Three Routes to Orange Petal Color via Carotenoid Components in 9 Compositae Species

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We analyzed the petals of orange- and yellow-flowered cultivars of 9 Compositae (Asteraceae) species for total anthocyanin content, total carotenoid content, and carotenoid composition to clarify the mechanisms responsible for the differences in color. Petals of both orange- and yellow-flowered cultivars of these species contained yellowish carotenoids in common. There were three different ways for orange-flowered cultivars to add redness to the yellow base in order to form orange petals. The first way to display orange is to accumulate more anthocyanins than yellow-flowered cultivars; orange petals of *Chrysanthemum morifolium*, *Gerbera jamesonii*, and *Zinnia elegans* are mainly formed in this way. The second is to accumulate more carotenoids than yellow-flowered cultivars; orange petals of *Helianthus annuus*, *Tagetes erecta*, and *Tagetes petula* are formed in this way. The third is to accumulate more reddish carotenoids than yellow-flowered cultivars; orange petals of *Calendula officinalis*, *Gazania* spp., and *Osteospermum ecklonis* are mainly formed in this way. These three ways could be combined through inbreeding to produce orange flowers that vary in color tones.

Key Words: anthocyanin, carotenoid, Compositae, HPLC, petal color.

Introduction

Carotenoids and anthocyanins are the major pigments in petals (Goodwin and Britton, 1988; Grotewold, 2006; Harborne, 1988; Hirschberg, 2001; Mol et al., 1998). Carotenoids are generally responsible for petal colors in the yellow to orange range, and anthocyanins for those in the orange to blue range. The wide range of petal colors in various plants originates mainly from combinations of these pigments.

Orange coloration is generated by a combination of yellow and red pigments in many cases. In *Alstroemeria* (Tatsuzawa et al., 2004), *Rosa hybrida* (Yokoi and Saito, 1973), and *Zinnia elegans* (Boyle and Stimart, 1989), orange petals contain both carotenoids and anthocyanins, as yellow and red pigments, respectively. On the other hand, orange is produced only with carotenoids in some species, such as *Calendula officinalis* (Kishimoto et al., 2005), *Eschscholzia californica* (Strain, 1938), *Lilium lancifolium* (Deli et al., 1999), and *Tagetes erecta* (Moehls et al., 2001).

Many plants belonging to the Compositae (Asteraceae) family, including chrysanthemum (*Chrysanthemum morifolium* Ramat.), which is one of the most important ornamental plants in the world, have both yellow- and orange-flowered cultivars. Carotenoids are responsible for the deep yellow coloration in these plants (Hayashi, 1988), so the same carotenoids are probably produced in both orange and yellow petals. We tried to confirm this hypothesis and to determine the pigments responsible for the red coloration in the orange petals of 9 species by qualitative and quantitative comparison of pigments between yellow and orange petals.

Materials and Methods

Plant materials

In greenhouses at the National Institute of Floricultural Science (Tsukuba, Ibaraki, Japan), we grew 37 cultivars of 9 Compositae species: *C. officinalis* L. (calendula), *C. morifolium* Ramat. (chrysanthemum), *Gazania* spp. (gazania), *Gerbera jamesonii* Bol. ex Adlam. (gerbera), *Helianthus annuus* L. (sunflower), *Osteospermum ecklonis* (DC.) Norl. (African daisy), *T. erecta* L. (African marigold), *Tagetes petula* L. (French marigold), and *Z. elegans* Jacq (zinnia). Petals of fully-opened flowers were harvested and stored at −80°C until use (Table 1).
Measurement of color parameters

The chromaticity of 3 fresh petals of each cultivar was measured with a spectrophotometer (CD-100, Yokogawa Co., Ltd., Tokyo, Japan). It was expressed by the CIELAB (CIE 1976) method: L*, a* and b* values indicated lightness, redness, and yellowness, respectively (Robertson, 1977). Color saturation and hue were expressed as chroma (C) and hue-angle (h), respectively, which were calculated from a* and b* values. The calculation formulas were as follows:

\[
C = \sqrt{(a^*)^2 + (b^*)^2}
\]

\[
h = \tan^{-1}\left(\frac{b^*}{a^*}\right)
\]

Zero degrees of hue-angle means red, and 90 degrees mean yellow.

