Inheritance and Epistasis Studies of Chlorophyll Deficiency in Lettuce

E.J. Ryder,1 Z.H. Kim,2 and W. Waycott3
U.S. Department of Agriculture, Agricultural Research Service, 1636 East Alisal Street, Salinas, CA 93905

ABSTRACT. Three mutant traits for chlorophyll deficiency in lettuce (Lactuca sativa L.), bleached bud, calico-2, and pale green, are inherited as single recessive alleles. Bleached bud is epistatic to another recessive allele, dappled. Calico-2 is epistatic to dappled. Pale green is hypostatic to chlorophyll deficient-3. The Vanguard cd mutant is the same as chlorophyll deficient-3. The light green mutant 8744-1 is the same as light green. Independent inheritance is shown for bleached bud and dappled, calico-2 and dappled, and pale green and chlorophyll deficient-3, respectively.

Genetic mapping in lettuce proceeds on two fronts. One is the generation of genetic markers of various types from a single cross by means of several molecular technologies (Kesseli et al., 1994). This enables the establishment of linkage between DNA-based markers and previously identified character genes (Waycott et al., 1999). The other is identification of new character genes, linkages, and epistatic effects by study of numerous crosses each involving relatively few traits. This report is of the latter type, and describes the inheritance of three new genes for chlorophyll deficiency, the epistatic relationship among these and other previously reported chlorophyll deficiency genes, and clarification of the identity of similar mutants.

The most recent publication on chlorophyll deficiency in lettuce described six mutants and their epistatic relationship to each other and to previously described mutants (Ryder, 1996). Of these, chlorophyll deficient-3 (cd-3) is recessive and epistatic to light green (lg); chlorophyll deficient-4 (cd-4) is recessive, hypostatic to virescent (vi), and linked to an anthocyanin gene; and chlorophyll deficient-5 (cd-5), chlorophyll deficient-6 (cd-6), and chlorophyll deficient-7 (cd-7) are unlinked recessive genes. It was shown that cd-5 and cd-6 are both epistatic to cd-7. The sixth gene, dappled (dap), is recessive, lethal in combination with sickly (si), epistatic to lg, and hypostatic to vi.

Materials and Methods

The trait bleached bud occurs in a genetic line 92-217. This line was one of several mutant types isolated from early flowering lettuce treated with ethyl methane sulfonate (EMS) for the study of gibberellin regulated dwarfs (Waycott et al., 1995). In two crosses, 92-217 × 'Salinas' and 92-217 × 'Prizehead', the male parents were a standard crispedhead cultivar and a standard red leaf cultivar, respectively. In four crosses, 92-163 × 92-217, 92-217 × 93-716-1 and reciprocal, and 93-716-28 × 92-217, the other parent was dappled.

Bleached bud may be described as follows: The cotyledons are green; the first true leaf and subsequent rosette leaves are blotchy green and yellow; cauline leaves are normal green; involucres are white when immature and turn light green with white tipped bracts as they mature.

Vanguard cd has yellow leaves, which are similar in appearance to those of 80-1277 (cd-3). The inheritance of Vanguard cd was studied in the cross Vanguard cd × 'Salinas 88'. Vanguard cd was found in a greenhouse population of 'Vanguard', a crisphead lettuce. The cross 80-1277 × Vanguard cd was made to test for alleleism.

Calico was selected from a field population of mutants derived from treatment with EMS. It was used in the following crosses for genetic analysis: Calico × 'Prizehead', Calico × Pseudo-tipburn, Calico × GL 77, Calico × 'Oakleaf', 92-163 × Calico, Calico × 80-1277 and reciprocal, and Calico × Vanguard cd and reciprocal. Pseudo-tipburn is a mutant type not reported here. GL 77 is a leaf type lettuce line from Korea. 'Oakleaf' is a leaf type cultivar.

Calico has green cotyledons with white margins. The true leaves show a mosaic of dark and light green, yellow-green, and white. Later appearing leaves, including the cauline leaves, are green with no mosaic effect. The involucres are white when immature, turning light green with maturity; this phenotype is very similar to bleached bud.

