks in all cases. The trees were planted at 12.2 × 12.2-m spacing and were pruned and fertilized annually with best management practices applied during their establishment as recommended in Reid et al. (2009). This repository is located near the Missouri River bluffs on a ridgetop with deep, loess-derived, well-draining Menfro silt-loam soil type, which is well-suited to growing black walnut based on the Missouri black walnut soil suitability index (Wallace and Young, 2008). In 2017, cultivar identities of all trees and ramets in this population were confirmed using microsatellite markers (Zhao et al., 2017).

**Evaluation methods.** In this study, a total of 20 traits were evaluated. Between 2001 and 2008, all cultivar ramets were evaluated for phenological traits as defined by the International Plant Genetics Research Institute, Descriptors of Walnut (IPGRI, 1994): leaf budbreak date, first female bloom date, last female bloom date, first male bloom date, last male bloom date, first bloom overlap (when >50% female and male blooms are both visible on an individual tree), bloom overlap percentage (percentage of days in bloom overlap), dichogamy habit (protogynous or protandrous), harvest date, and growing season length measured as the number of days from the first female bloom until nut harvest.

As the trees began producing nuts, nut and kernel quality data were collected in 2007–10, 2013, and 2015. The nuts were harvested and processed as recommended in Reid et al. (2009) before nut and kernel quality evaluations. During these evaluations, two replications of 25 nuts were randomly selected from the total yield per ramet. Each nut was measured (millimeters) for its length, width, and thickness as described by Koyuncu et al. (2004). Nut length was measured along the longitudinal axis of the shell, nut width was the diameter from one cheek to the other cheek of the shell, and nut thickness was the diameter along the position of the nut suture. Weight (grams) was measured for each in-shell nut. Each nut was cracked using a hand nut cracker (Master Nut Cracker; Master Cracker, Sarcoxie, MO, USA). The kernels were extracted from the shell and weighed (grams). Kernel weight and nut weight were used to calculate the kernel percentage for each cultivar. The kernel percentage calculation excluded blank nuts. Kernel color was evaluated using a 1 to 5 rating scale, with 1 as the lightest and 5 as the darkest. Kernel venation was evaluated using a 1 to 5 rating scale with 1 as veins not visible and 5 as veins highly visible. The values from the two samples were averaged for analysis each year for each cultivar. In addition, precocity was evaluated using the total number of nuts produced by each cultivar between the ages of 6 to 10 years (2002–06), and the alternate bearing index (ABI) was calculated each year as: $1/(year-1)\Sigma\{(yield_{year}-yield_{year-1})/yield_{year}+yield_{year-1})\}$ (Conner and Worley, 2000; Reid et al., 2004).

**Statistical analysis.** Data collected between 2001 and 2015 were compiled and curated in 2021. Before analysis, the data underwent quality control in the R statistical environment [R (R Foundation for Statistical Computing, Vienna, Austria), R Studio (RStudio, Inc., Boston, MA, USA)]. Normality was tested for each trait using Q-Q plot and Shapiro-Wilk tests. When normality was not met, box-cox transformation was applied with the MASS package (Venables and Ripley, 2002). After these steps, the data for 54 cultivars were used for further analysis. Pearson's correlation matrix was performed to assess correlations between traits using the cor() function in R. Principal components analysis (PCA) was performed using the factoextra package in R (Kassambara and Mundt, 2020). Thirteen traits were used to generate the principal components (PC). Highly positive correlated traits were excluded from the analysis, including nut width, first female bloom, last female bloom, first male bloom, last male bloom, first bloom overlap, and bloom overlap percentage. A biplot of PC1 and PC2 was constructed using ggplot2 in R (Wickham et al., 2016).

## Results and Discussion

**Phenological dates.** Considerable variation was observed for all phenological traits, with summary statistics reported (Table 1). Mean values for leaf budbreak, dichogamy habit, and harvest date are reported for all cultivars in Supplemental Table 1. Black walnut is among the latest leafing trees in the eastern deciduous forest, as newly emerged green tissue and floral initials cannot tolerate more than a light frost (−2 °C) without sustaining damage and crop loss. Early leafing and flowering dates increase the likelihood of frost damage, resulting in reduced nut production (Reid et al., 2004); however, early leafing is correlated with precocity and greater yields in Persian walnut (Germain, 1990; Solar et al., 2001). The range in leaf budbreak was 8 Apr to 17 May. The range in date of the first female was 17 Apr to 28 May. Early leafing cultivars are vulnerable to frost in some years, with a 10% probability of occurrence in central Missouri on or after 15 Apr (Guinan and Wiebold, 2016). Average first female bloom ranged from 17 Apr to 28 May, whereas average first male bloom dates occur between 19 Apr and 31 May. The dichogamy habit revealed that 41 cultivars are protogynous, and 13 are protandrous. Harvest dates ranged from 4 Sep to 16 Oct, and average season length ranged from 97 to 175 d.

