Heritability Estimates of L*a*b* Color Space Values in Winter Squash (*Cucurbita* spp.)

Rachel A. Itle, Eileen A. Kabelka, and James W. Olmstead

Horticultural Sciences Department, Institute of Food and Agricultural Sciences, University of Florida, P.O. Box 110690, 1253 Fifield Hall, Gainesville, FL 32611

Additional index words. broad-sense heritability, chroma, colorimeter, *Cucurbita moschata*, *Cucurbita pepo*, hue angle, mesocarp

Abstract. Carotenoids serve as protective antioxidants, and function in normal vision, bone growth, cell division and differentiation, and reproduction. Winter squash (*Cucurbita* spp.) is an excellent dietary source of carotenoids. The range of colors from yellow to red in *Cucurbita* species indicates that increasing carotenoid levels through plant breeding is possible. The objective of this research was to determine the heritability of flesh color in winter squash in both *Cucurbita moschata* Duchesne and *Cucurbita pepo* L. Segregating families representing F₂, BC_{1P1} and BC_{1P2} populations were created in two families of *C. pepo* ('Table Gold Acorn' × PI 314806 and 'Table King Bush' × PI 314806) and one family of *C. moschata* ('Butterbush' × 'Sucrine DuBerry'). Broad-sense heritabilities were calculated for the F₂, BC_{1P1}, and BC_{1P2} populations within each of the three families. Heritabilities ranged from 0.19 to 0.82 for L*, 0.28 to 0.97 for chroma, and 0.12 to 0.87 for hue across all families. Transgressive segregation for color space values L* was identified in the 'Table King Bush' × PI 314806 *C. pepo* population. Our results indicate that it is possible to breed for improved flesh color in *Cucurbita*, but the population size and number of test locations for evaluation need to be increased to provide better heritability estimates. *Cucurbita* species are grown throughout the world and their availability and low price makes them an important potential source of carotenoids for human nutrition and health for all ages.

The mesocarp tissue (flesh) of squash (*Cucurbita* spp.) genotypes ranges from white to orange, resulting from variation in the presence and concentrations of different carotenoid pigments. Carotenoids contribute to the nutritional value of squash (Gross, 1991), as an antioxidant source that protects the body against cancer and degenerative diseases (Murkovic et al., 2002). Certain carotenoids within squash can also serve as a source of provitamin A (Gross, 1991), a precursor of vitamin A available in plant tissues, which functions to promote normal vision, cell division and differentiation, and reproduction (ODS/NIH, 2013).

In developing countries, blindness, stunted growth, and mortality can be produced by the deficiency of vitamin A, especially in young children and pregnant women. Carotenoids also have been shown to improve cognitive function in preadolescent children and older adults. Among other known benefits from carotenoid consumption include improved cardiovascular and bone health, sun protection, weight management, and improved visual and brain development in infants (Eggersdorfer and Wyss, 2018). Carotenoids are important for human nutrition and health for all ages, and their availability in *Cucurbita* species make them an important and easily accessible source.

Bonina-Noseworthy et al. (2016) published a survey of carotenoid concentrations of different cultigens of winter squash grown in New Hampshire. It was reported by the authors that the major carotenoids observed were lutein, zeaxanthin, and β-carotene (41% to 63% of total carotenoid concentration) and flavoxanthin and neoxanthin (37% to 59% of total carotenoid concentration) in *C. maxima* hybrids. Similarly, lutein and β-carotene were the highest observed in three inbred lines of *C. moschata* and 'Waltham Butter-nut' cultigens, with violaxanthin and neoxanthin comprising 14% to 29% of the total carotenoid concentration for these cultigens. A small amount of α-carotene was detected in *C. moschata*, but was not observed in *C. maxima* cultigens.

Several studies have examined the genes and the inheritance of rind color within the *Cucurbita* genus, and are reported in the current species gene list (Paris and Padley, 2014), and in Ercan et al. (2012). To date, there have been only three pairs of alleles that have been reported to condition flesh color within *Cucurbita*. The first is the *Wf* (white flesh) gene in *C. pepo* L., which is dominant to *wf* (colored flesh) (Sinnott and Durham, 1922) and is complementary to *W* (weak fruit coloration) for white fruit rind color (Paris, 1995). The second is the *B* (bicolor) gene, dominant to *b* (green fruit coloration, previously known as *B*⁺), which can affect both the rind and the flesh color of squash fruit in *C. pepo*. The presence of *B* gene produces a precocious yellow pigmentation in the fruit rind that occurs before anthesis, unlike normal fruit yellowing which transitions from green to yellow after anthesis (Shifriss, 1988). The *B* gene, which was introduced into *C. moschata* from *C. pepo* using backcross breeding, has also been demonstrated to alter the flesh coloration from light yellow to light orange in winter squash (*C. moschata* Duchesne) (Paris et al., 1985).

The *B* gene has been noted to have variable phenotypic expression depending upon the environment, genetic background, and the combination of both. Color variation within fruit on the same plant and degree of precociousness across environments has also been noted in *Bb* heterozygotes of different genetic backgrounds and *B* allele strength, dosage, and presence of modifier genes have been hypothesized to affect the expression of the *B* alleles (Shifriss, 1981; Shifriss and Paris, 1981).

The third gene controlling color in *Cucurbita* is the *I-2* (light fruit coloration-2) gene, which is recessive to *L-2* (intense and dark fruit coloration). The dominant *B* and *L-2* alleles have complementary gene interaction in the fruit flesh in *C. pepo* (Globerson, 1969; Paris, 1988, 2002; Paris and Nerson, 1986) and in *C. maxima* (Lopez-Anido et al., 2003). This interaction alters flesh color from a light yellow to an intense orange color. The *B* gene of *C. maxima* *B*^{max} (Paris and Padley, 2014) was identified to have a stronger effect than the *B* gene of *C. pepo*, in depleting chlorophyll in the fruit, which may be caused by an additional linked gene which activates *B* expression (Shifriss et al., 1989). In *C. pepo*, the presence of the dominant *L-2* allele was reported to double the carotenoid content, while the presence of both the dominant *L-2* and *B* alleles were reported to increase carotenoid content by up to 15 times. This suggests that the *B* gene may be a regulatory element in the isoprenoid pathway (Tadmor et al., 2005).

Inheritance of rind and flesh color in squash was previously examined in *C. pepo* by Paris (1988) with a cross between a pale flesh variety 'Vegetable Spaghetti', *b/b l-2/l-2*, and a precocious orange flesh variety 'Precocious Fordhook Zucchini', *B/B L-2/L-2*. The F₂ population and the BC₁ populations segregated at a 9:7 and a 3:1 ratio for orange and pale fleshed fruits, respectively. These segregation ratios fit a complementary dominant

Received for publication 30 Apr. 2021. Accepted for publication 16 Oct. 2021.

Published online 10 January 2022.

We thank Kristen N. Young, Lauren B. Coleman, and Dario J. Chavez for their help in field and laboratory work. We also thank James C. Colee from the University of Florida Institute of Food and Agricultural Sciences Statistics Department for help in data analysis, and José X. Chaparro and Linda Wessel-Beaver for their help in data analysis and manuscript review.

Current address for R.A.I.: Department of Horticulture, College of Agricultural & Environmental Sciences, University of Georgia, Cowart Building, 210 Cowart Street, Griffin, GA 30223

R.A.I. is the corresponding author. E-mail: ritle@uga.edu.

This is an open access article distributed under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

two gene model. Other observations suggest inheritance of flesh color of squash is influenced by several genetic and environmental factors (Shifriss, 1981; Shifriss and Paris, 1981). Similarly, Sinnott and Durham (1922) reported that flesh color was easily classified into color categories; however, the intermediate forms of each color category were difficult to classify. In addition, research has shown a complex control of carotenoid biogenesis and variability in the expression of carotenoid genes in squash (Nakkanong et al., 2012; Obrero et al., 2013; Zhang et al., 2014), which likely is the result of the complex quantitative nature of color in squash.

Paris (1994) reviewed the advances made through plant breeding for increase in carotenoid content in cucurbits. Pedigree selection has been used to introgress major alleles affecting carotenoid content as previously described. Another method used has been backcross breeding to introgress major alleles related to carotenoid content in different genetic backgrounds. For example, Paris (1994) described the gain obtained for the cultigen ‘Spookie’ by backcrossing six generations to a source of *B* allele in terms of an increase in carotenoid content.

For intermediate color classifications that are difficult to categorize using subjective scoring, a method or tool that could be used to aid in discerning different categories would be useful. More precise objective color measurements can be made using a colorimeter, which is a tool that provides numeric color values based on an xyz coordinate system that can be converted into the widely used tristimulus CIE $L^*a^*b^*$ color space values. L^* (lightness) ranges from black (0) to white (100). Color space values a^* and b^* are color directions. For color space value a^* , positive values are in the red direction and negative values are in the green direction. For color space value b^* , positive values are in the yellow direction and negative values are in the blue direction. Chroma and hue are calculated using color space values a^* and b^* . Chroma (saturation or vividness) is chromaticity, which becomes more intense when color increases; as it decreases, color becomes duller. Hue (tint of color) is an angular measurement where 0° = red, 45° = orange-red, 90° = yellow, 180° = green, and 270° = blue.