Pale green also was derived from a population treated with the mutagen and was originally called EMS yellow. It was studied in the cross 80-1277 × EMS yellow. Cotyledons are green. True leaves on young plants are very light green with a yellowish cast, becoming greenish-yellow on new leaves forming on older plants. Involucres on the seed head are greenish-yellow. Pedicels are short, giving the seed head a compressed appearance.

‘Australian’ and 8744-1 are both light green in color, with no apparent color change as the plant matures. ‘Australian’ is a leaf lettuce with lobed leaves. Leaf color is controlled by light green (lg) (Lindqvist, 1960; Ryder unpublished; Thompson, 1938). 8744-1 is a mutant derived from seeds treated with thermal neutrons. The cross 8744-1 × ‘Australian’ was made to study gene identity.

All new genes reported herein were studied in F1 populations and F2 families. All segregations were analyzed using $\chi^2$ comparisons.

Results and Discussion

Two groups of populations were grown and analyzed for the inheritance of bleached bud. The first included two crosses of 92-217 with ‘Salinas’ and ‘Prizehead’, respectively. Segregation of the bleached bud trait only was tracked. The F1 populations were green. In both F2 populations, segregation of green:bleached bud approximated closely to a 3:1 ratio (Table 1). Among F2 families from green F1 plants, the ratio of green:segregating families approximated 1:2. Within segregating F2 families, the ratio of
green:bleached bud plants approximated 3:1. Anthocyanin content contributed by ‘Prizehead’ was not affected by the chlorophyll deficient trait, nor did it mask the deficiency.

The second group consisted of four populations from crosses between the bleached bud parent (92-217) and plants with the trait dappled (92-163, 93-716-1, 93-716-28). F1 plants were green. The F1 populations segregated approximately 9 green:3 dappled:4 bleached bud (Table 2). One population fit less well than the other three, but heterogeneity was not significant ($\chi^2 = 5.55, P = 0.1–0.2$). Among F2 families from green or dappled F1 plants, the expected distribution of families was 1:2:2:4:2:1 for all green; green and dappled; green and bleached bud; green, dappled, and bleached bud; dappled and bleached bud; and all dappled. This expectation was met (Table 2). Finally, segregation within segregating F2 families approximated 3:1 for single gene segregation. In those families where both genes were segregating, the expectation of 9:3:4 was barely met ($\chi^2 = 6.66, P = 0.02–0.05$), because of an excess of dappled plants and a shortage of bleached bud plants.

The preponderance of evidence indicates that bleached bud is inherited as a single recessive and the gene is named bleached bud ($Blbl$). It is epistatic to dappled.

The Vanguard cd mutant was studied in the cross Vanguard cd x ‘Salinas 88’. The F1 was green. In F2, the ratio of green: chlorophyll deficient approximated 3:1 (Table 3). F3 family segregation from green plants approximated 1 green:2 segregating. Segregation within segregating families approximated 3:1. Results indicate a single gene with chlorophyll deficiency recessive. The similarity of the phenotype to that of cd-3 suggested making the cross 80-1277 x Vanguard cd and its reciprocal. All F1 plants were yellow in both crosses and there was no segregation in F2. Therefore, it is highly likely that the Vanguard cd mutant is cd-3 and the latter designation will be used in the remainder of the paper.

