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Genes for "Reverse" Fruit Striping in Squash (*Cucurbita pepo*)

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Longitudinal striping is a common fruit color pattern in *Cucurbita pepo* and is conferred by any of several alleles at the *light coloration-1* locus. Normally, dark stripes appear over the fruit surface areas between the 10 main subepidermal vein tracts with the lighter background color over and adjacent to these vein tracts. Less commonly, the striping is "reversed," that is, lighter than the background color. The present work was conducted to elucidate the mode of inheritance of reverse striping. The results indicated that reverse striping is conferred by the complementary interaction of an allele for striping with a heretofore unidentified allele at the *light coloration-2* locus which is hereby designated $l\text{-}2^R$. Fruits of plants carrying an allele for striping and that are homozygous for $l\text{-}2^R$ have completely reversed striping, whereas those heterozygous for $l\text{-}2^R$ have striping that is completely normal or that is partially normal and partially reversed.

Key words: *complementary genes, fruit coloration, multiple allelism*

External longitudinal striping of the fruit is common in the Cucurbitaceae, both in wild taxa and in cultigens. Striping is expressed as alternating intense or dark stripes and light stripes, and the breadth and contiguity of the respective dark/intense and light-colored stripes is under genetic control. Genes conferring striping have been identified in watermelon, *Citrullus lanatus* (Thunb.) Matsum. & Nakai (Guner and Wehner 2003; Gusmini and Wehner 2006), melon, *Cucumis melo* L. (Burger et al. 2005–2006; Pitrat 2005–2006), and pumpkin, squash, and gourd, *Cucurbita pepo* L. (Paris and Brown 2005). Although the stripes, dark/intense and light, number more or less 20 of each on watermelon, there are 10 of each on melon and 10 or sometimes 12 or rarely 14 of each on pumpkin, squash, and gourd. The stripes are positioned on the exocarp according to the position of the underlying main carpellary vein tracts (Duchesne 1786; Paris 2002a, 2007).

Striping in *Cucurbita pepo* has several different types of expression. Most commonly, broad, contiguous dark/intense stripes alternate with narrow, light stripes of the background color. The dark/intense stripes are broad and contiguous along the length of the fruit, occupying most of its surface, and the light stripes are positioned immediately above the main carpellary vein tracts (Paris 2000b). Not as common is the phenotype of narrow, broken (noncontiguous) dark/intense stripes (Paris and Burger 1989). More rare are irregular stripes, which are distributed sporadically over the fruit surface (Paris 2003). Each of these types of stripes is conferred by a particular allele at the *l-1* (*light coloration-1*) locus. The allele for broad-contiguous stripes, $l\text{-}1^{B.St}$, is dominant to the allele for narrow, broken stripes, $l\text{-}1^{St}$. Both are dominant or codominant to $l\text{-}1^{i.St}$ for irregular stripes, meaning that on occasion both broad-contiguous stripes or narrow-broken stripes can be augmented with irregular stripes to produce a total of 5 striped phenotypes: broad contiguous, narrow broken, irregular, broad contiguous plus irregular, and narrow broken plus irregular (Paris 2003). All these alleles for striping are dominant to *l-1*, which confers light color over the entire fruit surface, and recessive to the top dominant allele, *L-1*, which confers dark/intense coloration over the entire fruit surface (Paris and Nerson 1986).

Fruit striping can be obvious throughout fruit development if the dominant allele at the *l-2* (*light coloration-2*) locus is present together with an allele for striping (Paris and Burger 1989; Paris et al. 2003). The *L-2* allele is quite common and, in combination with the $l\text{-}1^{B.St}$ allele, is responsible for the bold, developmentally constant striping so obvious on fruits of many 'cocozelle' cultivars (*Cucurbita pepo* subsp. *pepo* 'Cocozelle' Group) (Paris 1986, 2000b). However, fruit color of *C. pepo* is a function of fruit development, meaning that fruit coloration can change dramatically over time (Duchesne 1786; Shifriss 1949; Paris 2007) and that fruit color inheritance must be studied accordingly. For example, the fruits of most of the pumpkin cultivars (*C. pepo* subsp. *pepo* Pumpkin Group) grown in the United States are light green when young, becoming blackish green by 2½ weeks past anthesis, and then turn intense orange on ripening, 6 weeks after anthesis. As noted by Duchesne (1786) so long ago in regard fruit striping, the changes over development can be stunning, with stripes that

are dark becoming light and stripes that are nearly white becoming nearly black. In other words, the intensity or darkness of the coloration of the stripes can reverse.