The CIE $L^*a^*b^*$ color space values may be used as an indirect method of determining the levels of pigments present in plant tissues that produce various colorations. These objective color values have been correlated with carotenoids levels, which are the main compounds responsible for the determination of the flesh color in squash fruit (Itle and Kabelka, 2009). In this study, $L^*a^*b^*$ color space values were correlated with the major carotenoids present in squash (*C. moschata* and *C. pepo*) flesh: lutein [L^* ($r = -0.68$), a^* ($r = 0.84$), b^* ($r = 0.87$), chroma ($r = 0.87$), and hue ($r = -0.80$)], α -carotene [a^* ($r = 0.70$) and hue ($r = -0.62$)], β -carotene [a^* ($r = 0.77$) and hue ($r = -0.69$)], and total carotenoids [L^* ($r = -0.66$), a^* ($r = 0.91$), b^* ($r = 0.75$), chroma ($r = 0.76$), and hue ($r = -0.83$)].

Additional studies have also identified correlations between color space values and carotenoid content in *Cucurbita* flesh. In *C. maxima*, total carotenoid content was correlated with a^* , b^* , and Chroma, ($r = 0.76$ – 0.77) and with L^* ($r = -0.53$). Beta-carotene was also correlated with a^* , b^* , and Chroma, ($r = 0.66$ – 0.77) and with L^* ($r = -0.54$) (Seroczyńska et al., 2006). In *C. pepo*, lutein and total carotenoid content were both moderately correlated with b^* in the mesocarp tissue, $r = 0.61$ and $r = 0.58$, respectively (Martínez-Valdivieso et al., 2015). Furthermore, measurements of $L^*a^*b^*$ color space values have been related to carotenoid content in crops and food products including mangoes (Omela-Paz et al., 2008), apricots (Dóka et al., 2013), lemons (Conesa et al., 2019), olive oil (Moyano et al., 2008), orange juice (Meléndez-Martínez et al., 2003), goji berries (Patsilnakos et al., 2018), and cassava (Afonso et al., 2017), among others.

In squash, colorimeter values can be a useful tool in plant breeding to quickly and objectively screen populations and germplasm for a desired flesh coloration and nutritional value in terms of carotenoid content (Itle and Kabelka, 2009; Martínez-Valdivieso et al., 2015; Seroczyńska et al., 2006). Generally, squash is bred to be sold as varieties and hybrid seed. Squash is naturally outcrossing and varieties are developed by selection in segregating populations followed by inbreeding (self-pollination). Varieties tend to be highly homogenous and highly homozygous, and can be released as open pollinated varieties or inbred lines. Hybrid seed tends to be highly homogenous and highly heterozygous. The seed is normally produced by selecting two inbred lines that have traits of interest to cross them and to produce hybrids that will share superior characteristics of both parents. A colorimeter may enable faster and more accurate selection of desirable individuals at various stages in a breeding program for flesh color.

In addition, knowledge of the heritability of flesh color genes would give the plant breeder an idea of the population size and time frame needed to manipulate flesh color in squash. To aid in the color classification of intermediate color categories difficult to subjectively identify with the naked eye and to estimate heritability, the use of a colorimeter was tested in *Cucurbita* spp. segregating populations. To date, there are no known reported population studies examining the heritability estimates of genes controlling flesh color in squash (*C. moschata* and *C. pepo*). The objective of this study was to estimate the heritability of flesh color measured by $L^*a^*b^*$ color space values in *C. moschata* and *C. pepo* families.

Materials and Methods

Plant material. Crosses to determine the heritability of flesh color in squash were made based on an initial screening of *C. moschata* and *C. pepo* cultigens (Itle and Kabelka, 2009). From these crosses, two *C. pepo* [‘Table Gold Acorn’ (TGA, yellow-orange) $\times$ PI 314806 (light whitish-yellow)] and [‘Table King Bush’ (TKB, light yellow) $\times$ PI 314806],

and one *C. moschata* [‘Butterbush’ (BB, orange-red) $\times$ ‘Sucrine DuBerry’ (SDub, dull yellow-orange)] were created. F_1 individuals were randomly selected and self-pollinated to create three F_2 populations. Three additional F_1 individuals were reciprocally backcrossed to each parent (BC_{1P1} and BC_{1P2}). All crosses were made in a greenhouse, with a temperature range between 19 and 34 °C, located in Gainesville, FL.

Field trials. The field trial used for selecting the above parents was described in Itle and Kabelka (2009). F_2 and BC₁ screens were conducted 9 Apr.–3 July 2008 and 6 Apr.–4 July 2009, respectively. One hundred individuals for each F_2 and BC₁ population were planted in a completely random design at the University of Florida’s Plant Science Research and Education Unit (PSREU) in Citra, Florida. All seeds were sown directly into the field and were not started as transplants in the greenhouse. Within each F_2 population, eight individuals for each homozygous parent and F_1 progenitor were randomly planted, with the exception of P₂, PI 314806, in the *C. pepo* crosses. Five and six seeds were sown in the TGA and TKB F_2 populations, respectively, because of limited seed number. Within each BC₁ population, six individuals for each homozygous parent and F_1 progenitor were randomly planted. Fruit of the F_2 and related BC₁ populations were harvested along the same timeline in 2008 and 2009, with populations within the families of TGA $\times$ PI 314806, TKB $\times$ PI 314806, and BB $\times$ SDub harvested at 10, 11, and 12 weeks after field planting, respectively. Recommended conventional cultural practices and fertility rates for Florida squash production were followed for all field trials (Olson and Simonne, 2007).

Color analysis. Intermediate categories of flesh color were subjectively observed in segregating populations of the three plant families. These included, but were not limited to, shades of white, whitish-yellow, yellow, yellow-orange, orange, and orange-red. An objective color measurement was used to better understand and classify the categories within families and to study the heritability estimates of genes controlling flesh color in squash. CIE $L^*a^*b^*$ color values, with a reference to their subjective corresponding color classifications, have been correlated with carotenoids levels in squash fruit (Itle and Kabelka, 2009).

Color was recorded using a Minolta CR-400 Colorimeter (Minolta Camera Co., Ltd., Ramsey, NJ) tristimulus color analyzer, equipped with an 8 mm diameter measuring area and diffuse illumination of a 2° Standard Observer. Color measurements included $L^*a^*b^*$, chroma, and hue. Only, L^* , chroma, and hue were used for further analyses because chroma and hue are derived from color spaces values a^* and b^* . Three fruit were harvested from each plant and analyzed. Three random flesh color measurements of transversely sliced fruit were recorded in succession to avoid discoloration. A single fruit for each plant was selected for analyses because of variable fruit maturity within a genotype. Selection was

based upon the lowest L* average replicate measurements within a fruit. Within *Cucurbita*, it has been reported that there is fruit to fruit variation within a plant (Paris, 1994) and that levels of carotenoids increased with fruit maturation (Bonina-Noseworthy et al., 2016; Noseworthy and Loy, 2008). In addition, L* was negatively correlated with total carotenoid content (Itle and Kabelka, 2009; Seroczyńska et al., 2006), lutein content (Itle and Kabelka, 2009), beta-carotene content (Itle and Kabelka, 2009; Seroczyńska et al., 2006), and alpha-carotene content (Itle and Kabelka, 2009) in earlier studies in *Cucurbita*. Selection of the fruit with the lowest average fruit replicate measurements for color space value L* was based upon the concept that more mature flesh would have a darker pigmentation and thereby have a lower L* value. In addition, chroma and hue values from the same fruit were also used.

Variance, heritability, and standard error estimations by generation means analysis. Variances were calculated for each color space value within a generation for each of the nine populations. For heritability calculations within the F₂ populations, phenotypic variances for each trait were equal to the variance among plants of each F₂ population, $\sigma_p^2 = \sigma_{F_2}^2$ (Mahmud and Kramer, 1951). Environmental variances were calculated as $\sigma_E^2 = (1/2)\sigma_{F_1}^2 + (1/4)\sigma_{P_1}^2 + (1/4)\sigma_{P_2}^2$ (Cuevas et al., 2009). For heritability calculations within the BC₁ populations, phenotypic variances for each trait were equal to the variance among plants of each BC₁ population, $\sigma_p^2 = \sigma_{BC_{1P_1}}^2$ and $\sigma_p^2 = \sigma_{BC_{1P_2}}^2$. Environmental variances for BC_{1P₁} populations were calculated as $\sigma_E^2 = (1/4)\sigma_{F_1}^2 + (5/8)\sigma_{P_1}^2 + (1/8)\sigma_{P_2}^2$, and for BC_{1P₂} were calculated as $\sigma_E^2 = (1/4)\sigma_{F_1}^2 + (1/8)\sigma_{P_1}^2 + (5/8)\sigma_{P_2}^2$. Additionally, P₁, P₂, and F₁ plants used for environmental variance calculations were planted within each respective population.

Genetic variances were calculated as $\sigma_G^2 = \sigma_p^2 - \sigma_E^2$ (Warner, 1952; Wright, 1968). Variance estimates were used to calculate broad-sense heritabilities for each trait. Broad-sense heritabilities were calculated using genetic variance and phenotypic variance: $H = \sigma_G^2 / \sigma_p^2$. Standard errors were calculated as (Lynch and Walsh, 1998): $SE = \text{Sqrt}[\text{Var}(V_x)]$, where $\text{Var}(V_x)$ equals the variance of the desired parameter. Estimates of $\text{Var}(V_x)$ were calculated as: $\Sigma [2[\text{Var}(k)^2]/(n + 2)]$ where $\text{Var}(k)$ was each of the variance components used to calculate V_x . SE calculations for broad-sense heritability were calculated as: $SE(H) = [SE(\sigma_G^2)]/\sigma_p^2$ (Hallauer and Miranda, 1988).