Analysis of flavonoids

Fresh petal tissue (0.5 g) was extracted with 1% HCl–

Table 1. Chromaticity in petals of orange- and yellow-flowered cultivars of 9 Compositae species.

| Species          | Cultivar     | Petal color | L   | a*   | b*   | h°  | C°  |
|------------------|--------------|-------------|-----|------|------|-----|-----|
| C. officinalis   | Alice Orange | Orange      | 68.1 ± 1.2  | 41.7 ± 1.8 | 93.4 ± 1.6 | 65.9 | 102.3 |
|                  | Orange Star  | Orange      | 67.7 ± 0.8  | 49.2 ± 1.0 | 90.3 ± 0.8 | 61.4 | 102.8 |
|                  | Orange Gem   | Orange      | 71.5 ± 0.7  | 35.9 ± 1.9 | 118.7 ± 1.1 | 69.5 | 102.6 |
|                  | Ponpon Orange| Orange      | 73.7 ± 1.5  | 32.3 ± 1.3 | 97.8 ± 2.0 | 71.7 | 102.9 |
|                  | Alice Yellow | Yellow      | 83.4 ± 0.5  | 3.2 ± 1.1  | 122.0 ± 0.8 | 88.5 | 122.1 |
|                  | Gold Star    | Yellow      | 83.2 ± 0.2  | 11.1 ± 0.8 | 115.5 ± 0.8 | 84.5 | 116.0 |
|                  | Golden Gem   | Yellow      | 85.0 ± 0.1  | −1.7 ± 0.2 | 118.7 ± 1.1 | 90.8 | 102.6 |
|                  | Ponpon Yellow| Yellow      | 85.3 ± 0.4  | −4.2 ± 0.4 | 120.2 ± 0.4 | 92.0 | 120.3 |
| C. marifulium    | Dark Dramatic| Orange      | 50.1 ± 1.8  | 27.1 ± 1.1 | 48.2 ± 2.6 | 60.6 | 55.3 |
|                  | Florida Marble| Yellow     | 92.7 ± 0.1  | −15.2 ± 0.3 | 77.8 ± 2.4 | 101.0 | 79.3 |
|                  | Vodka Lime   | Yellow      | 89.2 ± 0.3  | −14.4 ± 0.1 | 66.3 ± 2.6 | 102.2 | 67.8 |
| Gazania          | Daybreak Orange| Orange   | 74.4 ± 1.1  | 23.8 ± 2.6 | 110.8 ± 1.3 | 77.9 | 113.3 |
|                  | Daybreak Yellow| Yellow   | 79.2 ± 0.5  | 8.9 ± 0.7  | 122.2 ± 2.2 | 85.8 | 122.5 |
| G. jamesonii     | Orphe Orange | Orange      | 53.4 ± 1.1  | 62.7 ± 1.0 | 53.1 ± 0.5 | 40.3 | 82.1 |
|                  | Dancer Orange| Orange      | 63.1 ± 0.9  | 50.1 ± 1.5 | 40.2 ± 0.5 | 38.7 | 64.3 |
|                  | Labyrinth Orange| Orange   | 73.3 ± 0.4  | 28.3 ± 1.4 | 85.9 ± 0.8 | 71.8 | 90.4 |
|                  | Lambada Orange| Orange      | 52.4 ± 1.1  | 64.6 ± 0.8 | 62.2 ± 0.9 | 43.9 | 89.7 |
|                  | Illusion Yellow| Yellow    | 84.1 ± 0.3  | 2.5 ± 0.5  | 85.7 ± 1.2 | 88.4 | 85.7 |
|                  | Espirit Yellow| Yellow     | 86.4 ± 0.6  | −4.3 ± 0.5 | 101.4 ± 0.6 | 92.4 | 101.5 |
|                  | Sega Yellow  | Yellow      | 92.6 ± 0.2  | −13.0 ± 0.5 | 53.7 ± 1.4 | 101.5 | 65.0 |