Correlations among traits are reported (Table 2). Budbreak is positively correlated ($r$) with dates of first female bloom (0.48), last female bloom (0.56), first male bloom (0.82), last male bloom (0.84), and first bloom overlap (0.82). The date of leaf budbreak was not correlated with any of the other traits studied. First female bloom date is positively correlated with last female bloom (0.94) and first bloom overlap (0.52), as one would expect, but negatively correlated with dichogamy habit (−0.36), season length (−0.32), and precocity (−0.39). Harvest date is

Table 1. Summary statistics on individual data points for phenological, horticultural, and nut quality traits collected 2001–15 from 54 eastern black walnut (*Juglans nigra*) cultivars in the University of Missouri Center for Agroforestry germplasm repository in New Franklin, MO, USA.

| Trait | Unit | Minimum | Maximum | Mean | *SD* |
|---|---|---|---|---|---|
| Budbreak | Date | 8 Apr | 19 May | – | – |
| First female bloom | Date | 17 Apr | 28 May | – | – |
| Last female bloom | Date | 28 Apr | 8 Jun | – | – |
| First male bloom | Date | 19 Apr | 31 May | – | – |
| Last male bloom | Date | 25 Apr | 6 Jun | – | – |
| First bloom overlap | Date | 24 Apr | 9 Jun | – | – |
| Bloom overlap percentage | % | 0 | 2.55 | 0.26 | 0.22 |
| Dichogamy habit | | 1 | 2 | – | – |
| Harvest date | Date | 4 Sep | 16 Oct | – | – |
| Season length | Days | 97.0 | 175.0 | 136.6 | 14.3 |
| Alternate bearing index | | 0.3 | 1.0 | 0.6 | 0.2 |
| Nut length | mm | 27.5 | 63.1 | 38.3 | 4.0 |
| Nut thickness | mm | 19.5 | 40.3 | 29.9 | 2.4 |
| Nut width | mm | 25.9 | 50.4 | 38.1 | 3.9 |
| Nut weight | g | 8.8 | 43.0 | 19.5 | 4.2 |
| Kernel weight | g | 1.1 | 8.6 | 5.3 | 1.2 |
| Kernel percentage | % | 5.3 | 39.2 | 26.8 | 5.9 |
| Precocity | No. | 16.0 | 1522.0 | 421.2 | 354.2 |
| Kernel color | Categorical | 1.0 | 4.2 | 1.9 | 0.7 |
| Kernel venation | Categorical | 0.0 | 3.5 | 1.5 | 0.6 |

Table 2. Pearson's correlation matrix of 20 phenology and kernel quality traits in 54 eastern black walnut (*Juglans nigra*) cultivars.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Budbreak | 1 | | | | | | | | | | | | | | | | | | | |
| 2. First female bloom | 0.48[ii] | 1 | | | | | | | | | | | | | | | | | | |
| 3. Last female bloom | 0.56[ii] | 0.94[ii] | 1 | | | | | | | | | | | | | | | | | |
| 4. First male bloom | 0.82[ii] | 0.13 | 0.22 | 1 | | | | | | | | | | | | | | | | |
| 5. Last male bloom | 0.84[ii] | 0.12 | 0.19 | 0.94[ii] | 1 | | | | | | | | | | | | | | | |
| 6. First bloom overlap | 0.82[ii] | 0.52[ii] | 0.62[ii] | 0.73[ii] | 0.77[ii] | 1 | | | | | | | | | | | | | | |
| 7. Bloom overlap % | 0.02 | −0.08 | −0.05 | −0.06 | 0.00 | 0.06 | 1 | | | | | | | | | | | | | |
| 8. Dichogamy habit | 0.23 | −0.36[ii] | −0.34[ii] | 0.61[ii] | 0.53[ii] | 0.24 | −0.03 | 1 | | | | | | | | | | | | |
| 9. Harvest date | 0.33 | 0.23 | 0.18 | 0.35[ii] | 0.41[ii] | 0.30[i] | 0.08 | 0.27[i] | 1 | | | | | | | | | | | |
| 10. Season length | −0.08 | −0.32[i] | −0.42[ii] | 0.11 | 0.12 | −0.15 | −0.03 | 0.48[ii] | 0.63[ii] | 1 | | | | | | | | | | |
| 11. Nut length | 0.15 | 0.03 | −0.05 | 0.16 | 0.20 | 0.17 | 0.11 | 0.22 | 0.39[ii] | 0.35[ii] | 1 | | | | | | | | | |
| 12. Nut thickness | 0.10 | −0.01 | −0.08 | 0.16 | 0.20 | 0.17 | 0.05 | 0.20 | 0.41[ii] | 0.28[i] | 0.62[ii] | 1 | | | | | | | | |
| 13. Nut width | 0.05 | −0.20 | −0.24 | 0.21 | 0.23 | 0.06 | −0.08 | 0.26 | 0.29[i] | 0.27[i] | 0.34[ii] | 0.82[ii] | 1 | | | | | | | |
| 14. Nut weight | 0.07 | −0.12 | −0.18 | 0.19 | 0.22 | 0.09 | 0.01 | 0.26 | 0.40[ii] | 0.38[ii] | 0.63[ii] | 0.92[ii] | 0.88[ii] | 1 | | | | | | |
| 15. Kernel weight | 0.21 | −0.04 | −0.07 | 0.28[i] | 0.27[i] | 0.28 | −0.06 | 0.34 | 0.24 | 0.23 | 0.21 | 0.24[i] | 0.37[ii] | 0.27[i] | 1 | | | | | |
| 16. Kernel percentage | 0.08 | 0.10 | 0.11 | 0.03 | −0.01 | 0.05 | −0.09 | 0.02 | −0.22 | −0.17 | −0.19 | −0.58[ii] | −0.53[ii] | −0.60[ii] | 0.51[ii] | 1 | | | | |
| 17. Kernel color | 0.00 | −0.25 | −0.26 | 0.00 | 0.05 | −0.07 | 0.01 | 0.13 | 0.00 | 0.07 | 0.17 | 0.07 | 0.08 | 0.05 | 0.32[ii] | 0.17 | 1 | | | |
| 18. Kernel venation | 0.22 | 0.00 | 0.02 | 0.12 | 0.20 | 0.20 | 0.07 | 0.03 | −0.18 | −0.22 | 0.24[i] | 0.05 | −0.10 | −0.02 | 0.04 | 0.12 | 0.23[i] | 1 | | |
| 19. Precocity | −0.23 | −0.39[ii] | −0.30[i] | −0.19 | −0.15 | −0.12 | −0.03 | 0.14 | −0.27 | 0.05 | 0.05 | −0.25 | −0.32[i] | −0.32[i] | 0.03 | 0.38[ii] | 0.36[ii] | 0.16 | 1 | |
| 20. Alternate bearing index | 0.04 | 0.05 | 0.04 | −0.21 | −0.28 | −0.08 | −0.07 | −0.3 | −0.15 | −0.21 | 0.09 | 0.04 | −0.08 | −0.09 | 0.18 | 0.29[i] | 0.18 | 0.09 | 0.18 | 1 |