Data analysis. Color space values for all F₂ and BC₁ populations were subjected to analysis of variance (ANOVA) by the GLM procedure of SAS (Statistical Analysis System version 9.2; SAS Institute, Cary, NC). Genotype and fruit within a genotype were considered as fixed effects. Additionally, color space values for P₁, P₂, and F₁ within each of the three families were analyzed using the NPAR1WAY procedure. The Kolmogorov–Smirnov test was used to examine the differences between P₁, P₂, and F₁ within a family within a year. Comparisons were also made for each genotype using the Kolmogorov–Smirnov test to examine variation between years, and using the Wilcoxon Rank Sum test to examine variation between fruit within a year. The Spearman's Rank Correlation Coefficient test, using the CORR procedure, was used to assess consistency of color values in the P₁, P₂, and F₁ individuals within each family between years. The presence of transgressive segregants for color space values L*, chroma, and hue within families were analyzed using a Tukey test at a 5% significance level for the F₂, BC_{1P₁}, and BC_{1P₂} populations. Additionally, all hue angle values were converted from degrees to radians for

mean and variance calculations for heritability estimates.

Results

L*a*b* color space measurements means and ranges. For the color space value L* (lightness), the *C. pepo* genotypes (TGA, TKB, and PI 314806) had consistently higher values indicating lighter flesh color than the *C. moschata* (BB and SDub) genotypes (Table 1). Similarly, the mean of L* for F₁ individuals, and for the F₂ and BC₁ populations among the *C. pepo* genotypes fell within the range of the parental genotypes. In contrast, the *C. moschata* F₁ individuals and F₂ and BC₁ populations showed higher values than the parents in their respective years as reported in Tables 1 and 2, indicating that these generations had lighter average flesh color than either BB or SDub. For color space value chroma (color saturation), TGA, TKB, BB, and SDub had similar values, while PI 314806 was substantially lower, indicating that with the exception of PI 314806 all parental material had more vivid flesh colors. The *C. pepo* F₁ individuals and F₂, and BC₁ populations were skewed toward PI 314806 indicating duller average flesh colors than the P₁ genotypes of TGA and TKB in both *C. pepo* families. Mean hue angles were over 90° (yellow) for all *C. pepo* genotypes except TGA (yellow-orange). The *C. moschata* genotypes had mean hue angles below 90° indicating yellow-orange and orange-red flesh.

Distributions within individual families for color space values (Figs. 1–9), represented by: P₁, P₂, F₁, F₂, BC_{1P₁}, and BC_{1P₂}; displayed the variation present within each population and family. The distributions of CIE L*a*b* color space values for each family did not demonstrate the presence of major genes segregating in a Mendelian fashion. Observed segregation patterns within the

Table 1. Means, standard deviations (SD), and ranges of colorimetric values (L*, chroma, and hue)^z of fruit mesocarp (flesh) from intraspecific F₁ individuals and F₂ populations of *Cucurbita pepo* and *Cucurbita moschata*^y families.

Genotype	n	L*		Chroma		Hue	
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
'Table Gold Acorn' (TGA)	8 (24) ^w	76.7 (1.9)	70.9–80.6	62.4 (5.4)	51.6–69.9	82.6 (1.8)	78.8–86.4
PI 314806	5 (15)	82.7 (2.1)	80.5–86.5	9.1 (1.2)	7.5–11.1	102.7 (1.4)	100.2–104.7
F ₁ (TGA × PI 314806)	8 (24)	80.5 (1.5)	78.2–83.1	12.7 (1.9)	10.1–16.7	103.1 (1.3)	100.6–105.6
F ₂ (TGA × PI 314806)	96 (276)	81.3 (3.0)	71.8–87.6	17.4 (8.3)	6.2–43.2	100.2 (3.2)	91.6–107.1
'Table King Bush' (TKB)	8 (22)	80.4 (2.0)	77.2–84.2	49.4 (4.7)	43.0–57.1	90.9 (1.9)	87.1–93.4
PI 314806	6 (16)	83.7 (1.8)	79.6–86.4	9.3 (1.5)	7.3–11.8	103.4 (1.8)	100.4–105.8
F ₁ (TKB × PI 314806)	8 (22)	81.3 (2.8)	75.8–85.4	12.4 (1.8)	8.4–15.7	101.7 (2.2)	97.4–104.5
F ₂ (TKB × PI 314806)	91 (260)	81.3 (3.4)	70.8–89.8	19.0 (7.4)	8.0–40.2	99.1 (3.5)	89.7–106.9
'Butterbush' (BB)	7 (21)	67.4 (3.1)	60.6–71.1	70.7 (2.3)	65.5–76.2	71.5 (3.3)	65.8–78.0
'Sucrine DuBerry' (SDub)	8 (23)	65.1 (2.1)	62.5–71.2	57.5 (3.2)	51.7–65.2	71.1 (4.2)	67.5–81.2
F ₁ (BB × SDub)	8 (23)	68.9 (1.9)	66.7–74.2	64.3 (4.2)	51.6–72.2	73.5 (3.6)	70.9–85.4
F ₂ (BB × SDub)	86 (249)	68.3 (2.5)	61.5–77.5	64.8 (4.7)	50.5–70.8	73.8 (3.4)	67.3–90.4

^zNumeric description of color using L*a*b* CIELAB color space. L* (lightness) ranges from black (0) to white (100). Range in our data: 60.6–89.8, light to light. Chroma (saturation or vividness)—as chromaticity increases a color becomes more intense; as it decreases a color becomes more dull. Range in our data: 6.2–35.0, more dull; 36–76.2, more vivid. Hue (tint of color)—an angular measurement where 0° = red, 45° = orange-red, 90° = yellow, 180° = green, and 270° = blue. Range in our data: 65.8–107.1, yellow-orange to whitish-yellow.

^y*C. pepo* families include 'Table Gold Acorn' (TGA) × PI 314806, and 'Table King Bush' (TKB) × PI 314806. 'Butterbush' (BB) × 'Sucrine DuBerry' (SDub) is the *C. moschata* family.

^xValues shown are based on three colorimetric measurements averaged within a fruit for all color space values.

^wSample value n preceding parentheses is plant number. Value within parentheses is fruit number collected from n plants.

Table 2. Means, standard deviations (SD), and ranges of colorimetric values (L*, chroma, and hue)^z of fruit mesocarp (flesh) from intraspecific F₁ individuals and backcross (BC) populations of *Cucurbita pepo* and *Cucurbita moschata*^y families.

Genotype	n	L* ^x		Chroma		Hue	
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
'Table Gold Acorn' (TGA)	9 (27) ^w	72.9 (3.1)	66.6–79.0	69.4 (4.6)	56.9–78.3	80.9 (2.9)	76.1–88.3
PI 314806	7 (19)	81.9 (2.5)	77.0–86.1	11.6 (1.8)	9.0–15.3	102.5 (1.3)	100.3–104.8
F ₁ (TGA × PI 314806)	10 (30)	80.3 (2.0)	77.1–84.2	15.8 (1.7)	13.2–19.3	102.8 (2.0)	99.5–106.1
BC _{1P1} (TGA × F ₁)	71 (210)	79.4 (4.0)	68.3–87.0	37.5 (16.1)	10.4–72.8	95.4 (6.6)	78.1–107.3
BC _{1P2} (F ₁ × PI 314806)	91 (260)	80.9 (2.2)	73.2–85.9	14.9 (2.0)	9.8–19.9	101.3 (1.8)	96.7–105.9
'Table King Bush' (TKB)	10 (26)	80.5 (1.6)	77.3–83.3	53.2 (2.8)	48.3–59.3	90.5 (1.4)	87.7–92.9
PI 314806	6 (18)	81.5 (2.0)	79.1–85.4	11.4 (1.1)	9.6–13.2	102.3 (1.2)	100.6–105.0
F ₁ (TKB × PI 314806)	11 (32)	80.7 (2.1)	76.8–84.5	15.2 (1.3)	13.2–17.4	100.9 (1.4)	97.6–103.7
BC _{1P1} (F ₁ × TKB)	75 (219)	81.2 (2.8)	72.9–88.4	33.1 (11.8)	13.3–57.5	96.6 (3.4)	88.2–104.9
BC _{1P2} (F ₁ × PI 314806)	84 (246)	80.3 (2.2)	74.9–85.2	13.4 (1.5)	9.5–18.0	100.8 (1.4)	96.5–103.9
'Butterbush' (BB)	12 (36)	69.6 (2.1)	66.6–74.9	71.2 (3.1)	62.9–78.0	76.3 (3.5)	69.8–85.0
'Sucrine DuBerry' (SDub)	11 (29)	66.8 (1.6)	64.2–70.7	55.3 (4.2)	47.8–62.5	74.9 (4.0)	69.2–83.2
F ₁ (BB × SDub)	10 (30)	69.8 (1.7)	66.0–73.1	65.5 (3.1)	60.4–71.6	75.4 (2.5)	69.8–80.6
BC _{1P1} (F ₁ × BB)	67 (201)	70.8 (2.1)	66.1–79.1	66.8 (4.5)	44.4–75.2	79.4 (3.8)	70.7–95.8
BC _{1P2} (F ₁ × SDub)	88 (232)	68.7 (2.1)	64.2–76.6	61.2 (4.8)	46.1–70.9	75.7 (3.6)	69.1–88.0

^zNumeric description of color using L*a*b* CIELAB color space. L* (lightness) ranges from black (0) to white (100). Range in our data: 64.2–88.4, light to light. Chroma (saturation or vividness)—as chromaticity increases a color becomes more intense; as it decreases a color becomes more dull. Range in our data: 9.0–35.0, more dull; 36–78.3, more vivid. Hue (tint of color)—an angular measurement where 0° = red, 45° = orange-red, 90° = yellow, 180° = green, and 270° = blue. Range in our data: 69.2–107.3, yellow-orange to whitish-yellow.