|                  | Friesbee Yellow| Yellow   | 87.3 ± 1.5  | −5.8 ± 0.3 | 104.2 ± 2.0 | 93.2 | 104.3 |
| H. annuus        | Sunrich Orange| Orange      | 78.7 ± 0.5  | 14.4 ± 0.6 | 122.7 ± 2.9 | 83.3 | 123.5 |
|                  | Sonia Orange  | Orange      | 75.2 ± 0.7  | 20.9 ± 0.6 | 113.6 ± 1.0 | 79.6 | 115.5 |
|                  | Sunrich Lemon | Yellow      | 86.4 ± 0.4  | −4.5 ± 0.8 | 106.2 ± 2.5 | 92.4 | 106.3 |
|                  | Valentine Yellow| Yellow   | 88.4 ± 0.7  | −13.5 ± 0.4 | 67.7 ± 3.5 | 101.3 | 69.0 |
| O. ecklonis      | Jury Orange  | Orange      | 78.0 ± 0.5  | 23.9 ± 1.0 | 67.6 ± 1.6 | 68.7 | 67.8 |
|                  | Mikey Yellow | Yellow      | 88.0 ± 0.2  | −23.3 ± 0.4 | 74.9 ± 1.2 | 89.2 | 63.5 |
| T. erecta        | Orange Isis  | Yellow      | 72.9 ± 0.7  | 34.6 ± 0.9 | 111.9 ± 2.0 | 72.8 | 117.1 |
|                  | Yellow Isis  | Yellow      | 93.0 ± 1.0  | −15.4 ± 0.1 | 55.7 ± 1.3 | 105.4 | 57.8 |
| T. petala        | Safari Tangerine| Orange   | 65.6 ± 0.6  | 45.0 ± 1.2 | 111.9 ± 1.0 | 68.1 | 120.6 |
|                  | Bonanza Orange| Orange      | 68.7 ± 2.3  | 41.1 ± 4.7 | 115.2 ± 2.3 | 70.4 | 122.3 |
|                  | Safari Yellow | Yellow      | 87.6 ± 0.3  | −7.8 ± 0.4 | 111.6 ± 2.6 | 94.0 | 111.8 |
|                  | Bonanza Yellow| Yellow      | 87.4 ± 0.2  | −9.3 ± 1.3 | 109.9 ± 4.1 | 94.8 | 110.2 |
| Z. elegans       | Dreamland Coral| Orange    | 58.9 ± 1.1  | 50.6 ± 1.4 | 48.2 ± 4.2 | 43.6 | 69.8 |
|                  | Bonita Red   | Orange      | 42.8 ± 1.9  | 50.4 ± 2.7 | 43.4 ± 0.4 | 40.7 | 66.5 |
|                  | Dreamland Yellow| Yellow   | 76.7 ± 1.1  | 7.9 ± 0.6  | 116.2 ± 2.8 | 86.1 | 116.4 |
|                  | Bonita Yellow| Yellow      | 69.3 ± 0.5  | 5.3 ± 0.7  | 82.1 ± 2.5 | 86.3 | 82.3 |

* Chroma (C) and hue-angle (h) were calculated from a* and b* values (Robertson, 1977).

\(\pm\) Mean values ± SE (n = 3).
MeOH, and the extract was washed with diethyl ether to remove carotenoids. The UV-visible spectrum of each extract was recorded with a spectrophotometer (UV-240, Shimadzu Co. Ltd., Tokyo, Japan). Components whose absorption maxima ranged from 480 to 600 nm were counted as anthocyanins, and those from 300 to 400 nm as flavones (including flavonols). Absorbance at absorption maxima for each was measured, and total levels were calculated as cyanidin and luteolin equivalents by referring to a standard curve prepared from authentic cyanidin and luteolin. Measurements were performed in triplicate.

