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

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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, BC1P1, and BC1P2 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’ × ‘Suirce DuBerry’). Broad-sense heritabilities were calculated for the F2, BC1P1, and BC1P2 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.

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, improved visual and brain development in infants (Egggersdorfer and Wyss, 2018). Carotenoids are important for human nutrition and health for all ages, and their availability in Cucurbita species makes 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 50% of total carotenoid concentration) in C. maxima hybrids. Similarly, lutein and zeaxanthin 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) (Simont and Durham, 1922) and is complementary to W (weak fruit coloration) for white fruit rind color (Paris, 1995). The second is the B (biclor) gene, dominant to b (green fruit coloration, previously known as B1'), 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 BmX-2 (Paris and Padley, 2014) was identified to have a stronger effect than the B gene of C. pepo, in deploiting 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 fleshed variety ‘Vegetable Spaghetti’ × Bb I-2, and a precocious orange flesh variety ‘Precocious Fordhook Zucchini’, B/B I-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...
two gene model. Other observations suggest inheritance of flesh color of squash is influenced by several genetic and environmental factors (Shiffriss, 1981; Shiffriss 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 biosynthesis and variability in the expression of carotenoid genes in squash (Nakkanong et al., 2012; 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 cultivar ‘Sookie’ 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 spatial 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) (Seroczynska 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 (Oremelas-Paz et al., 2008), apricots (Dőka et al., 2013), lemons (Conesa et al., 2019), olive oil (Moyano et al., 2008), orange juice (Meléndez-Martínez et al., 2003), goji berries (Patsilinakos 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-Valdívieso et al., 2015; Seroczynska 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 selfing (pollination). Varieties tend to be highly homogeneous and highly homozygous, and can be released as open-pollinated varieties or inbred lines. Hybrid seed tends to be highly homogeneous 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 cultivars (Itle and Kabelka, 2009). From these crosses, two C. pepo [‘Table Gold Acorn’ (TGA, yellow-orange) × PI 314806 (light whitish-yellow)] and [‘Table King Bush’ (TKB, light yellow) × PI 314806], and one C. moschata [‘Butterbush’ (BB, orange-red) × ‘Sueene DuBerry’ (SDuh, dull yellow-orange)] were created. F1 individuals were randomly selected and self-pollinated to create three F2 populations. Three additional F1 individuals were reciprocally backcrossed to each parent (BC1P1 and BC1P2). 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). F2 and BC1 screens were conducted 9 Apr.–3 May 2008 and 6 Apr.–4 July 2009, respectively. One hundred individuals for each F2 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 F2 population, eight individuals for each homozygous parent and F1 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 F2 populations, respectively, because of limited seed number. Within each BC1 population, six individuals for each homozygous parent and F1 progenitor were randomly planted. Fruit of the F2 and related BC1 populations were harvested along the same timeline in 2008 and 2009, with populations within the families of TGA × PI 314806, TKB × PI 314806, and BB × SDuh 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 these 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 space 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...
Genetic variances were calculated as $\sigma^2_L = \sigma^2_F - \sigma^2_F$ (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: $H = \sigma^2_L/\sigma^2_F$. Standard errors were calculated as $(\text{Lynch and Walsh, 1998})$: $SE = \sqrt{\text{Var}(V_X)/n + 2}$ where $Var(V_X)$ equals the variance of the desired parameter. Estimates of $Var(V_X)$ were calculated as: $\Sigma [2[Var(k)/n (n + 2)]$ where $k$ was each of the variance components used to calculate $V_X$. SE calculations for broad-sense heritability were calculated as: $\text{se}(H) = \text{se}(\sigma^2_L)/\sigma^2_F$ (Hallauer and Miranda, 1988).