[i] Correlation is significant at the 0.05 level.
[ii] Correlation is significant at the 0.01 level.

positively correlated with first and last male bloom (0.35 and 0.41, respectively) and season length (0.63). Knowledge of such correlations among traits informs crossing design to enhance genetic gain for multiple criteria simultaneously and inform selection strategies. Correlations can also be used as a proxy to estimate challenging to measure traits if an easier to measure trait is strongly correlated.

**Nut quality and bearing.** The range for each nut quality trait was considerable, with summary statistics reported in Table 1, including mean values for total nuts, kernel weight, kernel percentage, kernel venation, and kernel color in cultivars of the University of Missouri eastern black walnut repository. Average kernel percentage and weight ranged from 5.5% to 39.2% and 1.1 to 8.6 g, respectively. The average nut length, width, and thickness ranges were from 27.5 to 63.1 mm, 25.9 to 50.4 mm, and 19.5 to 40.3 mm, respectively. The total number of nuts produced between 2002 and 2006 (precocity) ranged from 16 to 1522 total number of nuts produced by each cultivar. Kernel color was assessed on a scale of 1 (lightest) to 5 (darkest), with average kernel color ranging from 1 to 4.2.

Correlations among nut quality and bearing characteristics are reported (Table 2). Nut weight was positively correlated with nut length (0.63), nut thickness (0.92), and nut width (0.88), suggesting nut length, nut width, and nut thickness each as parameters for increasing nut weight. The correlation between nut weight and nut length, nut width, and nut thickness has also been reported in Persian walnut, which as a species has undergone prolonged selection pressure through years of cultivation (Bujdoso and Cseke, 2021; Mahmoodi et al., 2019). Nut weight also showed a weak positive correlation with kernel weight (0.27) and a strong negative correlation with kernel percentage (−0.60), which are similar to results from Amiri et al. (2010), wherein Persian walnut kernel percentage was positively correlated with kernel weight (0.22), but negatively correlated with nut weight (−0.36). These data suggest that nut weight is an imperfect proxy for increasing kernel weight, and the two characters do not increase proportionally. This distinction is possibly a function of thicker nutshells (not measured) or the physiological requirements and resources needed to fill the internal chambers of the shell with the kernel. Kernel percentage displayed additional strong negative correlations with nut thickness (−0.58) and nut width (−0.53), although interestingly, there was no significant relationship with nut length (−0.19).

Also, kernel percentage, ABI (0.29), and precocity (0.38) were positive correlations. These results suggest the potential to cull trees that display low precocity, as it is likely that they will also exhibit a low kernel percentage. This proactive culling will reduce the number of offspring that need to be assessed for kernel quality and improve breeding resource use. Kernel weight also demonstrated an important relationship with kernel color (0.32), as well as with first and last male bloom (0.28 and 0.27, respectively), nut thickness (0.24), and nut width (0.37). Precocity was positively correlated with kernel color (0.36) but negatively with nut weight (−0.32). Season length was positively correlated with dichogamy habit (0.48) but negatively with first and last female bloom (−0.32 and −0.42, respectively). Harvest date and season length positively correlated with nut weight (0.40 and 0.38) and nut length (0.39 and 0.35). Harvest date was also positively correlated with nut thickness (0.41).