Analysis of carotenoids

An acetone extract of fresh petals (0.5 g) was partitioned between diethyl ether and aqueous NaCl. The organic layer was washed with water, and the residue was saponified with equivalent 5% KOH–MeOH for 1 h at room temperature. The saponified matter was then extracted with diethyl ether and washed with water. The organic layer was dried and dissolved in 1 mL MeOH and used in quantitative and HPLC analyses. The total content of carotenoids was estimated from the absorbance at absorption maxima using the E1% value of lutein (2550) (Britton, 1995), which was defined as the theoretical absorbance of a 1% solution in a cell of 1 cm pathlength. Measurements were performed in triplicate. Each extract was analyzed by HPLC with a Jasco MD-915 photodiode array detector (Jasco, Tokyo, Japan) under the following conditions: column, YMC Carotenoid (250 mm × 4.6 mm i.d., 5 μm; YMC, Kyoto, Japan); solvent A, methanol (MeOH)/methyl tert-butyl ether (MTBE)/H2O = 90 : 6 : 4 (v/v/v); solvent B, MeOH/MTBE/H2O = 25 : 71 : 4; gradient, 0/100, 12/100, 96/0 (min%/A); flow rate, 1.0 mL·min−1; column temperature, 35°C; UV/visible monitoring range, 200–600 nm. The percentages of each carotenoid component were calculated according to the peak area of HPLC chromatograms at a wavelength of 450 nm.

Effect of carotenoid concentration on chromaticity

Total carotenoid extract from petals of yellow-flowered chrysanthemum ‘Syu-ho no Chikara’ was adjusted to 3 g·L−1 and diluted with 99% MeOH sequentially from 10- to 100-fold. One hundred microlitres of each solution was added to a well of a 96-well plate. The plate was photographed with a digital camera (Camedia C-2500L, Olympus Co. Ltd., Tokyo, Japan), and the chromaticity was analyzed with software (Adobe Photoshop Elements 2.0, Adobe Systems Inc., San Jose, CA, USA).

Results

Measurement of color parameters

The hue-angles (h) of yellow-flowered cultivars ranged from 84 to 105 degrees, and those of orange-flowered cultivars ranged from 38 to 80 degrees (Table 1). The a* values of yellow-flowered cultivars ranged from −15 to 11, and those of orange-flowered cultivars ranged from 14 to 65. No remarkable difference in b* values was observed in most species, except in C. officinalis, G. jamesonii, and Z. elegans, in which yellow-flowered cultivars tended to have higher b* values than orange-flowered cultivars. L values of yellow-flowered cultivars were consistently higher than those of orange-flowered cultivars, and higher L values tended to be paired with lower a* values. Orange-flowered cultivars of C. officinalis, Gazania, H. annuus, T. erecta, and T. petula were brilliant orange, and their L and chroma (C) values tended to be higher than those of the other orange-flowered species.

Flavonoid content

Total anthocyanin extracts of each species showed the same absorption maxima (data not shown), indicating that there was no difference in their anthocyanin compositions. The total anthocyanin content of all the yellow-flowered cultivars was very low or undetectable. Orange-flowered cultivars of C. morifolium, Gazania, G. jamesonii, and Z. elegans contained a higher level of anthocyanins than yellow-flowered cultivars (Table 2). The other species showed no significant differences in anthocyanin content between orange- and yellow-flowered cultivars. In each species, there was no significant difference between orange- and yellow-flowered cultivars in yellowish flavone content (data not shown).