^y*C. pepo* families include 'Table Gold Acorn' (TGA) × PI 314806, and 'Table King Bush' (TKB) × PI 314806. 'Butterbush' (BB) × 'Sucrine DuBerry' (SDub) is the *C. moschata* family.

^xValues shown are based on three colorimetric measurements averaged within a fruit for all color space values.

^wSample value n preceding parentheses is plant number. Value within parentheses is fruit number collected from n plants.

three families supported the appropriateness of using quantitative inheritance analyses for these values as an objective measurement of flesh color in squash.

Analysis of variance. Comparison of genotypes within each of the three families detected significant differences among, and within genotypes, from the F₂, BC_{1P1}, and BC_{1P2} populations ($P \leq 0.001$) for all color space values. Fruit from the P₁, P₂, and F₁ generations within each of the three families were compared for variability of L* chroma and hue between generations within a year, generations across years, and fruit within a generation within a year (Table 3).

Within the 'Table Gold Acorn' × PI 314806 family, the P₁, P₂, and F₁ generations were significantly different within both years ($P < 0.0001$) for all three color

space values (Table 3). TGA fruit had significant differences between years for all color space values ($P < 0.0001$). TGA fruit within the first year were not significantly different for L* ($P = 0.08$), and chroma ($P = 0.07$), and were significantly different for hue ($P = 0.05$), however, fruit within the second year were significantly different ($P < 0.01$) for all three color space values. PI 314806 fruit had significant differences for chroma across years ($P < 0.0001$), and did not have significant differences for L* ($P = 0.20$) and hue ($P = 0.31$) across years. PI314806 fruit within both the first and the second years were significantly different for all three color space values ($P < 0.01$). F₁ fruit had significant differences across years for chroma ($P < 0.0001$), and did not have

significant differences for L* ($P = 0.37$) and hue ($P = 0.20$). F₁ fruit within both years were significantly different for all color space values ($P < 0.01$).

Within the 'Table King Bush' × PI 314806 family, generations were significantly different within the first year ($P < 0.0001$) for all three color space values. Generations were significantly different within the second year for color space values chroma and hue ($P < 0.0001$) and not significantly different for L* ($P = 0.12$). TKB fruit were not significantly different across years for L* ($P = 0.73$) and hue ($P = 0.06$), and were significantly different for chroma ($P < 0.0001$). TKB fruit within the first and second years were significantly different for all color space values ($P < 0.01$). PI 314806 fruit had significant differences across years for all color

Fig. 1. 'Table Gold Acorn' (TGA) × PI 314806 family fruit mesocarp (flesh) histogram for L* (lightness) ranges from black (0) to white (100). P₁ = TGA, P₂ = PI 314806.

Fig. 2. 'Table Gold Acorn' (TGA) × PI 314806 family fruit mesocarp (flesh) histogram for chroma (saturation or vividness). Chromaticity increases as a color becomes more intense and decreases as a color becomes duller. P1 = TGA, P2 = PI 314806.

space values ($P < 0.0001$). PI 314806 fruit within the first and second years were significantly different for all color space values ($P < 0.03$). F₁ fruit had significant differences across years for chroma and hue ($P < 0.0001$ for both), and did not have significant

differences for L* ($P = 0.22$). F₁ fruit within both years were significantly different for all color space values ($P < 0.01$).

Within the 'Butterbush' × 'Sucrine DuBerry' family, generations were significantly different within both years ($P < 0.02$)

for all three color space values. BB fruit were significantly different across years for L* and hue ($P < 0.0001$ for both), and were not significantly different for chroma ($P = 0.10$). BB fruit within the first year were significantly different for L* and hue ($P < 0.01$ for both), and

Fig. 3. 'Table Gold Acorn' (TGA) × PI 314806 family fruit mesocarp (flesh) family histogram for hue (tint of color). An angular measurement where 0° = red, 45° = orange-red, 90° = yellow, 180° = green, and 270° = blue. P1 = TGA, P2 = PI 314806.

Fig. 4. 'Table King Bush' (TKB) × PI 314806 fruit mesocarp (flesh) family histogram for L* (lightness) ranges from black (0) to white (100). P1 = TKB, P2 = PI 314806.

not significantly different for chroma ($P = 0.9583$). Within the second year, BB fruit were significantly different for all color space values ($P < 0.01$). SDub fruit were significantly different across years for all three color space values ($P < 0.01$). SDub fruit were also significantly different within both years for all

color space values ($P < 0.01$). F₁ fruit were significantly different across years for L* ($P = 0.0008$) and hue ($P < 0.0001$), and were not significantly different for chroma ($P = 0.28$). F₁ fruit within the first year were significantly different for chroma ($P = 0.03$) and hue ($P = 0.001$), and were not significantly different for

L* ($P = 0.25$). Fruit within the second year were significantly different for hue ($P < 0.0001$) and L* ($P = 0.05$), and were not significantly different for chroma ($P = 0.28$).

Broad-sense heritability estimates. Phenotypic variances for all color space values for each generation from the three families

Fig. 5. 'Table King Bush' (TKB) × PI 314806 family fruit mesocarp (flesh) histogram for chroma (saturation or vividness). Chromaticity increases as a color becomes more intense and decreases as a color becomes duller. P1 = TKB, P2 = PI 314806.

Fig. 6. 'Table King Bush' (TKB) × PI 314806 family fruit mesocarp (flesh) histogram for hue (tint of color). An angular measurement where 0° = red, 45° = orange-red, 90° = yellow, 180° = green, and 270° = blue. P1 = TGA, P2 = PI 314806.

are reported in Table 4. Variance estimates were used to calculate broad-sense heritability estimates in F₂, BC_{1P1} and BC_{1P2} populations within each of the three families (Table 5).

For the 'Table Gold Acorn' × PI 314806 (*C. pepo*) family, broad-sense heritability for

color space value L* was highest in the F₂ population (0.82) and was intermediate to low for BC_{1P1} (0.48) and BC_{1P2} (0.38). Heritabilities for chroma for both the F₂ and BC_{1P1} populations were both 0.89, and it was negative for BC_{1P2} and is thus considered to be zero. Hue heritabilities were moderate to high for the F₂

and BC_{1P1} (0.78 and 0.87, respectively). The heritability estimate was low for the BC_{1P2} population (0.12), and was the lowest positive heritability estimate obtained for all families, populations, and color space values.

In the 'Table King Bush' × PI 314806 (*C. pepo*) family, broad-sense heritability

Fig. 7. 'Butterbush' (BB) × 'Sucrine DuBerry' (SDub) family fruit mesocarp (flesh) histogram for L* (lightness) ranges from black (0) to white (100). P1 = BB, P2 = SDub.

Fig. 8. 'Butterbush' (BB) $\times$ 'Sucrine DuBerry' (SDub) family fruit mesocarp (flesh) histogram for chroma (saturation or vividness). Chromaticity increases as a color becomes more intense and decreases as a color becomes duller. P1 = BB, P2 = SDub.

estimates for L^* were intermediate to low for all three populations, F_2 (0.59), BC_{1P1} (0.66), and BC_{1P2} (0.47) (Table 5). Chroma heritability estimates for the F_2 and BC_{1P1} populations were high (0.83 and 0.97, respectively), and the estimate obtained for the BC_{1P1} population was the highest heritability estimate obtained for all families,

populations, and color space values. The heritability estimate for chroma in the BC_{1P2} population is considered to be zero. Estimates of heritability for hue angle in the TKB family ranged from 0.39 in the BC_{1P2} population to 0.80 in the BC_{1P1} population, with an intermediate value obtained for the F_2 population (0.64).

In the *C. moschata* 'Butterbush' $\times$ 'Sucrine DuBerry' family, heritabilities for L^* ranged from low in the F_2 (0.19) and BC_{1P1} (0.29) populations, to moderately high in the BC_{1P2} population (0.75). Heritabilities for color space value chroma were intermediate for the F_2 population (0.49) and the BC_{1P1} population (0.40), and low

Fig. 9. 'Butterbush' (BB) $\times$ 'Sucrine DuBerry' (SDub) family fruit mesocarp (flesh) histogram for hue (tint of color). An angular measurement where 0° = red, 45° = orange-red, 90° = yellow, 180° = green, and 270° = blue. P1 = BB, P2 = SDub.

Table 3. Comparisons^z of colorimetric values (L*, chroma, and hue)^y measured in fruit mesocarp (flesh) from the parent and intraspecific F₁ generations that were used to create F₂ and BC₁ populations in two families of *Cucurbita pepo* and one family of *Cucurbita moschata*.^x

		'Table Gold Acorn' Family ^{w,v}			'Table King Bush' Family ^u			'Butterbush' Family ^l		
		P ₁	P ₂	F ₁	P ₁	P ₂	F ₁	P ₁	P ₂	F ₁
L*	Year ^s	<0.0001 ^r	0.1995	0.3743	0.7280	<0.0001	0.2218	<0.0001	<0.0001	0.0008
	Fruit (Year 1)	0.0797	0.0021	0.0003	0.0007	0.001	0.0002	0.0031	0.0026	0.2539
	Fruit (Year 2)	0.0002	0.0006	0.0002	0.0104	0.0125	0.0135	0.0009	0.001	0.0499
	Geno (Year 1)		<0.0001 ^q			<0.0001			<0.0001	
	Geno (Year 2)		<0.0001			0.1177			<0.0001	
Chroma	Year	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1034	0.0089	0.2813
	Fruit (Year 1)	0.0686	0.003	0.0001	<0.0001	0.0009	<0.0001	0.9583	0.0004	0.0263
	Fruit (Year 2)	0.0016	<0.0001	0.0002	0.0054	0.0300	0.0022	0.0127	<0.0001	0.2761
	Geno (Year 1)		<0.0001			<0.0001			<0.0001	
	Geno (Year 2)		<0.0001			<0.0001			<0.0001	
Hue	Year	<0.0001	0.3062	0.2009	0.0623	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Fruit (Year 1)	0.0468	0.0008	0.0017	<0.0001	0.0002	<0.0001	0.0004	0.0002	0.0014
	Fruit (Year 2)	<0.0001	<0.0001	<0.0001	0.0005	0.002	0.0001	<0.0001	<0.0001	<0.0001
	Geno (Year 1)		<0.0001			<0.0001			<0.0001	
	Geno (Year 2)		<0.0001			<0.0001			0.0175	

^zComparisons were made using the NPAR1WAY procedure in SAS v.9.2. *P* values are reported.