The principal focus of the UMCA eastern black walnut breeding program is to develop new cultivars with spur-bearing, precocity, thinner shells, and increased kernel weight and kernel percentage. An important threshold is a kernel percentage greater than 30%, and there is potential for gain well beyond this threshold. Persian walnut cultivars can exceed kernel percentages of 60% (Ebrahimi et al., 2011; Shah et al., 2021). Challenges might arise in simultaneously improving kernel weight and kernel percentage. However, cultivars with high kernel weight and kernel percentage exist in the UMCA collection (e.g., Pound #2, Daniel), and indirect selection parameters might facilitate future genetic gain for both traits simultaneously (e.g., nut length). Kernel color is also important, as darker kernels are associated with rancidity and are less desired by consumers (Reid 1990; Warmund et al., 2009a). However, unlike Persian walnut, nuts of eastern black walnut mature within indehiscent husks. The timely harvest of the mature nuts followed by prompt removal of the husks is required to maximize kernel color attributes (Reid et al., 2009). High precocity, as often seen on spur-bearing trees, is also essential to substantially increase returns with high production in the first 10 to 15 years. Fortunately, these characters show potential for improvement simultaneously to kernel weight and percentage.

**Ranking.** Average overall nut quality (kernel weight and percentage) and precocity were weighted equally and combined in a ranking system based on percentile scores (Table 3). The maximum value for the sum of the percentiles is 3.0. 'Emma K' had the highest total percentile score (2.75). 'Thomas Meyer' had the highest kernel weight (7.52 g), which falls into the range of Persian walnut cultivars. Cultivars above the 85th percentile were Emma K, Sparks 127, Daniel, Thomas Meyer, Sparks 147, Pound #2, Football, and Kwik Krop. Breeding pedigrees at UMCA have thus far incorporated 'Sparks 127' and 'Daniel', and these data offer direction for how to diversify the parental founders. The cultivar with the highest kernel percentage and kernel weight of 5.60 g was Sparks 147, which has an index of 2.32. Jackson was the most precocious cultivar, producing more than 1500 nuts during the evaluation period; however, it indexed at 2.19, a score lowered by its moderate kernel weight (4.85 g).

**Principal component analysis.** PCA in plant breeding is used to reduce data complexity across a large set of variables, directing focus to the main differences among genotypes. This approach has been used in recent Persian walnut improvement studies to assess the diversity and relationships among phenology and nut traits in breeding populations and cultivars (Bükücü et al., 2020; Bujdoso and Cseke, 2021; Shah et al., 2021). PCA is a useful tool for genetic improvement as it can inform crossing scheme design and selection strategies.

In this study, the first five components combined (Table 4) have eigenvalues greater than 1.00 and account for 77.3% of variation. PC1 explains 24.1% of variation with a strong contribution from nut weight, nut thickness, and harvest date. PC2 explains 19.3% of variation and has contributions from kernel percentage, kernel weight, nut length, kernel color, and kernel venation, all of which share directionality. PC3, with an eigenvalue of 1.84, explains 14.2% of variation, and includes contributions from precocity, season length, dichogamy habit, and alternate bearing.

Biplot analysis was performed on PC1 and PC2 to visualize relationships between variables' eigenvector loadings and directionality. Smaller angles indicate stronger relationships or shared variation (Fig. 1). Kernel percentage and precocity share similar eigenvectors, which have similar directionality to those of ABI,

Table 3. Overall nut and precocity ranking for 54 eastern black walnut (*Juglans nigra*) cultivars from historic data collected between 2001 and 2015.