'Delicata' is an unusual cultivar of *Cucurbita pepo* subsp. *texana* (Scheele) Filov that is probably most closely related to the Acorn Group (Goldman 2004). During the several days immediately after anthesis, the young fruit, if examined closely, can be seen to have 10 broad stripes that are slightly darker and alternating with 10 narrow, slightly lighter stripes. Within a week past anthesis, the darker stripes fade and the lighter stripes accumulate pigmentation, and by 15 days past anthesis, the broader stripes have become lighter, whereas the narrow stripes of the background color have become darker, that is, the fruits of this age and afterward clearly show 10 broad lighter stripes alternating with 10 narrow darker stripes. That is, 'Delicata' exhibits what could be called "reverse" striping. Initial data have suggested that the reverse striping of 'Delicata' is conferred by 2 or more genes and that one of these genes is *l-1*^{BSt} (Paris 2002a). However, the identity of the gene or genes conferring the reversal of the striping has not as yet been revealed. The objective of the present work was to determine the mode of inheritance of reverse striping.

Materials and Methods

Seeds of 'Delicata' were obtained in 1981 from Stokes Seeds (Buffalo, NY). 'Delicata' plants have dark stems and their fruits, by 15 days past anthesis, display distinct reverse striping. Specifically, the fruits have broad light yellow stripes alternating with the narrow dark green stripes of the background. 'Delicata' was cross-pollinated in 1991 with a bush inbred nearly isogenic with 'Vegetable Spaghetti' (*C. pepo* subsp. *pepo* Vegetable Marrow Group). This inbred, designated 85a-30-45-17, like 'Vegetable Spaghetti' has genotype *d/d l-1/l-1 l-2/l-2* (Paris and Nerson 1986; Edelstein et al. 1989), the plants having light stems and fruits that are entirely light greenish yellow colored, referred to as "plain light," by 15 days past anthesis. F_1 plants were backcrossed to 85a-30-45-17. Backcrossing was repeated through to the sixth generation, with selection in each generation for the reverse striping and the dark stem characteristics of 'Delicata,' but it was noticed that the expression of the reversal of striping was always incomplete, that is, the stripes were lighter than the background color for only a part of their length. Sixth backcross generation plants were self-pollinated. The self-pollinated progeny were, in turn, self-pollinated, and some progeny were recovered that exhibited complete expression of reverse striping, that is, the stripes were lighter than the background over their entire length. These progeny were selected, and self-pollination and selection was repeated for 1–3 generations until 2 true-breeding reverse-stripe inbreds were developed, one designated 1349-8-10-9 having the light stems of the recurrent parent and another designated 1349-12-17-1-1-44 having the dark stems of the donor parent. These 2 new inbreds are thus nearly isogenic with the recurrent parent, differing only in the reverse-stripe characteristic, with the latter inbred differing also in stem color.

The mode of inheritance of reverse stripes was investigated by crossing these 2 new near-isogenic inbreds with 3 previously established near-isogenic bush inbreds (Table 1). The light-stem, reverse-stripe inbred 1349-8-10-9 was crossed with 85a-30-45-17, described above, and with an accession designated 676a-8-12-9, genotype *d/d l-1/l-1 L-2/L-2* (Paris 2003), which has light stems and fruits that are blue-gray "type 2" green by 15 days past anthesis (Paris and Nerson 1986). The reverse-stripe inbred 1349-12-17-1-1-44, which has the dark stem characteristic derived from 'Delicata,' was crossed with the accession designated 540a-1-1-2-4, genotype *D/D l-1/l-1 l-2/l-2*, which has dark stems and fruits that are dark green by 15 days past anthesis. The F_1 obtained by crossing 1349-8-10-9 with 676a-8-12-9 was also testcrossed on 85a-30-45-17, as was the F_1 obtained by crossing 1349-12-17-1-1-44 with 540a-1-1-2-4, in both instances to establish the genotype of 'Delicata' in regard to the *D* and *l-2* loci (Paris and Nerson 1986).

Parental-, filial-, backcross-, and testcross-generation plants were grown in the spring and summer seasons of 2005, 2007, and 2008 at Newe Ya'ar (northern Israel) using standard cultural practices (Paris and Burger 1989) including a within-row spacing of 50 cm. At the outset, external fruit color was to be examined at 3 developmental stages: young (2–5 days past anthesis), intermediate (15–18 days past anthesis), and mature (40–44 days past anthesis). However, these initial results indicated that mature fruits do not differ from intermediate-age fruits with regard to the presence or absence or degree of expression of reverse striping. Moreover, in these progenies, the young fruits were either entirely light colored or exhibited striping in various degrees of contrast, from obviously darker to barely discernable. This situation did not allow easy classification for color of young fruits, and hence, only the color of the intermediate-age fruits was scored for this investigation. Nonetheless, it

Table 1. Near-isogenic bush accessions used for crossing their phenotypes, and genotypes