^yNumeric description of color using L*a*b* CIELAB color space. L* (lightness) ranges from black (0) to white (100). Chroma (saturation or vividness)—as chromaticity increases a color becomes more intense; as it decreases a color becomes more dull. Hue (tint of color)—an angular measurement where 0° = red, 45° = orange-red, 90° = yellow, 180° = green, and 270° = blue.

^x*C. pepo* families include 'Table Gold Acorn' (TGA) × PI 314806, and 'Table King Bush' (TKB) × PI 314806. 'Butterbush' (BB) × Sucrine DuBerry' (SDub) is the *C. moschata* family.

^wCultivar used to denote family name was used as the female parent in the cross to make the F₁.

^vIn the 'Table Gold Acorn' (TGA) Family: P₁ = TGA, P₂ = PI 314806, F₁ = (TGA × PI 314806).

^uIn the 'Table King Bush' (TKB) Family: P₁ = TKB, P₂ = PI 314806, F₁ = (TKB × PI 314806).

^lIn the 'Butterbush' (BB) Family: P₁ = BB, P₂ = 'Sucrine DuBerry' (SDub), F₁ = (BB × SDub).

^sYear compares year 1 (2008) and year 2 (2009).

^rCorresponding *n* values can be found in Tables 1 [generations grown in year 1 (2008)] and 2 [generations grown in year 2 (2009)]. Values shown are based on three colorimetric measurements not averaged within a fruit for all color space values.

^qGeno (Year) compares P₁, P₂, and F₁ within a family within the indicated year.

in the BC_{1P2} population (0.28). The heritabilities for all three populations for hue angle were negative and are considered zero.

Discussion

*L*a*b* color space means and ranges.* Variation was observed across color space values for each family and generations within

a family (Tables 1 and 2; Figs. 1–9). This could be because of the genetic composition and genetic background of each genotype. Flesh color of squash was reported to be controlled by three interacting loci (Paris and Padley, 2014), which included *Wf*, *L-2*, and *B*. The effects of these genes were studied within near-isogenic lines (NILs) in *C. pepo* by Tadmor et al. (2005). Lack of dominant *D* (*dark stem*) or dominant *L-2* alleles produced

a yellow flesh color. Similarly, when either the dominant *D* or the *L-2* alleles were present, a yellow-orange flesh color was observed. Additional effects were reported when the *B* allele interacted with *L-2* producing an orange flesh color. The interaction of these genes may be responsible for the increased yellow-orange of TGA and yellow pigmentation of TKB, as each parent is compared with the F₂ and BC populations within

Table 4. Variance estimates for calculating heritability of colorimetric values (L*, chroma, and hue)^z measured in fruit mesocarp (flesh) in intraspecific F₁ individuals and F₂ and BC₁ populations in *Cucurbita pepo* and *Cucurbita moschata*^y families with one fruit per plant.^x

Family	Variance estimate	F ₂			BC _{1P1}			BC _{1P2}		
		L*	Chroma	Hue ^w	L*	Chroma	Hue	L*	Chroma	Hue
'Table Gold Acorn' (TGA) × PI 314806	σ^2_{P1}	4.56	25.93	0.0008	9.59	46.42	0.0023	7.98	23.45	0.0007
	σ^2_{P2}	0.13	1.28	0.0005	7.24	1.16	0.0002	0.91	4.51	0.0009
	σ^2_{F1}	0.56	3.51	0.0008	0.44	3.02	0.0016	2.49	2.30	0.0016
	$\sigma^2_{POPULATION}$	7.93	76.51	0.0032	13.55	278.24	0.0145	3.55	4.16	0.0012
	σ^2_{P1}	3.83	33.28	0.0017	2.20	5.62	0.0010	2.02	0.27	0.0001
'Table King Bush' (TKB) × PI 314806	σ^2_{P2}	2.57	2.01	0.0006	0.27	0.21	0.0004	1.08	3.86	0.0005
	σ^2_{F1}	5.98	1.89	0.0016	2.44	1.10	0.0004	3.63	1.00	0.0005
	$\sigma^2_{POPULATION}$	11.33	58.43	0.0039	6.00	149.69	0.0041	3.45	2.18	0.0007
	σ^2_{P1}	11.04	6.83	0.0023	2.66	10.90	0.0086	0.28	10.22	0.0040
	σ^2_{P2}	3.99	10.70	0.0051	1.99	6.49	0.0028	0.44	15.59	0.0065
'Butterbush' (BB) × 'Sucrine DuBerry' (SDub)	σ^2_{F1}	2.00	12.73	0.0053	0.80	4.39	0.0027	2.67	11.13	0.0010
	$\sigma^2_{POPULATION}$	5.84	20.89	0.0031	2.96	14.60	0.0046	3.89	19.13	0.0035

^zNumeric description of color using L*a*b* CIELAB color space. L* (lightness) ranges from black (0) to white (100). Chroma (saturation or vividness)—as chromaticity increases a color becomes more intense; as it decreases a color becomes more dull. Hue (tint of color)—an angular measurement where 0° = red, 45° = orange-red, 90° = yellow, 180° = green, and 270° = blue.

^y*C. pepo* families include 'Table Gold Acorn' (TGA) × PI 314806, and 'Table King Bush' (TKB) × PI 314806. 'Butterbush' (BB) × 'Sucrine DuBerry' (SDub) is the *C. moschata* family.

^xFruit with lowest L* values were selected as an indicator of the most mature fruit. Corresponding chroma and hue values of the same fruit were also used.

^wValues converted from degrees to radians for calculation.

^vRepresents the variance present in the F₂, BC_{1P1}, or BC_{1P2} population within the respective family.

Table 5. Genetic means estimates for heritability of colorimetric values (L^* , chroma, and hue)^z measured in fruit mesocarp (flesh) in intraspecific F_2 and BC_1 populations in *Cucurbita pepo* and *Cucurbita moschata*^z families with one fruit per plant.^x

Family	Genetic parameter ^w	F_2			BC_{1P1}			BC_{1P2}		
		L ±SE*	Chroma ±SE	Hue ±SE ^v	L ±SE*	Chroma ±SE	Hue ±SE	L ±SE*	Chroma ±SE	Hue ±SE
'Table Gold Acorn' (TGA) × PI 314806	σ^2_P	7.93	76.51	0.0032	13.55	278.24	0.0145	3.55	4.16	0.0012
	σ^2_E	±2.82	±8.75	±0.0569	±3.68	±16.68	±0.1202	±1.88	±2.04	±0.0349
	σ^2_G	1.45	8.56	0.0007	7.01	29.91	0.0019	2.19	6.32	0.0011
	H	±0.53	±3.01	±0.0002	±3.50	±16.76	±0.0009	±0.72	±2.39	±0.0004
	H	6.48	67.96	0.0025	6.54	248.33	0.0126	1.36	-2.16	0.0002
'Table King Bush' (TKB) × PI 314806	σ^2_P	±1.53	±12.40	±0.0005	±4.95	±50.70	±0.0026	±1.66	—	±0.0005
	σ^2_E	0.82	0.89	0.7796	0.48	0.89	0.8704	0.38	—	0.1246
	σ^2_G	±0.19	±0.16	±0.1642	±0.36	±0.18	±0.1819	±0.47	—	±0.3854
	H	11.33	58.43	0.0039	6.00	149.69	0.0041	3.45	2.18	0.0007
	H	±3.37	±7.64	±0.0622	±2.45	±12.23	±0.0640	±1.86	±1.48	±0.0265
'Butterbush' (BB) × 'Sucrine DuBerry' (SDub)	σ^2_P	4.59	9.77	0.0014	2.02	3.82	0.0008	1.83	2.70	0.0004
	σ^2_E	±1.44	±3.75	±0.0004	±0.75	±1.76	±0.0003	±0.70	±1.71	±0.0002
	σ^2_G	6.74	48.67	0.0025	3.99	145.87	0.0033	1.61	-0.51	0.0003
	H	±2.32	±11.36	±0.0008	±1.06	±2.25	±0.0004	±0.95	—	±0.0002
	H	0.59	0.83	0.6379	0.66	0.97	0.8040	0.47	—	0.3916
'C. pepo' families include 'Table Gold Acorn' (TGA) × PI 314806, and 'Table King Bush' (TKB) × PI 314806. 'Butterbush' (BB) × 'Sucrine DuBerry' (SDub) is the <i>C. moschata</i> family.	σ^2_P	±0.20	±0.19	±0.2007	±0.18	±0.02	±0.1051	±0.28	—	±0.3397
	σ^2_E	5.84	20.89	0.0031	2.96	14.60	0.0046	3.89	19.13	0.0035
	σ^2_G	±2.42	±4.57	±0.0558	±1.72	±3.82	±0.0677	±1.97	±4.37	±0.0593
	H	4.76	10.75	0.0045	2.11	8.72	0.0064	0.98	13.81	0.0048
	H	±1.39	±3.18	±0.0013	±0.85	±3.48	±0.0027	±0.38	±5.14	±0.0021
Values converted from degrees to radians for calculation.	σ^2_P	1.08	10.15	-0.0014	±1.17	±5.01	-0.0018	2.91	5.32	-0.0013
	σ^2_E	±2.70	±4.67	—	±1.17	±5.01	—	±0.70	±6.11	—
	σ^2_G	0.19	0.49	—	0.29	0.40	—	0.75	0.28	—
	H	±0.46	±0.22	—	±0.40	±0.34	—	±0.18	±0.32	—
	H	—	—	—	—	—	—	—	—	—

^zNumeric description of color using $L^*a^*b^*$ CIELAB color space. L^* (lightness) ranges from black (0) to white (100). Chroma (saturation or vividness)—as chromaticity increases a color becomes more intense; as it decreases a color becomes more dull. Hue (tint of color)—an angular measurement where 0° = red, 45° = orange-red, 90° = yellow, 180° = green, and 270° = blue.