| | Kernel wt (g) | | Kernel percentage (%) | | Total nuts (no.)[i] | | |
|---|---|---|---|---|---|---|---|
| Cultivar | Avg | Percentile | Avg | Percentile | Avg | Percentile | Total percentile |
| Emma K | 5.9 | 0.81 | 34.5 | 0.96 | 1473.0 | 0.98 | 2.75 |
| Sparks 127 | 5.6 | 0.75 | 31.7 | 0.89 | 988.2 | 0.94 | 2.58 |
| Daniel | 6.9 | 0.98 | 30.5 | 0.81 | 482.6 | 0.72 | 2.51 |
| Thomas Meyer | 7.5 | 1.00 | 33.0 | 0.91 | 404.4 | 0.57 | 2.48 |
| Sparks 147 | 5.6 | 0.74 | 35.0 | 1.00 | 412.5 | 0.58 | 2.32 |
| Pound #2 | 6.3 | 0.91 | 34.5 | 0.98 | 258.0 | 0.42 | 2.31 |
| Football | 6.1 | 0.87 | 26.3 | 0.51 | 870.5 | 0.89 | 2.27 |
| Kwik Krop | 5.5 | 0.72 | 31.6 | 0.87 | 413.1 | 0.60 | 2.19 |
| Jackson | 4.8 | 0.25 | 34.1 | 0.94 | 1522 | 1.00 | 2.19 |
| Neel | 6.3 | 0.89 | 30.1 | 0.75 | 360.0 | 0.53 | 2.17 |
| Surprise | 6.0 | 0.83 | 28.3 | 0.64 | 454.6 | 0.70 | 2.17 |
| Harc-Thomas | 6.1 | 0.85 | 29.1 | 0.74 | 328.0 | 0.49 | 2.08 |
| South Fork | 5.4 | 0.64 | 26.6 | 0.57 | 870.0 | 0.87 | 2.08 |
| Sparrow | 5.1 | 0.51 | 28.0 | 0.62 | 761.5 | 0.83 | 1.96 |
| Schessler | 5.3 | 0.58 | 26.6 | 0.55 | 618.2 | 0.79 | 1.92 |
| Bowser | 5.0 | 0.47 | 31.2 | 0.85 | 373.3 | 0.55 | 1.87 |
| Harney | 6.6 | 0.94 | 33.1 | 0.92 | 16.0 | 0.00 | 1.86 |
| Clermont(L) | 5.7 | 0.77 | 24.8 | 0.45 | 435.0 | 0.64 | 1.86 |
| Clermont(W) | 5.7 | 0.79 | 23.1 | 0.32 | 453.0 | 0.68 | 1.79 |
| Tomboy | 4.9 | 0.40 | 23.2 | 0.36 | 1160.3 | 0.96 | 1.72 |
| Brown Nugget | 4.2 | 0.11 | 28.5 | 0.68 | 977.0 | 0.92 | 1.71 |
| Mystry | 4.8 | 0.26 | 26.6 | 0.58 | 766.0 | 0.85 | 1.69 |
| Crosby | 4.9 | 0.42 | 23.1 | 0.34 | 973.0 | 0.91 | 1.67 |
| Beck | 5.0 | 0.43 | 30.6 | 0.83 | 190.0 | 0.34 | 1.60 |
| Kitty | 5.1 | 0.49 | 30.3 | 0.77 | 138.0 | 0.26 | 1.52 |
| Thomas | 5.4 | 0.62 | 22.0 | 0.13 | 566.5 | 0.75 | 1.50 |
| Cooksey | 2.7 | 0.00 | 28.8 | 0.70 | 577.0 | 0.77 | 1.47 |
| Hare | 5.2 | 0.55 | 22.7 | 0.26 | 426.0 | 0.62 | 1.43 |
| Ridgeway | 6.9 | 0.96 | 22.2 | 0.19 | 153.0 | 0.28 | 1.43 |
| McGinnis | 4.6 | 0.19 | 28.9 | 0.72 | 351.5 | 0.51 | 1.42 |
| Grundy | 5.2 | 0.57 | 22.0 | 0.15 | 435.0 | 0.66 | 1.38 |
| Cranz | 4.4 | 0.15 | 30.4 | 0.79 | 271.5 | 0.43 | 1.37 |
| Scrimger | 4.9 | 0.30 | 28.0 | 0.60 | 300.0 | 0.45 | 1.35 |
| Davidson | 4.9 | 0.32 | 22.7 | 0.28 | 522.8 | 0.74 | 1.34 |
| Cochrane | 3.0 | 0.02 | 25.8 | 0.49 | 667.0 | 0.81 | 1.32 |
| Ogden | 5.5 | 0.68 | 24.7 | 0.43 | 121.0 | 0.21 | 1.32 |
| Higbee Mill | 5.4 | 0.66 | 22.8 | 0.30 | 176.0 | 0.32 | 1.28 |
| Mintel | 5.5 | 0.70 | 24.6 | 0.42 | 82.0 | 0.09 | 1.21 |
| Shreve | 6.5 | 0.92 | 20.4 | 0.09 | 94.0 | 0.15 | 1.16 |
| Russel #3 | 4.9 | 0.36 | 23.6 | 0.40 | 191.0 | 0.36 | 1.12 |
| Teneyck | 4.5 | 0.17 | 23.4 | 0.38 | 327.0 | 0.47 | 1.02 |
| Wiard | 4.3 | 0.13 | 28.3 | 0.66 | 96.0 | 0.17 | 0.96 |
| Krause | 4.9 | 0.38 | 22.5 | 0.23 | 137.5 | 0.25 | 0.86 |
| Pritchett | 4.9 | 0.28 | 25.1 | 0.47 | 59.0 | 0.06 | 0.81 |
| Elmer Myer | 4.8 | 0.23 | 26.5 | 0.53 | 49.6 | 0.04 | 0.80 |
| Russel #1 | 5.3 | 0.60 | 21.9 | 0.11 | 75.5 | 0.08 | 0.79 |
| Ness | 4.9 | 0.34 | 22.3 | 0.21 | 123.0 | 0.23 | 0.78 |
| Knuvean | 4.7 | 0.21 | 22.0 | 0.17 | 239.5 | 0.40 | 0.78 |
| Philops | 5.1 | 0.53 | 15.9 | 0.00 | 101.5 | 0.19 | 0.72 |
| Dubois | 5.0 | 0.45 | 19.2 | 0.06 | 49.0 | 0.02 | 0.53 |
| Rupert | 3.1 | 0.06 | 19.3 | 0.08 | 212.0 | 0.38 | 0.52 |
| OK Selection | 3.5 | 0.09 | 17.4 | 0.04 | 160.0 | 0.30 | 0.43 |
| Purdue #41 | 3.0 | 0.04 | 22.5 | 0.25 | 89.5 | 0.13 | 0.42 |
| Purdue #137 | 3.2 | 0.08 | 16.1 | 0.02 | 88.0 | 0.11 | 0.21 |

[i] Total number of nuts produced by each cultivar between the ages of 6 to 10 years (2002–06).