Carotenoid content

Orange-flowered cultivars of H. annuus, T. erecta, and T. petula contained 3 to 40 times the concentrations of carotenoids in yellow-flowered cultivars, and more than 1 mg·g−1 FW in their petals. Orange-flowered cultivars of O. ecklonis, Gazania and C. officinalis also had higher carotenoid contents than yellow-flowered cultivars, but the differences were not as pronounced. Table 3 shows the correlation between the carotenoid content and color tone. Redness (a* value) tended to increase as the carotenoid content increased.

Carotenoid components

We analyzed carotenoid components by HPLC, and compared components of orange-flowered cultivars with those of yellow-flowered cultivars of each species (Figs. 1 and 2). Peaks were identified by comparing retention time and absorbance spectra with those previously identified in the petals of C. morifolium (Kishimoto et al., 2004) and C. officinalis (Kishimoto et al., 2005). There were differences in carotenoid components between orange- and yellow-flowered cultivars of C. officinalis and Gazania (Tables 4 and 5, Fig.2). These orange-flowered cultivars contained unique carotenoids that were not found in yellow-flowered cultivars. We previously identified 10 unique...
carotenoids in petals of orange-flowered cultivars of *C. officinalis*; (5'Z,9'Z)-rubixanthin, α-carotene, (5'Z)-rubixanthin, δ-carotene, (5Z,9Z,5'Z,9'Z)-lycopene, γ-carotene, (5'Z)-γ-carotene, (5Z,9Z,5'Z)-lycopene, (5Z,9Z)-lycopene and lycopene. We defined those unique carotenoids and β-carotene whose main absorption maxima ranged from 446 to 473 nm as reddish carotenoids in Tables 4, 5 and 6. Among these 10 carotenoids, (5'Z)-rubixanthin, (5Z,9Z,5'Z)-lycopene, and lycopene were also found in petals of orange-flowered *Gazania*. UV-visible absorption maxima of

### Table 2. Total anthocyanin content and total carotenoid content in petals of orange- and yellow-flowered cultivars of 9 Compositae species.

| Species          | Cultivars   | Petal color | Total anthocyanins (µg·g⁻¹ FW) | Total carotenoids (µg·g⁻¹ FW) |
|------------------|-------------|-------------|---------------------------------|-------------------------------|
| *C. officinalis* | Alice Orange | Orange      | ND                             | 1696.2 ± 35.1*                |
|                  | Orange Star | Orange      | ND                             | 1449.4 ± 34.0                |
|                  | Orange Gem  | Orange      | ND                             | 1072.7 ± 43.5                |
|                  | Ponpon Orange | Orange     | ND                             | 1190.1 ± 101.6               |
|                  | Alice Yellow | Yellow      | 159.3 ± 2.4*                   | 1249.9 ± 56.8                |
|                  | Gold Star   | Yellow      | ND                             | 1260.8 ± 28.3                |
|                  | Golden Gem  | Yellow      | ND                             | 1178.6 ± 26.0                |
|                  | Ponpon Yellow | Yellow     | ND                             | 1093.3 ± 33.9                |
| *C. morifolium*  | Dark Dramatic | Orange     | 729.7 ± 13.4                   | 343.4 ± 3.7                  |
|                  | Florida Marble | Yellow   | ND                             | 144.9 ± 1.9                  |
|                  | Vodka Lime  | Yellow      | ND                             | 121.9 ± 2.9                  |
| *Gazania*        | Daybreak Orange | Orange   | 54.7 ± 3.7                     | 3037.9 ± 122.9               |
|                  | Daybreak Yellow | Yellow | ND                             | 1535.2 ± 112.5               |
| *G. jamesonii*   | Orphe       | Orange      | 446.6 ± 82.2                   | 136.3 ± 1.0                  |
|                  | Dancer      | Orange      | 913.8 ± 28.8                   | 90.0 ± 2.6                   |
|                  | Labyrinth   | Orange      | 131.3 ± 4.9                    | 156.6 ± 5.6                  |
|                  | Lambada     | Orange      | 692.1 ± 14.0                   | 180.2 ± 9.0                  |
|                  | Illusion    | Yellow      | ND                             | 203.2 ± 6.1                  |
|                  | Esprit      | Yellow      | ND                             | 215.1 ± 7.0                  |
|                  | Sega        | Yellow      | ND                             | 37.9 ± 1.9                   |
|                  | Fresbee     | Yellow      | ND                             | 187.2 ± 3.0                  |
| *H. annuus*      | Sunrich Orange | Orange    | 1023.8 ± 86.2                  | 103.8 ± 29.0                 |
|                  | Sonia       | Orange      | 1599.6 ± 54.3                  | 143.8 ± 2.7                  |
|                  | Sunrich Lemon | Yellow   | ND                             | 305.2 ± 13.9                 |
|                  | Valentine   | Yellow      | ND                             | 143.8 ± 2.7                  |
| *O. ecklonis*    | Jury        | Orange      | ND                             | 360.8 ± 29.0                 |
|                  | Mikey       | Yellow      | ND                             | 244.8 ± 1.6                  |
| *T. erecta*      | Orange Isis | Orange      | ND                             | 2130.0 ± 58.1                |
|                  | Yellow Isis | Yellow      | ND                             | 48.4 ± 3.0                   |
| *T. petula*      | Safari Tangerine | Orange | ND                             | 2019.6 ± 53.6                |
|                  | Bonanza Orange | Orange   | ND                             | 1957.7 ± 84.5                |
|                  | Safari Yellow | Yellow     | ND                             | 312.3 ± 37.3                 |
|                  | Bonanza Yellow | Yellow  | ND                             | 270.3 ± 2.5                  |
| *Z. elegans*     | Dreamland Coral | Orange  | 517.7 ± 22.6                   | 82.5 ± 2.4                   |
|                  | Bonita Red  | Orange      | 985.0 ± 106.9                  | 252.6 ± 23.8                 |
|                  | Dreamland Yellow | Yellow | ND                             | 414.0 ± 15.0                 |
|                  | Bonita Yellow | Yellow     | ND                             | 266.6 ± 6.7                  |