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 $P_1$ 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: $P_1$, $P_2$, $F_1$, $BC_1P_1$, and $BC_2P_2$, 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) of fruit mesocarp (flesh) from intraintraspecific $P_1$ individuals and $F_2$ populations of $C$. pepo and $C$. moschata families.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table Gold Acorn’ (TGA)</td>
<td>8</td>
<td>76.7 (1.9)</td>
<td>70.9–80.6</td>
<td>62.4 (5.4)</td>
<td>51.6–69.9</td>
<td>82.6 (1.8)</td>
<td>78.8–86.4</td>
</tr>
<tr>
<td>PI 314806</td>
<td>5</td>
<td>82.7 (2.1)</td>
<td>80.5–86.5</td>
<td>91.1 (1.2)</td>
<td>75.6–11.1</td>
<td>102.7 (1.4)</td>
<td>100.2–104.7</td>
</tr>
<tr>
<td>$F_1$ (TGA × PI 314806)</td>
<td>8</td>
<td>80.5 (1.5)</td>
<td>78.2–83.1</td>
<td>12.7 (1.9)</td>
<td>10.1–16.7</td>
<td>103.1 (1.3)</td>
<td>100.6–105.6</td>
</tr>
<tr>
<td>$F_2$ (TGA × PI 314806)</td>
<td>96</td>
<td>81.3 (3.0)</td>
<td>71.8–87.6</td>
<td>17.4 (8.3)</td>
<td>6.2–43.2</td>
<td>100.2 (3.2)</td>
<td>91.6–107.1</td>
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<td>‘Table King Bush’ (TKB)</td>
<td>8</td>
<td>80.4 (2.0)</td>
<td>77.2–84.2</td>
<td>49.4 (4.7)</td>
<td>43.0–57.1</td>
<td>90.9 (1.9)</td>
<td>87.1–93.4</td>
</tr>
<tr>
<td>PI 314806</td>
<td>6</td>
<td>83.7 (1.8)</td>
<td>79.6–86.4</td>
<td>9.3 (1.5)</td>
<td>7.3–11.8</td>
<td>103.4 (1.8)</td>
<td>100.4–105.8</td>
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<td>$F_1$ (TKB × PI 314806)</td>
<td>8</td>
<td>81.3 (2.8)</td>
<td>75.8–88.5</td>
<td>12.4 (1.8)</td>
<td>8.4–15.7</td>
<td>101.7 (2.2)</td>
<td>97.4–104.5</td>
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<td>$F_2$ (TKB × PI 314806)</td>
<td>91</td>
<td>81.3 (3.4)</td>
<td>70.8–89.8</td>
<td>19.0 (7.4)</td>
<td>8.4–40.2</td>
<td>99.1 (3.5)</td>
<td>89.7–106.9</td>
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<td>‘Butterbush’ (BB)</td>
<td>7</td>
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<td>70.7 (2.3)</td>
<td>65.5–76.2</td>
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<td>68.5–78.0</td>
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<tr>
<td>‘Sucrine DuBerry’ (SDub)</td>
<td>8</td>
<td>65.1 (2.1)</td>
<td>62.5–71.2</td>
<td>57.5 (3.2)</td>
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<tr>
<td>$F_1$ (BB × SDub)</td>
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<td>68.9 (1.9)</td>
<td>66.7–75.5</td>
<td>64.3 (4.2)</td>
<td>51.6–72.2</td>
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<td>$F_2$ (BB × SDub)</td>
<td>86</td>
<td>68.3 (2.5)</td>
<td>61.5–77.5</td>
<td>64.8 (4.7)</td>
<td>50.5–70.8</td>
<td>73.8 (3.4)</td>
<td>67.3–90.4</td>
</tr>
</tbody>
</table>

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*Numeric 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.

*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.