Accession	Stem	Fruit at 15–18 days past anthesis	Genotype
85a-30-45-17	Light	Plain light yellow	*d/d l-1/l-1 l-2/l-2*
540a-1-1-2-4	Dark	Dark green	*D/D l-1/l-1 l-2/l-2*
676a-8-12-9	Light	Type 2, blue-gray green	*d/d l-1/l-1 L-2/L-2*
1349-8-10-9	Light	Reverse striped, light with type 2 background	To be determined
1349-12-17-1-1-44	Dark	Reverse striped, light with dark background	To be determined

was apparent that fruits on which broad, darker striping was discernable when they were young always exhibited broad striping, be it normal, partially reversed or completely reversed, on attaining intermediate age. The color of the plant stem was classified on the same day as intermediate-age fruit color (Paris and Nerson 1986).

Results

Crossing Light Stem Reverse Stripe with Genotype *d/d l-1/l-1 l-2/l-2*

Accession 1349-8-10-9 had light stems and its intermediate-age fruits had broad, contiguous reverse stripes, specifically broad, light yellow stripes alternating with the narrow, light blue-gray green stripes of the background (Figure 1). Inbred 85a-30-45-17, genotype *d/d l-1/l-1 l-2/l-2*, had light stems and plain light yellow fruits. The F_1 plants obtained from crossing these 2 inbreds, regardless of the direction of the cross, had light stems and their intermediate-age fruits were clearly striped. Quite often, however, the stripes were dark green for most of their length, that is, normal, and only near the peduncle end of the fruit were they light yellow, that is, reversed (Figure 1), with the background color being light blue-gray green over the entire length of the fruit, regardless of the adjacent stripe color.

Often as well, fruits borne on the same plant differed in the degree of expression of reversed stripes. For example, F_1 plants were found that bore both a normal, dark green striped fruit and a partly reverse, that is, a partly light yellow striped fruit. This necessitated considering plants that had normal-striped or partly reverse-striped fruits as the same phenotypic category. In extreme cases, fruit striping could be nearly completely normal on 1 fruit and nearly completely reversed on another (Figure 2). Such plants had to be classified into this same phenotypic category too, and they underscore the phenotypic plasticity of the heterozygotes and the difficulties encountered in correctly classifying individual plants. On the other hand, the reverse-stripe parents bore only completely reverse-stripe fruits, and hence, plants bearing completely reverse-striped fruits were considered as a separate phenotype. Given the phenotypes of the parents and the phenotypic range of the F_1, striping was considered to be dominant but complete reversal of striping was recessive.

Figure 1. Intermediate-age, 15–18 days past anthesis, fruits of inbreds 1349-8-10-9 (left) and 85a-30-45-17 (right) and their reciprocal F_1s. The fruit of 1349-8-10-9 has complete reverse striping, that is, its contiguous broad stripes are light yellow over their entire length and the narrow blue-gray green stripes are the background color. The fruit of 85a-30-45-17 is "plain light." The F_1 fruits exhibit striping, but the striping is normal, that is, darker than the background over most of the length of the fruit and is reversed, that is, lighter than the background, on only a small portion of the fruit, near the peduncle end.

In the F_2, 5 distinct phenotypes were observed (Table 2). Three resembled the 2 parents and the F_1. One of the 2 other phenotypes was fruits having broad, light yellow-green stripes on a plain light yellow background. The other phenotype was light blue-gray green fruits. The simplest explanation for the presence of these 5 phenotypes is to first assume that 2 genes are segregating. Accordingly, one of the genes accounts for the striping and the other accounts for the light blue-gray green background color. This by itself would result in the appearance of 4 phenotypes: 1) broad normal (dark green), partially reverse, or completely reverse (light yellow) striped with light blue-gray green background, 2) broad light yellow-green striped on plain light yellow background, 3) light blue-gray green, and 4) plain light yellow, in an expected ratio of 9:3:3:1. The first of these phenotypes actually embraces 2 of the 5 observed phenotypes, these being 1) normal or partially reverse striped and 2) completely reverse striped. Two alternative hypotheses could be offered for the splitting of the first phenotype. One would be that only 2 genes are interacting; one, for the striping itself, is dominant and the other, for the complete reversal, is recessive. It also means that the same gene conferring the light blue-gray green background color in a dominant fashion, that is, when heterozygous or homozygous, also confers complete reversal of striping, but in a recessive fashion, that is, only when homozygous. This would result in an expected 5-phenotype ratio of 6:3:3:3:1 for the F_2 progeny. The other hypothesis would be that a third, independent recessive gene is needed for complete reversal of striping. This would result in an expected 5-phenotype ratio of 27:9:12:12:4 for the F_2 progeny.