* Not detectable.

**Table 3.** Effect of carotenoid content on chromaticity.

| Carotenoid content (mg·L⁻¹)* | L       | a*      | b*       |
|------------------------------|---------|---------|----------|
| 30                           | 91.4 ± 0.8* | -15.6 ± 0.4 | 70.7 ± 0.9 |
| 300                          | 84.3 ± 0.4  | 3.0 ± 0.5  | 82.4 ± 0.3 |
| 3000                         | 58.8 ± 0.6  | 61.0 ± 0.5  | 63.4 ± 0.6 |

* Carotenoid solution was used at 100 µL per well of a 96-well plate.

**Mean values ± SE (n = 3).**
these carotenoids were at longer wavelengths than that of lutein (Table 5), a major carotenoid in the petals of 9 Compositae plants in this study (Figs. 1 and 2). We therefore consider that these carotenoids were responsible for the orange color formation of the petals. Although orange- and yellow-flowered cultivars of *O. ecklonis* showed the same composition, the proportions of three reddish carotenoids—(5'Z)-γ-carotene, (5Z,9Z,5'Z)-lycopene, and lycopene—were higher in the orange-flowered cultivar (Table 6, Fig. 2). These carotenoids have also been identified from petals of orange-flowered cultivars of *C. officinalis*. The other 6 species tested showed no difference in carotenoid composition or contents between orange- and yellow-flowered cultivars. Figure 1 shows an HPLC chromatogram of one cultivar as a representative of each species. The main carotenoid components in petals of *C. morifolium*, *H. annuus*, *T. erecta*, and *T. petula* were α-carotene derivatives, such as lutein and lutein-5,6-epoxide. By contrast, those of *G. jamesonii* and *Z. elegans* were β-carotene derivatives, such as zeaxanthin, antheraxanthin, violaxanthin, and neoxanthin, in addition to α-carotene derivatives. Almost all carotenoids detected from these 6 species were yellowish carotenoids whose main