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

*Sample size 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) of fruit mesocarp (flesh) from intraspecific F1 individuals and backcross (BC) populations of Cucurbita pepo and Cucurbita moschata families.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>Mean (SD)</th>
<th>Range</th>
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<tbody>
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<td>'Table Gold Acorn' (TGA)</td>
<td>9 (27)</td>
<td>72.9 (3.1)</td>
<td>66.6–79.0</td>
<td>69.4 (4.6)</td>
<td>56.9–78.3</td>
<td>80.9 (2.9)</td>
<td>76.1–88.3</td>
</tr>
<tr>
<td>PI 314806</td>
<td>7 (19)</td>
<td>81.9 (2.5)</td>
<td>77.0–86.1</td>
<td>11.6 (1.8)</td>
<td>9.0–15.3</td>
<td>102.5 (1.3)</td>
<td>100.3–104.8</td>
</tr>
<tr>
<td>F1 (TGA × PI 314086)</td>
<td>10 (30)</td>
<td>80.3 (2.0)</td>
<td>77.1–84.2</td>
<td>15.8 (1.7)</td>
<td>13.2–19.3</td>
<td>102.8 (2.0)</td>
<td>99.5–106.1</td>
</tr>
<tr>
<td>BC1P1 (TGA × F1)</td>
<td>71 (210)</td>
<td>79.4 (4.0)</td>
<td>68.3–78.7</td>
<td>37.5 (16.1)</td>
<td>10.4–72.8</td>
<td>95.4 (6.6)</td>
<td>78.1–107.3</td>
</tr>
<tr>
<td>BC1P2 (F1 × PI 314086)</td>
<td>91 (260)</td>
<td>80.9 (2.2)</td>
<td>73.2–85.9</td>
<td>14.9 (2.0)</td>
<td>9.8–19.9</td>
<td>101.3 (1.8)</td>
<td>96.7–105.9</td>
</tr>
<tr>
<td>'Table King Bush' (TKB)</td>
<td>10 (26)</td>
<td>80.5 (1.6)</td>
<td>77.3–83.3</td>
<td>53.2 (2.8)</td>
<td>48.3–59.3</td>
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<td>87.7–92.9</td>
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<td>9.6–13.2</td>
<td>102.3 (1.2)</td>
<td>96.0–105.0</td>
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<tr>
<td>F1 (TKB × PI 314806)</td>
<td>11 (32)</td>
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<td>76.8–84.5</td>
<td>15.2 (1.3)</td>
<td>13.2–17.4</td>
<td>100.9 (1.4)</td>
<td>97.6–103.7</td>
</tr>
<tr>
<td>BC1P1 (F1 × TKB)</td>
<td>75 (219)</td>
<td>81.2 (2.8)</td>
<td>72.9–88.4</td>
<td>33.1 (11.8)</td>
<td>13.3–57.5</td>
<td>96.6 (3.4)</td>
<td>88.2–104.9</td>
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<td>BC1P2 (F1 × PI 314086)</td>
<td>84 (246)</td>
<td>80.3 (2.2)</td>
<td>74.9–85.2</td>
<td>13.4 (1.5)</td>
<td>9.5–18.0</td>
<td>100.8 (1.4)</td>
<td>96.5–103.9</td>
</tr>
<tr>
<td>'Sucrine DuBerry' (SDub)</td>
<td>12 (36)</td>
<td>69.6 (2.1)</td>
<td>66.6–74.9</td>
<td>71.2 (3.1)</td>
<td>62.9–78.0</td>
<td>76.3 (3.5)</td>
<td>69.8–85.0</td>
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<tr>
<td>'Butterbush' (BB)</td>
<td>11 (29)</td>
<td>66.8 (1.6)</td>
<td>64.2–70.7</td>
<td>55.3 (4.2)</td>
<td>47.8–62.5</td>
<td>74.9 (4.0)</td>
<td>69.2–83.2</td>
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<tr>
<td>BC1P1 (BB × SDub)</td>
<td>10 (30)</td>
<td>69.8 (1.7)</td>
<td>66.0–73.1</td>
<td>65.5 (3.1)</td>
<td>60.4–71.6</td>
<td>75.4 (2.5)</td>
<td>69.8–80.6</td>
</tr>
<tr>
<td>BC1P2 (F1 × SDub)</td>
<td>67 (201)</td>
<td>70.8 (2.1)</td>
<td>66.1–79.1</td>
<td>66.8 (4.5)</td>
<td>44.4–75.2</td>
<td>79.4 (3.8)</td>
<td>70.7–95.8</td>
</tr>
<tr>
<td>BC1P2 (F1 × SDub)</td>
<td>88 (232)</td>
<td>68.7 (2.1)</td>
<td>64.2–76.6</td>
<td>61.2 (4.8)</td>
<td>46.1–70.9</td>
<td>75.7 (3.6)</td>
<td>69.1–88.0</td>
</tr>
</tbody>
</table>

*Numeric 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.

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.