The results for the total F_2 population did not deviate significantly from the expected 6:3:3:3:1 ratio of the first hypothesis (Table 2). The results obtained from the F_2 population derived from the F_1 using the reverse-stripe parent, 1349-8-10-9, as the female fit this ratio quite well. The F_2 population derived from the F_1 using the reverse-stripe parent as the male did deviate significantly, having proportionally too few reverse striped and too many individuals with a plain light yellow background. With regard to the second hypothesis, the results for the total F_2 population deviated highly significantly from the expected 27:9:12:12:4 ratio (chi square = 16.320, $P < 0.01$).

Figure 2. Fruits approximately 2 (left) and 3 (right) weeks past anthesis on a single plant. The younger fruit, the one on the left, shows stripes nearly entirely light in color (reversed), and the older fruit, the one on the right, shows stripes nearly entirely dark in color (normal).

Of the plants bearing fruits that were striped and had a light blue-gray green background, those having normal or partially reversed stripes versus those having completely reversed stripes should segregate according to the 2-gene 6:3 ratio of the first hypothesis. The results for the total F_2 population fit quite well ($n = 193$, chi square = 0.065, $P = 0.80$) and regardless of the direction of the cross ($n = 122$, chi square = 1.480, $P = 0.22$ and $n = 71$, chi square = 1.380, $P = 0.23$). On the other hand, the results for the total F_2 population deviated highly significantly from the expected 3-gene 27:9 ratio of the second hypothesis (chi square = 8.706, $P < 0.01$).

If 2 complementary genes, one dominant and one recessive, interact to confer complete reverse striping, then the expected condensed F_2 ratio of all other phenotypes versus the completely reverse-stripe phenotype would be 13:3. The results tallied for the total F_2 population (Table 2) fit this expectation well (chi square = 0.444, $P = 0.50$) but not for the F_2 derived from the F_1 using the reverse-stripe parent, 1349-8-10-9, as the male, for which there was a lack of completely reverse-stripe individuals (chi square = 4.690, $P = 0.03$). If the results are tested against the expected 3-gene 55:9 ratio, however, the F_2 population derived from the F_1 using the reverse-stripe parent as the female deviated highly significantly (chi square = 9.492, $P < 0.01$).

If, for the total F_2 population, the number of individuals having a striped phenotype is tallied ($n = 278$) against the number individuals having a nonstriped phenotype ($n = 101$) (Table 2), the result does not deviate significantly from the expected 3:1 ratio of striped versus nonstriped (chi square = 0.550, $P = 0.46$) regardless of the direction of the cross (chi square = 0.038, $P = 0.86$ and chi square = 1.899, $P = 0.17$). Hence, striping is conferred by a single dominant gene.

However, if the number of individuals having a light blue-gray green background phenotype is tallied ($n = 261$) against the number of individuals having a plain light yellow background phenotype ($n = 118$) (Table 2), the result for the total F_2 population does deviate significantly from the expected 3:1 ratio of blue-gray green versus plain light yellow (chi square = 7.607, $P < 0.01$), the results for one direction of the cross being nonsignificant (chi square = 1.449, $P = 0.23$) but for the other, again in which 1349-8-10-9 was the male parent for the F_1, highly significant (chi square = 8.110, $P < 0.01$). The deviations from the

Table 2. Segregation for reverse striping and its components, fruits 15–18 days past anthesis, in crossing the plain light, *d/d l-1/l-1 l-2/l-2*, 85a-30-45-17 accession with the nearly isogenic light-stem, reverse-stripe accession designated 1349-8-10-9

	Number of plants								
Generation	Total	Normal + partially reversed stripes[a]	Completely reversed stripes[b]	Light yellow-green stripes[c]	Light blue-gray[d]	Plain light[e]	Expected ratio	Chi square	*P*
P_1, 1349-8-10-9	32	0	32	0	0	0	—	—	—
P_2, 85a-30-45-17	44	0	0	0	0	44	—	—	—
F_1, Total	95	95	0	0	0	0	—	—	—
F_2, $(P_1 \times P_2) \otimes$	221	75	47	45	36	18	6:3:3:3:1	3.784	0.44
F_2, $(P_2 \times P_1) \otimes$	158	52	19	40	32	15	6:3:3:3:1	11.181	0.02
F_2, Total	379	127	66	85	68	33	6:3:3:3:1	8.497	0.07
BC_1 (P_1), Total	117	69	48	0	0	0	1:1	3.769	0.05
BC_1 (P_2), Total	326	70	0	80	77	99	1:1:1:1	5.657	0.12

[a] Stripes darker than the light "type 2" blue-gray green background over their entire length or darker for a portion of their length, lighter for the rest.