Table 1. Segregation ratios of the chlorophyll deficient mutant bleached bud in two lettuce populations.

| F2 (Exp. 3:1) | Green | Dappled | bb | $\chi^2$ | P |
|---------------|--------|---------|----|----------|---|
| 92-217 x ‘Salinas’ | 51 | 20 | 0.39 | 0.5–0.7 |
| 92-217 x ‘Prizehead’ | 62 | 21 | 0.004 | 0.95 |

F2 families

| Green | Segr. | bb | $\chi^2$ | P |
|-------|-------|----|----------|---|
| Among families from green F2 (Exp. 1:2) | | | | |
| 92-217 x ‘Salinas’ | 18 | 22 | 2.46 | 0.1–0.2 |
| 92-217 x ‘Prizehead’ | 11 | 26 | 0.21 | 0.5–0.7 |
| Within segregating families (Exp. 3:1) | | | | |
| 92-217 x ‘Salinas’ | 260 | 81 | 0.28 | 0.5–0.7 |
| 92-217 x ‘Prizehead’ | 279 | 95 | 0.03 | 0.8–0.9 |

Table 2. Segregation ratios of chlorophyll deficient mutants, bleached bud and dappled, in four lettuce populations.

| F2 (Exp. 9:3:4) | Green | Dappled | bb | $\chi^2$ | P |
|----------------|--------|---------|----|----------|---|
| 92-163 x 92-217 | 166 | 52 | 68 | 0.38 | 0.8–0.9 |
| 92-217 x 93-716-1 | 135 | 50 | 80 | 4.18 | 0.1–0.2 |
| 93-716-1 x 92-217 | 163 | 52 | 68 | 0.18 | 0.9–0.95 |
| 93-716-28 x 92-217 | 164 | 59 | 66 | 0.98 | 0.5–0.7 |

Among F2 families, 92-163 x 92-217 (Exp. 1:2:2:4:2:1)

| From green F2 plants | Green | Dappled | bb | $\chi^2$ | P |
|----------------------|--------|---------|----|----------|---|
| All green | 6 | 7.92 | 0.47 |
| Green:dappled | 18 | 15.83 | 0.30 |
| Green:bleached bud | 10 | 15.83 | 2.15 |
| Green:dappled:bleached bud | 33 | 31.67 | 0.06 |
| From dappled F2 plants | | | | |
| Dappled:bleached bud | 21 | 18.67 | 0.29 |
| All dappled | 7 | 9.33 | 0.58 |
| Total | | 4.78 | 0.3–0.5 |

Within segregating families (Exp. 3:1, 9:3:4)

| Green | Dappled | bb | $\chi^2$ | P |
|-------|---------|----|----------|---|
| 245 | 68 | 1.79 | 0.1–0.2 |
| 334 | 115 | 0.09 | 0.7–0.8 |
| 569 | 210 | 1.59 | 0.2–0.3 |
| 693 | 256 | 273 | 6.66 | 0.02–0.05 |
Three groups of populations were grown and analyzed for the inheritance of calico. The first group included populations from the crosses: Calico × ‘Prizehead’, Calico × Pseudo-tipburn, Calico × GL 77, and Calico × ‘Oakleaf’. Only the calico trait was studied. F1 plants were green. In three F2 populations, segregation for green:calico gave acceptable fits to a 3:1 ratio (Table 4). No heterogeneity was shown ($\chi^2 = 1.72, P = 0.3-0.5$). In the fourth F2 population, there was a severe shortage of calico plants. The only explanation that could be advanced was the failure of 25 seeds to germinate; if all or nearly all were calico, the fit to 3:1 would be improved. Segregation among F3 families approximated 1:2 or 1:2:1, and within segregating families approximated 3:1, as expected.

The second group consisted of populations segregating for calico and a second trait. One, 92-163 x Calico, segregated for dappled as well as calico. The F1 was green. The F2 segregated approximately 9 green:3 dappled:4 calico, suggesting that calico is inherited as a single recessive and is epistatic to dappled (Table 5). Among F3 families, family distribution approximated 1:2:2:4:2:1:4 for all green; green and dappled; green and calico; green, dappled, and calico; dappled and calico; all dappled; and all calico. Segregation within segregating F3 families approximated 9 green:3 dappled:4 calico; 3 green:1 dappled; 3 green:1 calico; and 3 dappled:1 calico, respectively, as expected.