^x*C. pepo* families include 'Table Gold Acorn' (TGA) × PI 314806, and 'Table King Bush' (TKB) × PI 314806. 'Butterbush' (BB) × 'Sucrine DuBerry' (SDub) is the *C. moschata* family.

^yFruit with lowest L^* values were selected as an indicator of the most mature fruit. Corresponding chroma and hue values of the same fruit were then used.

^w σ^2_P , σ^2_E , σ^2_G , H respectively are phenotypic variance = $\sigma^2_{F_2}$; F_2 environmental variance = $(1/2)\sigma^2_{F_1} + (1/4)\sigma^2_{F_1} + (1/4)\sigma^2_{F_1} + (1/4)\sigma^2_{F_1}$; BC_{1P1} environmental variance = $(1/4)\sigma^2_{F_1} + (1/8)\sigma^2_{F_1} + (5/8)\sigma^2_{F_1} + (1/8)\sigma^2_{F_2}$; BC_{1P2} environmental variance = $(1/4)\sigma^2_{F_1} + (1/8)\sigma^2_{F_1} + (5/8)\sigma^2_{F_1} + (1/8)\sigma^2_{F_2}$; σ^2_P , σ^2_E , σ^2_G , H respectively are phenotypic variance = $\sigma^2_{F_2}$; F_2 environmental variance = $(1/2)\sigma^2_{F_1} + (1/4)\sigma^2_{F_1} + (1/4)\sigma^2_{F_1} + (1/4)\sigma^2_{F_1}$; BC_{1P1} environmental variance = $(1/4)\sigma^2_{F_1} + (1/8)\sigma^2_{F_1} + (5/8)\sigma^2_{F_1} + (1/8)\sigma^2_{F_2}$; BC_{1P2} environmental variance = $(1/4)\sigma^2_{F_1} + (1/8)\sigma^2_{F_1} + (5/8)\sigma^2_{F_1} + (1/8)\sigma^2_{F_2}$; σ^2_P , σ^2_E , σ^2_G , H respectively are phenotypic variance = $\sigma^2_{F_2}$; F_2 environmental variance = $(1/2)\sigma^2_{F_1} + (1/4)\sigma^2_{F_1} + (1/4)\sigma^2_{F_1} + (1/4)\sigma^2_{F_1}$; BC_{1P1} environmental variance = $(1/4)\sigma^2_{F_1} + (1/8)\sigma^2_{F_1} + (5/8)\sigma^2_{F_1} + (1/8)\sigma^2_{F_2}$; BC_{1P2} environmental variance = $(1/4)\sigma^2_{F_1} + (1/8)\sigma^2_{F_1} + (5/8)\sigma^2_{F_1} + (1/8)\sigma^2_{F_2}$.

^vValues converted from degrees to radians for calculation.

their respective families. However, CIE $L^*a^*b^*$ values distributions for each family did not demonstrate the presence of major genes segregating in a Mendelian fashion (Figs. 1–9).

Cucurbita pepo had a lighter and duller pigmented flesh (lower L^* and chroma, respectively) than the *C. moschata* generations as presented in Tables 1 and 2. Lighter and duller pigmented flesh could be affected by genetic variation for the trait and/or environmental effect of fruit maturity when harvested from the field.

In both years, TGA had a lower hue angle indicating a more orange flesh color, and TKB had a higher hue angle indicating a more yellow flesh color. White flesh (*Wf*) in *C. pepo* is documented as dominant to colored flesh and prevents accumulation of yellow pigments in mesocarp tissues (Paris and Padley, 2014). This is evident in the distribution of mean hue angles for F_1 and BC_{1P2} generations in both the TGA and TKB families.

For the BB family, mean hue angle values were highest in the F_1 and F_2 generations in 2008, and BC_{1P1} had the highest hue angle in 2009, indicating that these generations had the most yellow-orange hue within the family. Additionally, these three generation means had higher hue angle values than did BB for each respective year, indicating that BB has more of an orange-red flesh than the means of the F_1 , F_2 , and BC_{1P1} generations. All generation means for hue angles were lower than the TGA and TKB families. This indicates that the *C. moschata* family had the most orange-colored flesh.

Rationale for selecting single fruit color space value measurements. All heritability estimates were calculated based on a maximum of three fruit per genotype with three color measurements per fruit within the F_2 , BC_{1P1} and BC_{1P2} populations. However, ANOVA indicated highly significant differences between fruit within a genotype for all color space values in all families. Therefore, estimates were calculated using one fruit per genotype due to variation in fruit maturity within a genotype. Fruit within a genotype were selected based upon the lowest L^* color space value based on the average replicate measurements within a fruit, as previously described in the Materials and Methods section. Selection of the fruit with the lowest average fruit replicate measurements for color space value L^* will represent the most mature fruit sampled and therefore the darkest pigmented flesh.

Within *Cucurbita*, fruit to fruit variation within a plant has been reported (Paris, 1994). Similarly, the levels of carotenoids increase with fruit maturation (Bonina-Noseworthy et al., 2016; Noseworthy and Loy, 2008). In addition, L^* was negatively correlated with total carotenoid content ($r = -0.53$; $r = -0.66$) (Seroczynska et al., 2006; Itle and Kabelka, 2009, respectively), lutein content ($r = -0.68$) (Itle and Kabelka, 2009), beta-carotene content ($r = -0.54$; $r = -0.49$) (Seroczynska et al., 2006; Itle and Kabelka, 2009, respectively), and alpha-carotene

content ($r = -0.49$) (Itle and Kabelka, 2009) in earlier studies in *Cucurbita*. In citrus, L^* values decreased with fruit maturation and carotenoid synthesis (Jiménez-Cuesta et al., 1981). In tomato, L^* values have decreased with fruit ripening. It was noted that an increase in pigmentation synthesis from mature green to red stages resulted in decreasing L^* values (López Camelo and Gómez, 2004). Similarly, Goisser et al. (2020) observed both an exponential and a linear decrease in L^* values during ripening with an increase in lycopene concentration ($R^2 = 0.94$ and $R^2 = 0.79$, respectively). Strong to moderate linear regressions between color space value L^* and tomato fruit ripening and lycopene content were observed in Arias et al. (2000) ($R^2 = 0.84$), D'Souza et al. (1992) ($R^2 = 0.64-0.82$), and Carvalho et al. (2005) ($R^2 = 0.67$). Arias et al. (2000) also reported a very strong, negative correlation between L^* and lycopene content with tomato fruit maturity ($r = -0.95$).

Therefore, within this study, variation in fruit maturity may have contributed to the fruit to fruit variation measured by the color space values observed within a plant. Tagging flowers at anthesis and harvesting fruits at the same number of days after pollination (DAP) may have served as a measure to reduce the variability observed. Since this was not done, fruit selection per genotype was based on the lowest average L^* value to reduce the variability observed.

Variation for fruit color within a genotype is problematic for obtaining accurate heritability estimates. This is because of the error variance being confounded within the genotypic variance estimate, and will result in inaccurate estimates of heritability. All color space values within each population of each family were found to be significantly different for fruit within a genotype, as described earlier. This is likely because of the difficulty in obtaining three fruit from an individual plant that were of similar maturity, as squash blooms repeatedly and flowers can vary by several days in maturity along the vine. Flesh color development continues with fruit development and past fruit maturity, and this nongenetic variation could be problematic for our analyses. Because of this variation, single fruit selections for each color space value within a genotype were made and used for heritability analyses. Selected single fruit measures of heritability are thus reported for the F_2 and BC_1 populations.

The year to year variation was examined by using Spearman's rank correlation coefficient by comparing genotypes across years, 2008 and 2009 seasons, for TGA, TKB, PI 314806, BB, SDub, and the F_1 , created from each cross of the former. Spearman's rank correlation coefficient revealed no significant change in rank of genotypes for L^* ($r = 0.97$, $P \leq 0.0001$), chroma ($r = 0.97$, $P \leq 0.0001$), and hue ($r = 0.93$, $P = 0.0002$) between years. These results indicate that although the variation in fruit measurements within a genotype was significantly different for some color space values within generations (as reported earlier), the

relative differences among genotypes were not altered when examined in two growing seasons.