[b] Stripes lighter than the light type 2 blue-gray green background over their entire length.

[c] Yellow-green stripes with "plain light" yellow background.

[d] Not striped, light type 2 blue-gray green.

[e] Not striped, plain light yellow.

6:3:3:3:1 and 13:3 ratios in this direction of the cross, noted above, can be attributed mainly to this lack of blue-gray green background individuals.

In the backcross to the plain light parent, 85a-30-45-17, 4 phenotypes were recovered (Table 2). These included all the phenotypes seen in the F_2 except for completely reverse striped. No such individuals would be expected, however, if this phenotype is indeed recessive. Segregation was in accordance with the expected 1:1:1:1 ratio for 2 genes. The striped versus nonstriped phenotypes as well as the blue-gray green versus plain light yellow phenotypes did not deviate significantly from expectations (chi square = 2.074, $P = 0.16$ and chi square = 3.141, $P = 0.08$, respectively).

In the backcross to the reverse-striped parent, only plants bearing striped fruits were recovered, those with normal and/or partially reverse-stripe fruits outnumbering those with completely reverse-stripe fruits, the results barely remaining within the 95% confidence interval for the single-gene 1:1 ratio (Table 2).

Crossing Light Stem Reverse Stripe with Genotype *d/d l-1/l-1 L-2/L-2*

Inbred 676a-8-12-9-41, genotype *d/d l-1/l-1 L-2/L-2*, had light stems and type 2 blue-gray green fruits. The F_1 plants obtained from crossing it with the broad, reverse-stripe inbred 1349-8-10-9, regardless of the direction of the cross, had light stems and broad-striped intermediate-age fruits. Quite often, however, the stripes were darker for most of their length, that is, normal, and only near the peduncle end of the fruit were they lighter, that is, reversed (Figure 3). Once again, striping was dominant.

Often, fruits borne on the same plant differed in the degree of expression of reversed stripes. For example, F_1 plants were found that bore both, a normal-striped fruit and a partly reverse-striped fruit, again making it necessary to consider plants having normal stripes and/or partly reverse stripes in the same phenotypic category. Plants bearing completely reverse-striped fruits were again considered as a separate phenotype (Table 3). Hence, complete reversal of striping was recessive in this cross too.

In the F_2, 3 distinct phenotypes were recovered, broad normal/partial reverse striping, broad reverse striping, and blue-gray green, nonstriped (Table 3). No plants bearing fruits that were plain light yellow or light yellow-green striped with plain light yellow background were observed. The total F_2 population deviated from the 9:3:4 ratio not because of the proportion of normal/partial reverse striped to complete reverse striped ($n = 187$, expected 3:1, chi square = 0.515, $P = 0.47$) but because of a lack of nonstriped individuals ($n = 227$, expected 3:1, chi square = 6.592, $P = 0.01$).

The expected ratio of normal/partial reverse striped combined with nonstriped versus the complete reverse striped would, again, be 13:3, the ratio expected for the complementary interaction of a dominant gene with a recessive gene. The F_2 populations did not deviate significantly from this expectation (for the total F_2 population, $n = 227$, chi square = 2.059, $P = 0.16$) (Table 3).

Figure 3. Intermediate-age, 15–18 days past anthesis, fruits of inbreds 1349-8-10-9 (left) and 676a-8-12-9 (right) and their reciprocal F_1s. The fruit of 1349-8-10-9 has complete reverse striping, that is, its contiguous broad stripes are light yellow over their entire length and the narrow blue-gray green stripes are the background color. The fruit of 676a-8-12-9 is type 2 blue-gray green. The F_1 fruits exhibit striping, but the striping is normal, that is, darker than the background over most of the length of the fruit and is reversed, that is, lighter than the background, on only a small portion of the fruit, near the peduncle end.

In the backcross to the reverse-stripe parent, only plants bearing striped fruits were obtained. Segregation was in good accordance with the expected 1:1 ratio of normal/partial reverse striped to complete reverse striped (Table 3). In the backcross to the blue-gray green nonstriped parent, only the normal/partial reverse-striped and blue-gray green nonstriped phenotypes were obtained. No complete reverse-stripe fruits were observed. This time, the nonstriped phenotype had a greater number of individuals than expected but the deviation from the 1:1 ratio was nonsignificant.

The F_1 was also testcrossed on the plain light yellow, 85a-30-45-17 (*d/d l-1/l-1 l-2/l-2*) inbred. No individuals were obtained that bore fruits that were plain light yellow or light yellow-green striped with plain light yellow background; all were blue-gray green or striped with a blue-gray green background, and the proportion of these 2 phenotypes did not deviate significantly from the expected 1:1 ratio (Table 3). It was noticed, however, that the intensity of the blue-gray green background did vary markedly among individual plants, and there appeared to be an association of intenser background color with dark stripes and lighter background color with partially reversed stripes.