Table 4. Eigenvector loadings in principal components (PCs) of 13 phenological, horticultural, and nut quality traits for eastern black walnut (*Juglans nigra*) cultivars in the University of Missouri Center for Agroforestry germplasm repository in New Franklin, MO, USA.

| Trait | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| Kernel percentage | −0.32 | 0.35 | −0.17 | −0.34 | 0.00 |
| Kernel weight | 0.21 | 0.42 | −0.12 | −0.05 | 0.17 |
| Precocity | −0.28 | 0.19 | −0.35 | 0.33 | −0.02 |
| Nut thickness | 0.48 | −0.01 | 0.16 | 0.28 | 0.12 |
| Nut weight | 0.51 | 0.01 | 0.07 | 0.29 | 0.11 |
| Nut length | 0.22 | 0.45 | −0.03 | 0.00 | −0.17 |
| Kernel color | −0.07 | 0.35 | −0.09 | 0.44 | −0.21 |
| Kernel venation | −0.06 | 0.45 | 0.05 | 0.12 | 0.22 |
| Alternate bearing index | −0.08 | 0.22 | 0.38 | 0.07 | −0.44 |
| Budbreak | 0.18 | 0.26 | 0.21 | −0.48 | 0.23 |
| Dichogamy habit | 0.10 | −0.02 | −0.53 | −0.02 | 0.48 |
| Harvest date | 0.36 | 0.06 | −0.21 | −0.41 | −0.40 |
| Season length | 0.22 | −0.12 | −0.54 | 0.00 | −0.44 |
| | | | | | |
| Eigenvalue | 3.14 | 2.51 | 1.84 | 1.41 | 1.15 |
| Variance % | 24.12 | 19.27 | 14.16 | 10.87 | 8.85 |
| Cumulative variance % | 24.12 | 43.39 | 57.55 | 68.42 | 77.27 |

kernel venation, and kernel color. Interestingly, kernel percentage is nearly directionally opposed to nut weight with an obtuse relationship. Nut weight loads tightly with nut thickness and similarly with harvest date and season length. Interestingly, eigenvectors for nut weight and kernel weight display a relatively wide angle. Further, kernel weight is orthogonal to kernel percentage, suggesting different variation explains these three traits and that increasing nut weight is not a linear route for achieving simultaneous genetic gain in both kernel weight and kernel percentage. The tight relationship between kernel weight and nut length provides a possible indirect selection parameter, which might prove helpful in selecting individuals with both high kernel weight and kernel percentage.

## Conclusions

The UMCA eastern black walnut repository provides a critical resource for long-term breeding efforts. Eastern black walnut orchard production will benefit from planting cultivars with improved nut quality and yield through improving returns to growers and facilitating industry growth in the midwestern United States. Efforts to develop cultivars with precocity, spur-bearing habit, and exceptional nut quality (higher kernel percentage, larger kernels,

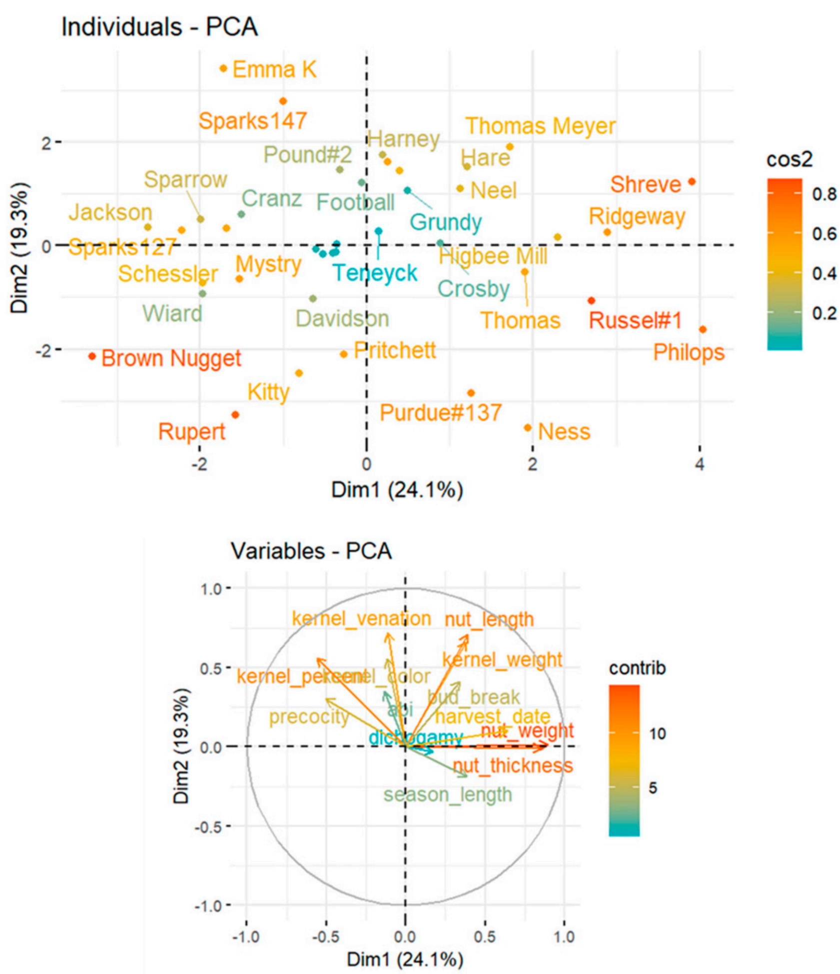


Fig. 1. Genetic variation for phenological and morphological characters of eastern black walnut (*Juglans nigra*) cultivars in the University of Missouri repository (New Franklin, MO, USA) as shown through the individual principal component analysis [PCA (top)] and variables PCA (bottom).