The third group consisted of four populations, Calico × 80-1277 and its reciprocal and Calico × Vanguard cd and its reciprocal, which segregated for calico and cd-3. F1 plants were green. Two F2 populations segregated approximately 9 green:3 cd-3:3 calico:1 light green calico (Table 6). The F2 of 80-1277 x Calico segregated with an excess of both the cd-3 and light green calico phenotypes. The other reciprocal population, Vanguard cd x Calico, showed a severe shortage of the light green calico class.

F3 family data were taken from the Calico x Vanguard cd and reciprocal populations. In F3, families the light green calico and calico classes were difficult to separate and were counted as one class, calico. Among F3 families from green plants, both sets approximated 1:2:2.4 for all green; green and cd-3; green and calico; green, cd-3, and calico. Within segregating families, for both populations, segregation was as expected. As calico and light green calico were counted together as one class, segregation in those families, where both mutants were segregating, approximated 9 green:3 cd-3:4 calico. This is not epistasis, since the traits can be distinguished.

Whitaker (1968) described a chlorophyll deficient mutant, which he did not name. It was named calico later (Robinson et al., 1983) and symbolized Clcl. This mutant was subsequently lost and could not be compared to the one we describe. However, the description differed from the one described in this study; the cotyledons were light green and true leaves had patches of green and white tissue. Therefore, it was likely not the same mutant. Our calico is inherited as a single recessive and is named calico-2 (Cl-2cl-2).

Inheritance of pale green was studied, together with cd-3, in the cross 80-1277 x EMS yellow. The F1 was green. In one F2 population, segregation of green:pale green approximated 3:1, and segregation of green:pale green:cd-3 approximated 9:3:4 (Table 7). In a second F2 population, the plants were crowded in the flat and many died, which probably accounts for a poor fit to the expected 9:3:4. Among F3 families from green plants of both F2 populations, the ratio approximated 1:2:2:4 for all green; green and pale green; green and cd-3; and green, pale green, and cd-3. Among families from pale green plants, the ratio approximated 1:2:1 for all pale green; pale green and cd-3; and all cd-3. Within segregating families, ratios approximated 3:1 for green and pale green; green and cd-3; and pale green and cd-3 and approximated

### Table 3. Segregation ratios of chlorophyll deficiency in the cross Vanguard cd x ‘Salinas 88’.

| Observed no. | Green | Segregating | $\chi^2$ | $P$ |
|--------------|-------|-------------|----------|-----|
| F2 (Exp. 3:1) | 78 | 20 | 1.11 | 0.2–0.3 |
| Among F3 families from green plants (Exp. 1:2) | 12 | 39 | 2.21 | 0.1–0.2 |
| Within segregating F3 families (Exp. 3:1) | 603 | 177 | 2.21 | 0.1–0.2 |

### Table 4. Segregation ratios of chlorophyll deficient mutant calico, in four lettuce populations.

| Observed no. | Green | Calico | $\chi^2$ | $P$ |
|--------------|-------|-------|----------|-----|
| F3 (Expect 3:1) | | | | |
| Calico × ‘Prizehead’ | 110 | 25 | 3.03 | 0.05–0.1 |
| Calico × Pseudo-tipburn | 81 | 26 | 0.03 | 0.9–0.95 |
| Calico × GL 77 | 186 | 37 | 9.41 | <.01 |
| Calico × ‘Oakleaf’ | 168 | 54 | 0.05 | 0.8–0.9 |
| F3 families | Green | Segr. | Calico | | |
| Among families from green F2 (Exp. 1:2) | | | | |
| Calico × Pseudo-tipburn’ | 54 | 127 | 0.99 | 0.3–0.5 |
| Among families from F2 plants (Exp. 1:2:1) | | | | |
| Calico × ‘Prizehead’ | 13 | 23 | 11 | 0.19 | 0.9–0.95 |
| Within segregating families (Exp. 3:1) | | | | |
| Calico × Pseudo-tipburn | 2049 | 692 | 0.09 | 0.7–0.8 |
| Calico × ‘Prizehead’ | 194 | 81 | 2.91 | 0.05–0.1 |