Crossing Dark Stem Reverse Stripe with Genotype *D/D l-1/l-1 l-2/l-2*

Accession 1349-12-17-1-1 had dark stems and its intermediate-age fruits had broad, contiguous reverse stripes,

Table 3. Segregation for reverse striping, fruits 15–18 days past anthesis, in crossing the type-2, *d/d l-1/l-1 L-2/L-2*, 676a-8-12-9 accession with the nearly isogenic light-stem, reverse-stripe accession designated 1349-8-10-9

	Number of plants						
Generation	Total	Normal + partially reversed stripes[a]	Completely reversed stripes[b]	Blue-gray[c]	Expected ratio	Chi square	*P*
P_1, 1349-8-10-9	32	0	32	0	—	—	—
P_3, 676a-8-12-9-41	34	0	0	34	—	—	—
F_1, $P_1 \times P_3$	39	38	1	0	—	—	—
F_1, $P_3 \times P_1$	35	35	0	0	—	—	—
F_1, Total	74	73	1	0	—	—	—
F_2, $(P_1 \times P_3) \otimes$	55	29	15	11	9:3:4	2.802	0.24
F_2, $(P_3 \times P_1) \otimes$	172	107	36	29	9:3:4	6.080	0.04
F_2, Total	227	136	51	40	9:3:4	7.158	0.03
BC_1, $F_1 \times P_1$	177	92	85	0	1:1	0.277	0.59
BC_1, $F_1 \times P_3$	234	103	0	131	1:1	3.350	0.07
Test, $P_2 \times (P_1 \times P_3)$	176	78	0	98	1:1	2.273	0.13

[a] Stripes darker than the "type 2" blue-gray green background over their entire length or darker for a portion of their length, lighter for the rest.

[b] Stripes lighter than the type 2 blue-gray green background over their entire length.

[c] Not striped, type 2 blue-gray green.

specifically broad, light yellow stripes alternating with narrow, dark green stripes (Figure 4). Inbred 540a-1-1-2-4, genotype *D/D l-1/l-1 l-2/l-2*, had dark stems and dark green fruits. The F_1 plants obtained from crossing these 2 inbreds, regardless of the direction of the cross, had dark stems and their intermediate-age fruits usually had some light yellow striping, at least near the peduncle end, but occasionally no striping was visible, that is, the fruits were entirely dark green.

Figure 4. Intermediate-age, 15–18 days past anthesis, fruits of inbreds 1349-12-17-1-1-44 (left) and 540a-1-1-2-4 (right) and their reciprocal F_1s. The fruit of 1349-12-17-1-1 has complete reverse striping, that is, its contiguous broad stripes are light yellow over their entire length and the narrow dark green stripes are the background color. The fruit of 540a-1-1-2-4 is dark green. Notice that the F_1 fruits exhibit reversal of striping, that is, are of a lighter color than background, most strongly near the peduncle end.

Completely dark fruits and fruits showing partial reversal of striping were sometimes found on the same plant. Plants bearing fruits that fit one or both of these descriptions could be distinguished fairly readily from those bearing completely reverse-striped fruits, which were again classified as a separate phenotype (Table 4). Hence, complete reversal of striping was recessive in this cross too. The F_2 results fit a 13:3 ratio, consistent with the idea that reverse striping is conferred by the interaction of 2 genes, 1 dominant and 1 recessive. The backcross to the reverse-stripe parent had significantly more plants bearing reverse-stripe fruits than expected for the single-gene, 1:1 ratio; however, the result did not fit the 1:3 2-gene ratio either (chi square = 9.503, $P < 0.01$). No completely reverse-stripe individuals were found in the progeny of the backcross to the nonstriped parent nor were any found in the progeny of the testcross with 85a-30-45-17. All testcross progeny had dark stems and dark, nonstriped or partially striped fruits.

Discussion

From an initial investigation, it appeared that at least 2 genes were needed to confer reverse striping (Paris 2002a). One of the genes implicated was $l\text{-}1^{BSt}$ but the identity of the other gene or genes could not be determined from the limited amount of data.