The environmental variation was estimated to be present because of significant differences detected between year 1 and year 2 within all generations for the majority of color space values comparisons ($P < 0.01$). One fruit from each plant of each genotype in each season was used to capture the environmental variance across years. The fruit selected was the fruit with the lowest average L^* value, which was consistent with the fruit selection criteria that was used for the F_2 and BC_1 populations.

Negative broad-sense heritability estimates. Negative estimates of broad-sense heritabilities were obtained for at least one estimate in each of the three families. Negative estimates of broad-sense heritability arose from negative genetic variance estimates, and were reported as zero values (Robinson et al., 1955). Obtaining negative variance components for heritability calculations may occur due to failure to meet assumptions of the model, the presence of additional data correlations, or due to sampling error (Thompson and Moore, 1963). In addition, negative heritability estimates can occur if true heritability is low to moderate (0.10–0.25) and the number of observations are limited (Gill and Jensen, 1968). All variance estimates used to calculate broad-sense heritability are reported to aid in explaining the source(s) of variation causing the negative estimates and prevent reporting bias. Because of obtaining negative heritability estimates, calculation of narrow-sense heritability and epistatic gene effects are not reported.

Flesh color heritability estimates obtained in other cucurbit crops, namely cucumber (*Cucumis sativus* L.) (Cuevas et al., 2009), and watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] (Bang et al., 2010) were able to be evaluated using chi-square goodness of fit test for gene segregation ratios. Flesh color in cucumber associated with beta-carotene indicated a two recessive gene model, and variation for flesh color in watermelon associated with lycopene also supported a two gene model. Data obtained in this study could not be easily classified into distinct groups and this calculation method was therefore not pursued as supported by the distributions of the CIE $L^*a^*b^*$ color values (Figs. 1–9).

Breeding for increased flesh color in winter squash. Transgressive segregation was examined using Tukey's test for the fruit with the lowest average replicate measurements for L^* color space values and its corresponding chroma and hue values for each plant of F_2 and BC_{1P1} populations of all three families. Evidence for transgressive segregation was not detected for color space values L^* , chroma, and hue for both TGA populations. TGA had the highest chroma and hue color space values in the F_2 generation. No individuals had L^* lower values than TGA for the F_2 and BC_{1P1} populations, and chroma and hue in the BC_{1P1} population. This lack of transgressive segregation could be a reflection of the limited homogenous data collected for

each genotype, the necessity for a larger number of individuals within the populations, or the limited diversity sampled. These data suggest that progeny within these populations cannot be used to exceed the values of their respective parental genotypes for lightness of color, color saturation, or tint of color.

There was an absence of transgressive genotypes in the TKB family for color space values chroma and hue. There was one potentially transgressive segregant for L^* in the F_2 population, but it is difficult to establish if this was due to differences of maturity levels or other environmental effects. Moderate broad-sense heritabilities for color space value L^* suggest that breeding for lower lightness of color values may be difficult in the TKB family.

No transgressive segregation was observed for color space values L^* , chroma, and hue for both the F_2 and BC_{1P1} populations within the BB family, as no individuals within a population significantly exceed BB. Additionally, there were low to intermediate heritabilities (0.19–0.49) for color space values L^* , chroma, and hue for all three populations within the BB family suggest, with the only exception of L^* in the BC_{1P2} population (0.75). This suggests that it may be difficult to increase flesh color in the BB family and their corresponding carotenoid levels as previously identified (Itle and Kabelka, 2009).

To obtain more accurate heritability estimates, more measurements per genotype could be used if individual flower anthesis was recorded, thereby controlling individual fruit maturity. Additionally, beta-carotene levels in some muskmelon cultivars have been shown to be influenced by soil type in different locations (Lester and Eischen, 1995). This suggests that accumulation of other carotenoid pigments in other cucurbit crops may also be affected by environmental conditions. Planting F_2 and BC_1 populations in either multiple years and/or multiple locations, in addition to increasing the number of individuals examined within a population, may enable partitioning out the environmental variation and aid in the calculation of heritability estimates. Similarly, it has been reported that the effects of modifier genes, the instability of *B* gene, and ranges of fruit pigmentation could cause variation for flesh color in *Cucurbita*. Shiffriss and Paris (1981) classified color in *C. pepo* as a quantitative trait with variation within each class categories (when measured as a qualitative trait), where yellow or other pigment did not present a clear limit among classes.

Conclusion

The data suggest that flesh color within *C. moschata* and *C. pepo* was not a simply inherited trait as measured by color space values L^* , chroma, and hue. Within plant and year to year variability for these color space values suggests the presence of other factors (environment, modifier genes, epistatic interactions, and others) which need to be further examined. Increasing population size and planting over additional locations and/or

years, and clonal replication, may help in calculating a better estimate of heritability for flesh color, which in turn will represent a gain in carotenoid levels. The presence of moderate to high heritability estimates within some populations may indicate the potential to improve upon flesh color in populations with more variability. The need for increasing the variability within a breeding population is also reflected in the low number of transgressive segregants in the F_2 populations, and the lack of transgressive segregants in the back-cross populations in all three families. If more variability existed in populations with moderate to high heritability estimates, there would be an increased opportunity to improve upon the parental phenotypes for the color space values in future populations within the respective family. The creation and use of hybrids with wild species and landraces may increase overall population variability, and may also prove beneficial in increasing flesh color in *Cucurbita* in future squash breeding efforts.