*Number of F3 families include 79 from 81 green F2 plants shown plus 112 F3 families from another F2 not evaluated.*
Table 5. Segregation ratios of calico and dappled in the cross 92-163 x Calico.

|                     | Observed no. |       |       | $\chi^2$ | $P$  |
|---------------------|--------------|-------|-------|----------|------|
|                     | Green        | Dappled | Calico |          |      |
| $F_2$ (Exp. 9:3:4)  | 175          | 42     | 73     | 3.68     | 0.1–0.2 |
| Among $F_2$ families (Exp. 1:2:2:4:2:1:4) | Observed | Expected | $\chi^2$ |       |      |
| From green $F_2$ plants | From green $F_2$ plants | All green | 2 | 5.75 | 2.45 |
|                       | Green:dappled | 14 | 11.5 | 0.54 |
|                       | Green:calico | 15 | 11.5 | 1.07 |
|                       | Green:dappled:calico | 27 | 23.0 | 0.70 |
| From dappled $F_2$ plants | Dappled:calico | 11 | 11.5 | 0.02 |
|                       | All dappled | 7 | 5.75 | 0.27 |
| From calico $F_2$ plants | All calico | 16 | 23.0 | 2.13 |
| Total                |               |     |       | 7.18     | 0.3–0.5 |

Table 6. Segregation ratios for calico and cd-3 in four lettuce populations.

|                     | Green | cd-3 | Calico | Both | $\chi^2$ | $P$  |
|---------------------|-------|------|--------|------|----------|------|
| $F_2$ (Exp. 9:3:3:1) |       |      |        |      |          |      |
| Calico x 80-1277    | 127   | 55   | 43     | 16   | 2.84     | 0.3–0.5 |
| 80-1277 x Calico    | 159   | 72   | 43     | 25   | 10.20    | 0.01–0.02 |
| Calico x Vanguard cd| 156   | 48   | 47     | 12   | 1.78     | 0.5–0.7 |
| Vanguard cd x Calico| 160   | 40   | 48     | 7    | 8.20     | 0.02–0.05 |

Calico x Vanguard cd and reciprocal

|                     | Cal x Van cd | Reciprocal |
|---------------------|--------------|------------|
| Among $F_2$ families from green $F_2$ plants (Exp. 1:2:2:4) | Obs. | Exp. | $\chi^2$ | Obs. | Exp. | $\chi^2$ |
| All green           | 4   |      | 3.56   | 0.05 | 3    | 3.89   | 0.02 |
| Green:cd-3         | 6   |      | 7.11   | 0.17 | 6    | 7.78   | 0.03 |
| Green:calico      | 8   |      | 7.11   | 0.11 | 4    | 7.78   | 0.92 |
| Green:cd-3:calico  | 14  |      | 14.22  | 0    | 16   | 15.56  | 0.75 |
| Total $\chi^2$     |      |      | 0.33   | 1.72 |       |        |      |
| $P$                 |      |      | 0.95–0.98 |     | 0.5–0.7 |     |      |

Within segregating families (Exp. 9:3:4, 3:1)

|                     | Obs. no. | $\chi^2$ | $P$  |
|---------------------|----------|----------|------|
| Green               | 237      | 0.77     | 0.11 |
| cd-3                | 60       | 2.86     | 0.23 |
| Calico              | 101      | 0.02     | 1.35 |
| Total               | 292      | 0.3–0.5  | 2.68 |
| Green               | 137      | 0.09     | 0.14 |
| cd-3                | 41       | 0.27     | 0.42 |
| Total               | 178      | 0.5–0.7  | 0.56 |
| Green               | 172      | 0        | 0.11 |
| Calico              | 57       | 0        | 0.34 |
| Total               | 0        | >0.99    | 0.45 |

$^3$Includes calico and light green calico classes due to difficulty in separating the two classes.
The conclusion from these results is that pale green is inherited as a single recessive. The gene is named *pale green* and is symbolized *Pgpg*. It is hypostatic to *cd-3*.