easier cracking, and lighter color) remain the primary target of the UMCA program. A comprehensive analysis of the historical phenological and nut quality data can give breeders a deeper understanding of available phenotypic diversity. The UMCA black walnut cultivars described here represent a breadth of variation for commercially important traits and can be readily used as parents. Trait relationships can guide breeding decisions, especially those associated with precocity and kernel quality since these are strongly related. Another potential proxy for precocity and kernel percentage is budbreak, which could even be assessed in the nursery early in the life of the trees. Immediate goals for the UMCA eastern black walnut breeding program will be to design crossing schemes based on the results presented here. Such information will be applied to the evaluation of selections and development of new full-sibling populations that were started in 2020.

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Supplemental Table 1. Mean values for leaf budbreak, dichogamy habit, harvest date, total nuts, kernel weight, kernel percentage, kernel venation, and kernel color in cultivars of the University of Missouri eastern black walnut (*Juglans nigra*) repository in New Franklin, MO, USA.

| Cultivar | Leaf budbreak | Dichogamy habit | Harvest date | Total nuts | Alternate bearing index | Kernel wt (g) | Kernel percentage (%) | Kernel venation | Kernel color |
|---|---|---|---|---|---|---|---|---|---|
| Beck | 26 Apr | Protogynous | 25 Sep | 190.0 | 0.55 | 5.0 | 30.6 | 1.5 | 1.4 |
| Bowser | 29 Apr | Protogynous | 19 Sep | 373.3 | 0.73 | 5.0 | 31.2 | 1.3 | 1.2 |
| Brown Nugget | 16 Apr | Protogynous | 9 Sep | 977.0 | 0.51 | 4.2 | 28.5 | 1.4 | 1.4 |
| Clermont(L) | 30 Apr | Protogynous | 29 Sep | 435.0 | 0.72 | 5.7 | 24.8 | 1.7 | 1.5 |
| Clermont(W) | 30 Apr | Protogynous | 26 Sep | 453.0 | 0.68 | 5.7 | 23.1 | 1.6 | 1.1 |
| Cochrane | 15 Apr | Protandrous | 8 Sep | 667.0 | 0.60 | 3.0 | 25.8 | 1.2 | 1.1 |
| Cooksey | 20 Apr | Protandrous | 12 Sep | 577.0 | 0.83 | 2.7 | 28.8 | 1.2 | 1.5 |
| Cranz | 29 Apr | Protandrous | 27 Sep | 271.5 | 0.75 | 4.4 | 30.4 | 2.0 | 1.2 |
| Crosby | 1 May | Protogynous | 2 Oct | 973.0 | 0.56 | 4.9 | 23.1 | 1.7 | 1.4 |
| Daniel | 26 Apr | Protogynous | 22 Sep | 482.6 | 0.79 | 6.9 | 30.5 | 2.0 | 1.6 |
| Davidson | 15 Apr | Protogynous | 10 Sep | 522.8 | 0.66 | 4.9 | 22.7 | 2.0 | 1.4 |
| Dubois | 20 Apr | Protogynous | 25 Sep | 49.0 | 0.47 | 5.0 | 19.2 | 1.4 | 1.5 |
| Elmer Myer | 1 May | Protogynous | 20 Sep | 49.6 | 0.69 | 4.8 | 26.5 | 1.6 | 1.5 |
| Emma K | 16 Apr | Protogynous | 24 Sep | 1473.0 | 0.76 | 5.9 | 34.5 | 2.8 | 1.8 |
| Football | 15 Apr | Protogynous | 27 Sep | 870.5 | 0.77 | 6.1 | 26.3 | 2.6 | 1.9 |
| Grundy | 19 Apr | Protandrous | 18 Sep | 435.0 | 0.85 | 5.2 | 22.0 | 1.7 | 1.8 |
| Harc- Thomas | 16 Apr | Protogynous | 23 Sep | 328.0 | 0.78 | 6.1 | 29.1 | 1.2 | 1.2 |
| Hare | 29 Apr | Protogynous | 30 Sep | 426.0 | 0.57 | 5.2 | 22.7 | 2.8 | 1.7 |