**Fig. 1.** HPLC analysis of carotenoids of petal extracts of Compositae species that showed no difference in carotenoid composition between orange- and yellow-flowered cultivars. A, *C. morifolium* ‘Florida Marble’; B, *G. jamesonii* ‘Dancer’; C, *H. annuus* ‘Sunrich Orange’; D, *T. erecta* ‘Orange Isis’; E, *T. petula* ‘Safari Tangerine’; F, *Z. elegans* ‘Dreamland Coral’. The data on *C. morifolium* come from Kishimoto et al. (2004). DL, (3S,5S,6R,3'R,6'R)-5,6-dihydro-5,6-dihydroxylyutein; V, violaxanthin; N, (9Z)-neoxanthin; L, lutein-5,6-epoxide; 9Z-V, (9Z)-violaxanthin; 9Z,9Z-Le, (9Z,9Z)-lutein-5,6-epoxide; 9Z-Le, (9Z)-lutein-5,6-epoxide; 9Z-Le, (9Z)-lutein-5,6-epoxide; L, lutein; 9Z-L, (9Z)-lutein; 9Z-L, (9Z)-lutein; Ax, antheraxanthin; Z, zeaxanthin; 9Z-Z, (9Z)-zeaxanthin; L, lutein; 9Z-L, (9Z)-lutein; 9Z-L, (9Z)-lutein; Ax, antheraxanthin; Z, zeaxanthin; 9Z-Z, (9Z)-zeaxanthin; β, β-carotene.

**Fig. 2.** HPLC analysis of carotenoids of petal extracts of Compositae species that showed differences in carotenoid composition between orange- and yellow-flowered cultivars. A, *C. officinalis* ‘Alice Orange’ and ‘Alice Yellow’; B, *Gazania* ‘Daybreak Orange’ and ‘Daybreak Yellow’; C, *O. ecklonis* ‘Jury’ and ‘Mikey’. The data on *C. officinalis* come from Kishimoto et al. (2005). V, violaxanthin; Lx, luteoxanthin; Le, lutein-5,6-epoxide; 9Z-V, (9Z)-violaxanthin; F, flavoxanthin; Au, auroxanthin; 9Z-Le, (9Z)-lutein-5,6-epoxide; L, lutein; Ax, antheraxanthin; 9Z-L, (9Z)-lutein; 9Z-Z-R, (9Z,9Z)-rubixanthin; α, α-carotene; β, β-carotene; 5Z-R, (5Z)-rubixanthin; δ, δ-carotene; cis-Ly, (5Z,9Z,5Z,9Z)-lycopene; γ, γ-carotene; 5Z-Z, (5Z)-Z-carotene; 5Z,5Z,5Z-Ly, (5Z,5Z,5Z)-lycopene; 5Z,5Z-Ly, (5Z,5Z)-lycopene; Ly, lycopene. Single numbers show unknown carotenoids.
Table 4. Carotenoid composition in petals of *C. officinalis*.