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

Sample size n preceding parentheses is plant number. Values 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, BC1P1, and BC1P2 populations (P ≤ 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 314086 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 = 0.40) across years. PI 314086 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 314086 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 314086 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.
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

![TGA Populations Chroma](image)

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.

![TGA Populations Hue](image)

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

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**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.

**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.
are reported in Table 4. Variance estimates were used to calculate broad-sense heritability estimates in $F_2$, $BC_{1P1}$ 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 $F_2$ population (0.82) and was intermediate to low for $BC_{1P1}$ (0.48) and $BC_{1P2}$ (0.38). Heritabilities for chroma for both the $F_2$ 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 $F_2$ 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...
estimates for L* were intermediate to low for all three populations, F2 (0.59), BC1P1 (0.66), and BC1P2 (0.47) (Table 5). Chroma heritability estimates for the F2 and BC1P1 populations were high (0.83 and 0.97, respectively), and the estimate obtained for the BC1P1 population was the highest heritability estimate obtained for all families, populations, and color space values. The heritability estimate for chroma in the BC1P2 population is considered to be zero. Estimates of heritability for hue angle in the TKB family ranged from 0.39 in the BC1P2 population to 0.80 in the BC1P1 population, with an intermediate value obtained for the F2 population (0.64).

In the C. moschata ‘Butterbush’ × ‘Sucrine DuBerry’ family, heritabilities for L* ranged from low in the F2 (0.19) and BC1P1 (0.29) populations, to moderately high in the BC1P2 population (0.75). Heritabilities for color space value chroma were intermediate for the F2 population (0.49) and the BC1P1 population (0.40), and low.

Fig. 8. ‘Butterbush’ (BB) × ‘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.

Fig. 9. ‘Butterbush’ (BB) × ‘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 of colorimetric values (L*, chroma, and hue) 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.

<table>
<thead>
<tr>
<th>Family</th>
<th>L*</th>
<th>Chroma</th>
<th>Hue</th>
<th>L*</th>
<th>Chroma</th>
<th>Hue</th>
<th>L*</th>
<th>Chroma</th>
<th>Hue</th>
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<tr>
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<td>F1</td>
<td>P1</td>
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<td>F1</td>
<td>P1</td>
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<td>'Butterbush' Family</td>
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</table>

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

<table>
<thead>
<tr>
<th>Family</th>
<th>Variance estimate</th>
<th>L*</th>
<th>Chroma</th>
<th>Hue</th>
<th>L*</th>
<th>Chroma</th>
<th>Hue</th>
<th>L*</th>
<th>Chroma</th>
<th>Hue</th>
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</thead>
<tbody>
<tr>
<td>'Table Gold Acorn' (TGA) × PI 314806</td>
<td>σ²_p1</td>
<td>4.56</td>
<td>25.93</td>
<td>0.0008</td>
<td>9.59</td>
<td>46.42</td>
<td>0.0023</td>
<td>7.98</td>
<td>23.45</td>
<td>0.0007</td>
</tr>
<tr>
<td>'Table King Bush' (TKB) × PI 314806</td>
<td>σ²_p1</td>
<td>3.83</td>
<td>33.28</td>
<td>0.0017</td>
<td>2.20</td>
<td>5.62</td>
<td>0.0010</td>
<td>2.02</td>
<td>0.47</td>
<td>0.0001</td>
</tr>
<tr>
<td>'Butterbush' (BB) × 'Sucrine DuBerry' (SDub)</td>
<td>σ²_p1</td>
<td>11.04</td>
<td>6.83</td>
<td>0.0023</td>
<td>2.66</td>
<td>10.90</td>
<td>0.0086</td>
<td>0.28</td>
<td>10.22</td>
<td>0.0040</td>
</tr>
</tbody>
</table>

Comparison of L* values for each family and generations within each 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

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

in the BC1P2 population (0.28). The heritabilities for all three populations for hue angle were negative and are considered zero.