F1 and F2 progeny of the cross 8744-1 x ‘Australian’ were all light green in color with no apparent variation. It was concluded that the mutant allele in 8744-1 was *light green (lglg)*, identical to that in ‘Australian’. The joint segregations of *dappled* and *bleached bud*, *calico-2* and *dappled*, and *cd-3* and *pale green* showed no deviations from independent inheritance. The number of individuals was low for a linkage study, so at this point the tentative conclusion is that there is no linkage between these pairs.

Information on the inheritance of these traits impacts primarily in their contribution to the lettuce genetic map and in potential physiology studies. As chlorophyll deficient mutants, they are less likely to have usefulness in a breeding program except as novelties.

### Table 7. Segregation ratios for pale green and chlorophyll deficient-3 in the cross 80-1277 (cd-3) x EMS yellow (pale green).

| F2 population | Green | pale green | cd-3 | χ² | P       |
|---------------|-------|------------|------|----|---------|
| Exp. 3:1      | 98    | 23         |      | 2.32 | 0.1–0.2 |
| Exp. 9:3:4    | 98    | 23         | 31   | 4.18 | 0.1–0.2 |
| Exp. 9:3:4    | 311   | 72         | 96   | 14.66 | <0.01  |

Among F3 families

From green plants (Exp. 1:2:2:4) | Observed | Expected | χ² | P       |
|----------------------------------|----------|----------|----|---------|
| All green                        | 2        | 3.89     | 0.92 |         |
| Green:pale green                 | 9        | 7.78     | 0.19 |         |
| Green:cd-3                       | 6        | 7.78     | 0.41 |         |
| Green:pale green:cd-3            | 18       | 15.56    | 0.38 |         |
| Total                            |          |          | 1.90 | 0.5–0.7 |

From pale green plants (Exp. 1:2) | Observed | Expected | χ² | P       |
|----------------------------------|----------|----------|----|---------|
| All pale green                   | 24       | 23.67    | 0.33 |         |
| Segregating                      | 47       | 47.33    | 0.33 |         |
| Total                            |          |          | 0.66 | 0.3–0.5 |

Within segregating families (Exp.9:3:4, 3:1) | Observed | Green | pale gr. | cd-3 | χ² | P       |
|--------------------------------------|----------|-------|----------|------|----|---------|
| Green:pale green:cd-3                | 216      | 86    | 102      |      | 1.96 | 0.3–0.5 |
| Green:pale green                     | 166      | 58    | 45       |      | 2.81 | 0.05–0.1 |
| Green:cd-3                          | 100      | 680   | 245      |      | 1.08 | 0.2–0.3 |

### Literature Cited

Kesseli, R.V., I. Paran, and R.W. Michelmore. 1994. Analysis of a detailed genetic linkage map of *Lactuca sativa* (lettuce) constructed from RFLP and RAPD markers. Genetics 136:1435–1446.

Lindqvist, K. 1960. Inheritance studies in lettuce. Hereditas 46:387–470.

Robinson, R.W., J.D. McCreight, and E.J. Ryder. 1983. The genes of lettuce and closely related species. Plant Breeding Rev. 1:267–293.

Ryder, E.J. 1996. Inheritance of chlorophyll deficiency traits in lettuce. J. Hered. 87:314–318.

Thompson, R.C. 1938. Genetic relations of some color factors in lettuce. USDA Tech. Bul. 620.

Waycott, W., S.B. Fort, and E.J. Ryder. 1995. Inheritance of dwarfing genes in *Lactuca sativa* L. J. Hered. 86:39–44.

Waycott, W., S.B. Fort, E.J. Ryder, and R.W. Michelmore. 1999. Mapping morphological genes relative to molecular markers in lettuce (*Lactuca sativa* L.) Heredity 82:245–251.

Whitaker, T.W. 1968. A chlorophyll-deficient mutant in lettuce. Veg. Improvement Nwsl. 10:5.