One of the nonparental fruit color phenotypes recovered in the F_2 populations of the cross between 1349-8-10-9 (reverse striped) and 85a-30-45-17 (*d/d l-1/l-1 l-2/l-2*) (Table 2) was strongly reminiscent of one seen in previous investigations. This was the one having broad, yellow-green or "type 1" stripes on a plain light yellow background (Paris and Burger 1989; Paris 2003; Paris et al. 2003). This phenotype, conferred by genotype $l\text{-}1^{BSt}$/— *l-2/l-2*, was

Table 4. Segregation for reverse striping, fruits 15–18 days past anthesis, in crossing the dark, *D/D l-1/l-1 l-2/l-2*, 540a-1-1-2-4 accession with the nearly isogenic dark-stem, reverse-stripe accession designated 1349-12-17-1-1

Generation	Number of plants			Expected ratio	Chi square	*P*
	Total	Dark or partially reversed stripes[a]	Completely reversed stripes[b]			
P_4, 1349-12-17-1-1	32	0	32	—	—	—
P_5, 540a-1-1-2-4	46	46	0	—	—	—
F_1, $P_4 \times P_5$	43	43	0	—	—	—
F_1, $P_5 \times P_4$	47	47	0	—	—	—
F_1, Total	90	90	0	—	—	—
F_2, $(P_4 \times P_5) \otimes$	123	96	27	13:3	0.827	0.37
F_2, $(P_5 \times P_4) \otimes$	183	145	38	13:3	0.488	0.48
F_2, Total	306	241	65	13:3	1.247	0.26
BC_1 (P_4)	167	59	108	1:1	14.377	<0.01
BC_1 (P_5)	309	309	0	—	—	—
Test, $P_2 \times (P_4 \times P_5)$	257	257	0	—	—	—

[a] Completely dark green or dark green with light yellow stripes over a portion of the length of the fruits.

[b] Stripes lighter than the dark green background over the entire length of the fruit.

recovered in filial and backcross populations of the cross between 'Cocozelle,' *d/d* $l\text{-}1^{BSt}/l\text{-}1^{BSt}$ *L-2/L-2* and 85a-30-45-17, *d/d l-1/l-1 l-2/l-2* (Paris et al. 2003). Apparently, 'Delicata' does indeed carry the same allele as 'Cocozelle,' $l\text{-}1^{BSt}$ (Paris 2000b), and this was the dominant gene for broad striping observed segregating in filial and backcross progenies.

Another of the 5 fruit color phenotypes recovered in the F_2 populations, the light blue-gray green (Table 2), was reminiscent of the type 2 phenotype, conferred by allele *L-2* (Paris and Nerson 1986; Paris and Burger 1989), but in this investigation, it appeared to be of a shade lighter than that which had been observed previously. In this respect, the lightness of the blue-gray green resembled more the phenotype recovered in filial and backcross populations of the cross between a wild gourd from Mexico, *Cucurbita pepo* subsp. *fraterna* (Bailey) Lira, Andres & Nee, and inbred 85a-30-45-17. This similar phenotype, found to be conferred by an allele having a weaker expression, $L\text{-}2^{w}$ (Paris 2002b), had no association with reverse striping. No reverse-stripe individuals, complete or partial, were found in filial and backcross generations derived from that cross. In the present investigation, none of the 176 plants of the testcross, inbred 85a-30-45-17, *l-2/l-2*, crossed with the F_1 of inbred 676a-8-12-9 (*L-2/L-2*) and 1349-8-10-9 (reverse striped), had fruits that were plain light or had plain light background color (Table 3). This indicates that 1349-8-10-9 and therefore the donor of its reverse striping, 'Delicata,' carry an allele for light blue-gray green background color at the *l-2* locus. This allele, though dominant to *l-2*, is weaker in its effect on the background color than *L-2*. In filial and backcross progenies of the cross between 1349-8-10-9 and 85a-30-45-17, all reverse-stripe individuals had this light blue-gray green background color. Therefore, the allele conferring this background color is necessary for the expression of reverse striping, and it is hereby designated $l\text{-}2^{R}$.

The results presented in Table 2 fit the 2-gene hypothesis for the total F_2 population, whereas at the same time rendering the 3-gene hypothesis untenable. The 5 phenotype 6:3:3:3:1 ratio for the segregation of 2 loci can be explained by having the first phenotype conferred by genotype $l\text{-}1^{BSt}/$— $l\text{-}2^{R}/l\text{-}2$, the second by $l\text{-}1^{BSt}/$— $l\text{-}2^{R}/l\text{-}2^{R}$, the third by $l\text{-}1^{BSt}/$— *l-2/l-2*, the fourth by *l-1/l-1* $l\text{-}2^{R}/$—, and the fifth by *l-1/l-1 l-2/l-2*. When considering background color only, $l\text{-}2^{R}$ is dominant, but when considering full expression of reverse striping, $l\text{-}2^{R}$ is recessive. To produce the complete reverse phenotype, 2 complementary genes interact, the one for the striping itself, $l\text{-}1^{BSt}$, is dominant and the other for complete reversal of the striping, $l\text{-}2^{R}$, is recessive. Hence, in the backcross to the plain light parent, genotype *l-1/l-1 l-2/l-2*, only 4 phenotypes were recovered, the phenotype of complete reverse striping being absent.