| Harney | 26 Apr | Protogynous | 26 Sep | 16.0 | 0.48 | 6.6 | 33.1 | 1.6 | 2.0 |
| Higbee Mill | 5 May | Protogynous | 2 Oct | 176.0 | 0.80 | 5.4 | 22.8 | 1.3 | 1.7 |
| Jackson | 15 Apr | Protogynous | 25 Sep | 1522.0 | 0.60 | 4.8 | 34.1 | 2.2 | 2.0 |
| Kitty | 14 Apr | Protogynous | 21 Sep | 138.0 | 0.58 | 5.1 | 30.3 | 1.0 | 1.1 |
| Knuvean | 18 Apr | Protandrous | 29 Sep | 239.5 | 0.80 | 4.7 | 22.0 | 1.5 | 1.4 |
| Krause | 16 Apr | Protandrous | 13 Sep | 137.5 | 0.74 | 4.9 | 22.5 | 1.9 | 1.6 |
| Kwik Krop | 25 Apr | Protogynous | 26 Sep | 413.1 | 0.55 | 5.5 | 31.6 | 1.7 | 1.6 |
| McGinnis | 16 Apr | Protandrous | 10 Sep | 351.5 | 0.76 | 4.6 | 28.9 | 1.3 | 1.1 |
| Mintel | 17 Apr | Protandrous | 25 Sep | 82.0 | 0.47 | 5.5 | 24.6 | 1.9 | 1.5 |
| Mystry | 18 Apr | Protogynous | 19 Sep | 766.0 | 0.66 | 4.8 | 26.6 | 1.9 | 1.5 |
| Neel | 2 May | Protogynous | 1 Oct | 360.0 | 0.78 | 6.3 | 30.1 | 1.5 | 1.6 |
| Ness | 16 Apr | Protogynous | 9 Oct | 123.0 | 0.74 | 4.9 | 22.3 | 1.0 | 1.3 |
| Ogden | 22 Apr | Protogynous | n/a | 121.0 | 0.60 | 5.5 | 24.7 | 1.6 | 0.7 |
| OK Selection | 20 Apr | Protogynous | 17 Oct | 160.0 | 0.51 | 3.5 | 17.4 | 1.1 | 1.4 |
| Philops | 22 Apr | Protogynous | 24 Sep | 101.5 | 0.65 | 5.1 | 15.9 | 2.0 | 1.0 |
| Pound #2 | 24 Apr | Protogynous | 29 Sep | 258.0 | 0.92 | 6.3 | 34.5 | 1.7 | 1.6 |
| Pritchett | 18 Apr | Protogynous | 24 Sep | 59.0 | 0.48 | 4.9 | 25.1 | 1.5 | 1.2 |
| Purdue #137 | 22 Apr | Protogynous | 2 Oct | 88.0 | 0.45 | 3.2 | 16.1 | 1.3 | 1.4 |
| Purdue #41 | 30 Apr | Protogynous | 28 Sep | 89.5 | 0.45 | 3.0 | 22.5 | 1.5 | 1.2 |
| Ridgeway | 19 Apr | Protogynous | 25 Sep | 153.0 | 0.64 | 6.9 | 22.2 | 2.0 | 1.1 |
| Rupert | 18 Apr | Protogynous | 20 Sep | 212.0 | 0.71 | 3.1 | 19.3 | 1.6 | 1.0 |
| Russel #1 | 21 Apr | Protogynous | 2 Oct | 75.5 | 0.59 | 5.3 | 21.9 | 1.6 | 1.2 |
| Russel #3 | 16 Apr | Protogynous | 24 Sep | 191.0 | 0.25 | 4.9 | 23.6 | 1.8 | 1.4 |
| Schessler | 13 Apr | Protogynous | 5 Sep | 618.2 | 0.71 | 5.3 | 26.6 | 1.7 | 1.5 |
| Shreve | 1 May | Protogynous | 2 Oct | 94.0 | 0.78 | 6.5 | 20.4 | 1.6 | 2.0 |
| South Fork | 13 Apr | Protogynous | 8 Oct | 870.0 | 0.76 | 5.4 | 26.6 | 1.7 | 1.1 |
| Sparks 127 | 23 Apr | Protogynous | 10 Sep | 988.2 | 0.71 | 5.6 | 31.7 | 1.6 | 1.3 |
| Sparks 147 | 2 May | Protogynous | 26 Sep | 412.5 | 0.68 | 5.6 | 35.0 | 2.1 | 1.9 |
| Sparrow | 26 Apr | Protogynous | 10 Sep | 761.5 | 0.58 | 5.1 | 28.0 | 2.1 | 1.9 |
| Surprise | 24 Apr | Protogynous | 29 Sep | 454.6 | 0.71 | 6.0 | 28.3 | 2.2 | 1.8 |
| Teneyck | 3 May | Protogynous | 18 Sep | 327.0 | 0.70 | 4.5 | 23.4 | 1.0 | 1.7 |
| Thomas | 1 May | Protogynous | 1 Oct | 566.5 | 0.68 | 5.4 | 22.0 | 1.7 | 1.2 |
| Thomas Meyer | 3 May | Protogynous | 28 Sep | 404.4 | 0.70 | 7.5 | 33.0 | 1.7 | 1.9 |
| Tomboy | 18 Apr | Protogynous | 15 Sep | 1160.3 | 0.76 | 4.9 | 23.2 | 1.7 | 1.7 |
| Wiard | 27 Apr | Protandrous | 24 Sep | 96.0 | 0.76 | 4.3 | 28.3 | 1.4 | 1.1 |

n/a = not applicable.