Literature Cited

- Afonso, T., R. Moresco, V.G. Uarotta, B.B. Navarro, E.D.C. Nunes, M. Maraschin, and M. Rocha. 2017. UV-Vis and CIELAB based chemometric characterization of *Manihot esculenta* carotenoid contents. *J. Integr. Bioinform.* 14(4):1–13, <https://doi.org/10.1515/jib-2017-0056>.
- Arias, R., T.C. Lee, L. Logendra, and H. Janes. 2000. Correlation of lycopene measured by HPLC with the L^* , a^* , b^* color readings of a hydroponic tomato and the relationship of maturity with color and lycopene content. *J. Agr. Food Chem.* 48(5):1697–1702, <https://doi.org/10.1021/jf990974e>.
- Bang, H., A.R. Davis, K. Sunggil, D.L. Leskovar, and S.R. King. 2010. Flesh color inheritance and gene interactions among canary yellow, pale yellow, and red watermelon. *J. Amer. Soc. Hort. Sci.* 135:362–368, <https://doi.org/10.21273/JASHS.135.4.362>.
- Bonina-Noseworthy, J., J.B. Loy, J. Curran-Celentano, R. Sideman, and D.A. Kopsell. 2016. Carotenoid concentration and composition in winter squash: Variability associated with different cultigens, harvest maturities, and storage times. *HortScience* 51(5):472–480, <https://doi.org/10.21273/HORTSCI.51.5.472>.
- Carvalho, W., M.E.D.N. Fonseca, H.R.D. Silva, L.S. Boiteux, and L.D.B. Giordano. 2005. Estimativa indireta de teores de licopeno em frutos de genótipos de tomateiro via análise colorimétrica. *Hortic. Bras.* 23:819–825, <https://doi.org/10.1590/S0102-05362005000300026>.
- Conesa, A., F.C. Manera, J.M. Brotons, J.C. Fernandez-Zapata, I. Simón, S. Simón-Grao, M. Alfósea-Simón, J.J. Martínez Nicolá, J.M. Valverde, and F. García-Sánchez. 2019. Changes in the content of chlorophylls and carotenoids in the rind of Fino 49 lemons during maturation and their relationship with parameters from the CIELAB color space. *Scientia Hort.* 243:252–260, <https://doi.org/10.1016/j.scienta.2018.08.030>.
- Cuevas, H.E., H. Song, J.E. Staub, and P.W. Simon. 2009. Inheritance of beta-carotene-associated flesh color in cucumber (*Cucumis sativus* L.) fruit. *Euphytica* 171:301–311, <https://doi.org/10.1007/s10681-009-0017-2>.
- D'Souza, M.C., S. Singha, and M. Ingle. 1992. Lycopene concentration of tomato fruit can be estimated from chromaticity values. *HortScience* 27(5):465–466, <https://doi.org/10.21273/HORTSCI.27.5.465>.
- Dóka, O., G. Ficzek, S. Luterotti, D. Bicanic, R. Spruijt, J.G. Buijsters, L. Szalay, and G. Végvári. 2013. Simple and rapid quantification of total carotenoids in lyophilized apricots (*Prunus armeniaca* L.) by means of reflectance colorimetry and photoacoustic spectroscopy. *Food Technol. Biotechnol.* 51(4):453–459.
- Eggersdorfer, M. and A. Wyss. 2018. Carotenoids in human nutrition and health. *Arch. Biochem. Biophys.* 652:18–26, <https://doi.org/10.1016/j.abb.2018.06.001>.
- Ercan, N., A.S. Sensoy, N. Sari, I. Solmaz, and V. Aras. 2012. Inheritance of fruit skin colour in summer squash (*Cucurbita pepo* L.). *Cucurbitaceae* 2012. *Proc. X EUCARPIA Mtg. Genet. and Breeding of Cucurbitaceae*. p. 693–696.
- Gill, J.T. and E.L. Jensen. 1968. Probability of obtaining negative estimates of heritability. *Biometrics* 24:517–526, <https://doi.org/10.2307/2528315>.
- Globerson, D. 1969. The inheritance of white fruit and stem color in summer squash, *Cucurbita pepo* L. *Euphytica* 18:249–255, <https://doi.org/10.1007/BF00035697>.
- Goisser, S., S. Wittmann, M. Fernandes, H. Mempel, and C. Ulrichs. 2020. Comparison of colorimeter and different portable food-scanners for non-destructive prediction of lycopene content in tomato fruit. *Postharvest Biol. Technol.* 167:111232, <https://doi.org/10.1016/j.postharvbio.2020.111232>.
- Gross, J. 1991. Pigments in vegetables: Chlorophylls and carotenoids. VanNostrand Reinhold, New York, NY.
- Hallauer, A.R. and J.B. Miranda. 1988. Quantitative genetics in maize breeding. Iowa State University Press, Ames, IA.
- Itle, R.A. and E.A. Kabelka. 2009. Correlation between L^* , a^* , b^* color space values and carotenoid content in pumpkins and squash (*Cucurbita* spp.). *HortScience* 44:633–637, <https://doi.org/10.21273/HORTSCI.44.3.633>.
- Lester, G.E. and F. Eischen. 1995. Beta-carotene content of postharvest orange-fleshed muskmelon fruit: Effect of cultivar, growing location and fruit size. *Plant Foods Hum. Nutr.* 49:191–197, <https://doi.org/10.1007/BF01093215>.
- Jiménez-Cuesta, M., J. Cuquerella, and J.M. Martínez-Jávaga. 1981. Determination of a color index for citrus fruit degreening. *Proc. Intl. Soc. Citricult* 2:750, 753.
- Lopez-Anido, F., V. Cravero, P. Asprelli, E. Cointy, I. Firpo, and S.M. Garcia. 2003. Inheritance of immature fruit color in *Cucurbita maxima* var. Zapallito (Carrière). *Millan. Cucurbit Genet. Coop. Rpt.* 26:48–50.
- López Camelo A.F., P.A. Gómez. 2004. Comparison of color indexes for tomato ripening. *Hort. Bras.* 22:534–537, <https://doi.org/10.1590/S0102-05362004000300006>.
- Lynch, M. and B. Walsh. 1998. Genetic and analysis of quantitative traits. Sinauer Association, Sunderland, MA.
- Mahmud, I. and H.H. Kramer. 1951. Segregation for yield, height and maturity following a soybean cross. *Agron. J.* 43:605–608, <https://doi.org/10.2134/agronj1951.00021962004300120005x>.
- Martínez-Valdivieso, D., P. Gómez, R. Font, Á. Alonso-Moraga, and M. Del Río-Celestino. 2015. Physical and chemical characterization in fruit from 22 summer squash (*Cucurbita pepo* L.) cultivars. *Lebensm. Wiss. Technol.* 64(2):1225–1233, <https://doi.org/10.1016/j.lwt.2015.07.023>.
- Meléndez-Martínez, A.J., I.M. Vicario, and F.J. Heredia. 2003. Application of tristimulus colorimetry to estimate the carotenoids content in ultrafrozen orange juices. *J. Agr. Food Chem.* 51(25):7266–7270, <https://doi.org/10.1021/jf034873z>.
- Moyano, M.J., A.J. Meléndez-Martínez, J. Alba, and F.J. Heredia. 2008. A comprehensive study on the colour of virgin olive oils and its relationship with their chlorophylls and carotenoids indexes (II): CIELUV and CIELAB uniform colour spaces. *Food Res. Int.* 41(5):513–521, <https://doi.org/10.1016/j.foodres.2008.03.007>.
- Murkovic, M., U. Mülleder, and H. Neunteufl. 2002. Carotenoid content in different varieties of pumpkins. *J. Food Compos. Anal.* 15:633–638, <https://doi.org/10.1006/jfca.2002.1052>.
- Nakkanong, K., J.H. Yang, and M.F. Zhang. 2012. Carotenoid accumulation and carotenogenic gene expression during fruit development in novel interspecific inbred squash lines and their parents. *J. Agr. Food Chem.* 60(23):5936–5944, <https://doi.org/10.1021/jf3007135>.
- Noseworthy, J.B. and J.B. Loy. 2008. Improving eating quality and carotenoid content of squash. *Cucurbitaceae* 2008. *Proc. IX EUCARPIA Mtg. Genet. and Breeding of Cucurbitaceae*. p. 521–528.
- Obrero, Á., C.I. González-Verdejo, J.V. Die, P. Goimez, M. Del Río-Celestino, and B. Romain. 2013. Carotenogenic gene expression and carotenoid accumulation in three varieties of *Cucurbita pepo* during fruit development. *J. Agr. Food Chem.* 61(26):6393–6403, <https://doi.org/10.1021/jf4004576>.
- ODS/NIH. 2013. Vitamin A. Fact Sheet for Health Professionals. 24 July 2015. <<https://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/#en2>>.
- Olson, S.M. and E.H. Simonne. 2007. Vegetable production handbook for Florida 2007–2008. Vance Publishing, Lenexa, KS.
- Ornelas-Paz, J.J., E.M. Yahia, and A.A. Gardea. 2008. Changes in external and internal color during postharvest ripening of ‘Manila’ and ‘Ataulfo’ mango fruit and relationship with carotenoid content determined by liquid chromatography-APCI+–time-of flight mass spectrometry. *Postharvest Biol. Technol.* 50:145–152, <https://doi.org/10.1016/j.postharvbio.2008.05.001>.
- Patsilinos, A., R. Ragno, S. Carradori, S. Petralito, and S. Cesa. 2018. Carotenoid content of Goji berries: CIELAB, HPLC-DAD analyses and quantitative correlation. *Food Chem.* 268:49–56, <https://doi.org/10.1016/j.foodchem.2018.06.013>.
- Paris, H.S., H. Nerson, and Y. Burger. 1985. Precocious PI 165561 and Precocious PI 165561R pumpkin breeding lines. *HortScience* 20:778–779.
- Paris, H.S. and H. Nerson. 1986. Genes for intense pigmentation of squash. *J. Hered.* 77:403–409, <https://doi.org/10.1093/oxfordjournals.jhered.a110270>.
- Paris, H.S. 1988. Complementary genes for orange fruit flesh color in *Cucurbita pepo*. *HortScience* 23:601–603.
- Paris, H.S. 1994. Genetic analysis and breeding of pumpkins and squash for high carotene content, p. 93–115. In: H.F. Linskens and J.F. Jackson (eds.) *Vegetables and vegetable products*. Springer, Berlin, Heidelberg, https://doi.org/10.1007/978-3-642-84830-8_5.
- Paris, H.S. 1995. The dominant *Wf* (White flesh) allele is necessary for expression of “white” mature fruit color in *Cucurbita pepo*. *Cucurbitaceae* 1994. *Mtg. Genet. and Breeding of Cucurbitaceae*. p. 219–220.
- Paris, H.S. 2002. Multiple allelism at a major locus affecting fruit coloration in *Cucurbita pepo*. *Euphytica* 125:149–153, <https://doi.org/10.1023/A:1015898305507>.
- Paris, H.S. and L.D. Padley. 2014. Gene list for *Cucurbita* species, 2014. *Cucurbit Genet. Coop. Rpt.* 37. Oct. 20 2021. <<https://cucurbit>

- info/wp-content/uploads/2018/10/gene14squash.pdf>.
- Robinson, H.F., R.E. Comstock, and P.H. Harvey. 1955. Genetic variances in open pollinated varieties of corn. *Genetics* 40:45–60, <https://doi.org/10.1093/genetics/40.1.45>.
- Seroczyńska, A., A. Korzeniewska, J. Sztangret-Wisniewska, K. Niemirówicz-Szczytt, and M. Gajewski. 2006. Relationship between carotenoids content and flower or fruit flesh colour of winter squash (*Cucurbita maxima* Duch.). *Folia Hort.* 18(1):51–61.
- Shifriss, O. 1981. Origin, expression, and significance of gene B in *Cucurbita pepo* L. *J. Amer. Soc. Hort. Sci.* 106:220–232.
- Shifriss, O. and H.S. Paris. 1981. Identification of modifier genes affecting the extent of precocious fruit pigmentation in *Cucurbita pepo* L. *J. Amer. Soc. Hort. Sci.* 106:653–660.
- Shifriss, O. 1988. On the emergence of B cultivars in squash. *HortScience* 23:238.
- Shifriss, O., R.B. Volin, and T.V. Williams. 1989. Relationship between the B genes of two *Cucurbita* species, II. *Cucurbit Genet. Coop. Rpt.* 12:75–77.
- Sinnott, E.W. and G.B. Durham. 1922. Inheritance in the summer squash. *J. Hered.* 13:177–186, <https://doi.org/oxfordjournals.jhered.a102197>.
- Tadmor, Y., H.S. Paris, A. Meir, A.A. Schaffer, and E. Lewinsohn. 2005. Dual role of the pigmentation gene B in affecting carotenoid and vitamin E content in squash (*Cucurbita pepo*) mesocarp. *J. Agr. Food Chem.* 53:9759–9763, <https://doi.org/10.1021/jf0520591>.
- Thompson, W.A. and J.R. Moore. 1963. Non-negative estimates of variance components. *Technometrics* 5:441–449, <https://doi.org/10.1080/00401706.1963.10490122>.
- Warner, J.N. 1952. A method for estimating heritability. *Agron. J.* 44:427–430, <https://doi.org/10.2134/agronj1952.00021962004400080007x>.
- Wright, S. 1968. The genetics of quantitative variability, p. 373–420. In: S. Wright (ed.) *Evolution and genetics of populations*. Univ. of Chicago Press, Chicago, IL.
- Zhang, M.K., M.P. Zhang, M. Mazourek, Y. Tadmor, and L. Li. 2014. Regulatory control of carotenoid accumulation in winter squash during storage. *Planta* 240(5):1063–1074, <https://doi.org/10.1007/s00425-014-2147-6>.