None of the 257 plants of the testcross, inbred 85a-30-45-17, *l-2/l-2*, crossed with the F_1 of inbred 540a-1-1-2-4 (*D/D*) and 1349-12-17-1-1 (dark stem, reverse striped), had light stems (Table 4). This indicates that 1349-12-17-1-1 carries an allele for dark stem color at the *D* locus, probably the same one, *D*, carried by 540a-1-1-2-4. 'Delicata,' then, has genotype *D/D* $l\text{-}1^{BSt}/l\text{-}1^{BSt}$ $l\text{-}2^{R}/l\text{-}2^{R}$. Only reverse striping, partial or complete, was easily observed in the filial and backcross *D/D* progenies. Normal striping was sometimes discernable but not easily and readily so because allele *D* is epistatic (Paris and Nerson 1986).

Deviations from expected F_2 and backcross ratios were encountered, as noted earlier. The number of light type 2 blue-gray green background, $l\text{-}2^{R}$, individuals was less, and the number of plain light background, *l-2*, individuals was more than expected (Table 2). However, some fruits were obtained in which the blue-gray green background was so light as to be barely discernable, that is, the fruits were almost plain light in appearance. It is quite possible, therefore, that a few $l\text{-}1^{R}/$— plants were misclassified as plain light. In a second case, there was an excess of striped, $l\text{-}1^{BSt}/$—, and lack of nonstriped, *l-1/l-1*, individuals in the F_2 of the cross between 676a-8-12-9 and 1349-8-10-9 (Table 3).

Such a deviation was not encountered previously in a number of crosses involving segregation at the *l-1* locus, and therefore, this seems to have been merely a chance occurrence. In a third case, proportionally more complete reverse-striped individuals were observed in a backcross involving the dark stem (*D/D*) inbreds (Table 4). Possibly, this could be attributable to misclassification, as the contrast of the light-colored reversed stripes would be more striking on a dark green background than on a light blue-gray green background (compare Figure 4 with Figure 1 and Figure 3), leading to the interpretation that the reverse striping was complete. Only the few outer cell layers of the fruit are responsible for its external appearance, which could result in the sensitivity of fruits, especially on heterozygous plants, to fluctuations in external or internal environmental factors (Figure 2). Nonetheless, segregation distortion has been encountered in *C. pepo* and, although not in a predictable fashion (Wilson and Payne 1994; Paris 1996, 2000a, 2002c; Zraidi et al. 2007), there seems to be a tendency toward elimination of genes from a donor parent when they enter a foreign genetic background.

Normal striping can be broad and contiguous, narrow and noncontiguous, irregular, combined irregular with broad and contiguous and combined irregular with narrow and non-contiguous (Paris 2003). By backcrossing, it has been possible to obtain reversals of all 5 of these types of striping (Paris 2008). Moreover, when 'Delicata' was crossed with 'Fordhook Zucchini,' *L-1/L-1 L-2/L-2*, the intermediate-age F_1 fruits were almost entirely light colored (Paris HS, unpublished data).

The reverse-stripe characteristic has been known for some time from cultivars of *C. pepo* subsp. *texana*, especially of the Acorn Group. Nonetheless, the intensity and the hue of the reverse stripes seem to differ among cultivars (Baggett and Kean 1990; Goldman 2004; Paris 2007). Reverse striping is rarely encountered in cultivars of *C. pepo* subsp. *pepo* (Paris 2001, 2007; Goldman 2004). The $l\text{-}2^R$ allele, although infrequent in *C. pepo* subsp. *pepo*, may be present in some cultivars of the Vegetable Marrow Group and possibly also in the 'Cocozelle' Group, as I have seen mature fruits in some segregating populations that have reverse striping. The fruits of the cultivars of these 2 groups are almost always harvested when very young, several days past anthesis, before the reverse striping is expressed, and hence, this characteristic would not be expected to have been noticed in these cultivars.

The fruits of 'Delicata' are used when mature. The broad reverse striping of the mature fruits is quite striking, being light yellow on a dark green background. In the present investigation, the difference in colors of the stripes of the mature fruits was less stark as the dark green background turned yellow-orange before maturity. Evidently, 'Delicata' has the same dominant *Mo-1* and *Mo-2* alleles for retention of chlorophyll as do cultivars of the Acorn Group (Paris 1997), resulting in the green background striping of the mature fruits, whereas 'Vegetable Spaghetti' and, quite likely, the inbreds derived from it are *mo-1/mo-1 mo-2/mo-2*, resulting in the change from green to yellow-orange prior to fruit maturity.

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