<table>
<thead>
<tr>
<th>Family</th>
<th>Variance estimate</th>
<th>L*</th>
<th>Chroma</th>
<th>Hue</th>
<th>L*</th>
<th>Chroma</th>
<th>Hue</th>
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Table 4. Variance estimates for calculating heritability of colorimetric values (L*, chroma, and hue) measured in fruit mesocarp (flesh) in intraspecific F1 individuals and F2 and BC1 populations in Cucurbita pepo and Cucurbita moschata families with one fruit per plant.

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</table>

Numeric 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.

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.

Fruit 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.

Values converted from degrees to radians for calculation.

Represents the variance present in the F2, BC1P1, or BC1P2 population within the respective family.
Table 5. Genetic means estimates for heritability of colorimetric values (L*, chroma, and hue) measured in fruit mesocarp (fl) of F2 and BC1 populations in Cucurbita pepo and Cucurbita moschata families with one fruit per plant.x

<table>
<thead>
<tr>
<th>Family</th>
<th>Genetic parameter</th>
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<th>$\sigma_e^2$</th>
<th>$H$</th>
<th>$\sigma_g^2$</th>
<th>$\sigma_p^2$</th>
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<th>$\sigma_E^2$</th>
<th>$\sigma_G^2$</th>
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<td>'Table Gold Acorn' (TGA) × PI 314806</td>
<td>$L$</td>
<td>7.93</td>
<td>±0.82</td>
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<td>Chroma</td>
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<td>±6.68</td>
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<td>±0.0015</td>
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<td>±0.0231</td>
<td>4.57</td>
<td>±0.0085</td>
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<tr>
<td></td>
<td>Hue</td>
<td>13.94</td>
<td>±0.29</td>
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<td>1.59</td>
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<td>1.88</td>
<td>±0.0086</td>
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<td>±0.0162</td>
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<tr>
<td></td>
<td>Chroma</td>
<td>13.55</td>
<td>±1.24</td>
<td>0.048</td>
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<td>9.16</td>
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<td>±0.0624</td>
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Numeric description of color using L*ab* 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) — red, 0°; orange-red, 45°; yellow, 90°; green, 135°; blue, 180°.

C. pepo families include ‘Table Gold Acorn’ (TGA) × PI 314806 and ‘Table King Bush’ (TKB) × PI 314806.

C. moschata families include ‘Buttershish’ (BB) × ‘Sucrine DuBerry’ (SDob) and ‘Table Gold Acorn’ (TGA) × PI 314806.

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 F2, BC1P1 and BC1P2 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 replicates 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 ($r = -0.66$) (Seroczyńska et al., 2006; Ile and Kabelka, 2009, respectively), lutein content ($r = -0.68$) (Ile and Kabelka, 2009), beta-carotene content ($r = -0.54$; $r = -0.49$) (Seroczyńska et al., 2006; Ile and Kabelka, 2009, respectively), and alpha-carotene their respective families. However, CIE L*ab* 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 F1 and BC1P2 generations in both the TGA and TKB families.

For the BB family, mean hue angle values were highest in the F1 and F2 generations in 2008, and BC1P1 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 F1, F2, and BC1P1 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.
content ($r = -0.49$) (Ilie and Kabelka, 2009) in earlier studies in *Cucurbita*. In citruss, 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 (Lopez Camelo and Gomez, 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 values 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 F₂ and BC₁ 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 F₁, created from each cross of the former. Spearman’s rank correlation coefficient revealed no significant change in rank of genotypes for L* ($r = 0.97, P ≤ 0.0001$), chroma ($r = 0.97, P ≤ 0.0001$), 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 variation 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 F₂ and BC₁ 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 (*Citrus crrus lanata* (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*ab* 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 F₂ and BC₁ 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 F₂ generation. No individuals had L* lower values than TGA for the F₂ and BC₁ populations, and chroma and hue in the BC₁ 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 F₂ population, but it is difficult to establish if this was due to differences of maturity levels or other environmental effects. Moderate broad-sense heritability 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 F₂ and BC₁ 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 BC₁ 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 (Ilie 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 (Leser and Eischen, 1995). This suggests that accumulation of other carotenoid pigments in other cucurbit crops may also be affected by environmental conditions. Planting F₂ and BC₁ 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 in flesh color in *Cucurbita*. Shiffris 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 cal-
culating 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 transgres-
sive segregants in the F2 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.

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