| Carotenoid | Absorption maxima (nm) | Orange cultivar | | | Yellow cultivar | | |
|------------|------------------------|-----------------|---|---|-----------------|---|---|
| | | Alice Orange | % of total carotenoid | Carotenoid content (µg·g⁻¹ FW) | | Alice Yellow | % of total carotenoid | Carotenoid content (µg·g⁻¹ FW) | |
| Luteoxanthin | 398, 411, 448 | 11.0 | 186.6 | | 15.6 | 195.0 | |
| Lutein-5,6-epoxide | 416, 438, 469 | 1.6 | 27.1 | | 3.2 | 40.0 | |
| Flavoxanthin | 398, 420, 448 | 28.5 | 483.4 | | 42.6 | 532.5 | |
| Auroxanthin | 380, 401, 425 | 7.1 | 120.4 | | 10.7 | 133.7 | |
| (9Z)-Lutein-5,6-epoxide | 413, 435, 463 | 5.0 | 84.8 | | 8.5 | 106.2 | |
| Lutein | 444, 473 | 2.0 | 33.9 | | 5.0 | 62.5 | |
| Antheraxanthin | 444, 474 | 1.0 | 17.0 | | 2.5 | 31.2 | |
| (9Z)-Lutein | 440, 467 | 0.6 | 10.2 | | 1.5 | 18.7 | |
| (5Z,9Z)-Rubixanthin | 455, 485 | 4.0 | 67.8 | | — | — | |
| α-Carotene | 446, 475 | 0.8 | 13.6 | | — | — | |
| β-Carotene | 452, 479 | 3.4 | 57.7 | | 1.0 | 12.5 | |
| δ-Carotene | 433, 457, 488 | 1.4 | 23.7 | | — | — | |
| (5Z,9Z,5Z,9Z)-Lycopene | 437, 461, 491 | 4.1 | 69.5 | | — | — | |
| γ-Carotene | 461, 493 | 2.0 | 33.9 | | — | — | |
| (5Z)-β-Carotene | 463, 493 | 4.4 | 74.6 | | — | — | |
| (5Z,9Z)-Lycopene | 442, 467, 497 | 3.5 | 59.4 | | — | — | |
| (5Z,9Z)-Lycopene | 442, 467, 497 | 4.1 | 69.5 | | — | — | |
| Lycopene | 446, 473, 505 | 8.7 | 147.6 | | — | — | |

| Yellowish carotenoids | 56.8 | 963.4 | 89.6 | 1119.9 | |
| Reddish carotenoids | 39.4 | 668.3 | 1.0 | 12.5 | |
| Total carotenoids (µg·g⁻¹ FW) | 1696.2 | 1249.9 | |

* Percentage of peak area in the HPLC chromatogram at 450 nm. Data of ‘Alice Orange’ come from Kishimoto et al. (2005).
* Lutein equivalent.
* Range of main absorption maxima from 401 to 445 nm.
* Range of main absorption maxima from 446 to 473 nm.

Table 5. Carotenoid composition in petals of *Gazania*.

| Carotenoid | Absorption maxima (nm) | Orange cultivar | | | Yellow cultivar | | |
|------------|------------------------|-----------------|---|---|-----------------|---|---|
| | | Daybreak Orange | % of total carotenoid | Carotenoid content (µg·g⁻¹ FW) | | Daybreak Yellow | % of total carotenoid | Carotenoid content (µg·g⁻¹ FW) | |
| Violaxanthin | 416, 439, 469 | 2.8 | 85.1 | | 8.1 | 124.4 | |
| Lutein-5,6-epoxide | 416, 438, 469 | 3.1 | 94.2 | | 26.6 | 408.4 | |
| (9Z)-Violaxanthin | 412, 436, 464 | 4.5 | 136.7 | | 9.7 | 148.9 | |
| Unknown carotenoid 1 | 422, 445, 473 | 5.4 | 164.0 | | 3.3 | 50.7 | |
| (9Z)-Lutein-5,6-epoxide | 413, 435, 463 | 2.5 | 75.9 | | 13.4 | 205.7 | |
| Lutein | 444, 473 | 20.4 | 619.7 | | 19.8 | 304.0 | |
| Antheraxanthin | 444, 474 | 10.0 | 303.8 | | 4.7 | 72.2 | |
| (9Z)-Lutein | 440, 467 | 10.0 | 303.8 | | 4.7 | 72.2 | |
| (9Z)-Zeaxanthin | 445, 471 | 5.2 | 158.0 | | 1.6 | 24.6 | |
| β-Carotene | 452, 479 | 2.3 | 69.9 | | 1.7 | 26.1 | |
| (5Z)-Rubixanthin | 461, 491 | 25.0 | 759.5 | | — | — | |
| (5Z,9Z,5Z)-Lycopene | 442, 467, 497 | 2.5 | 75.9 | | — | — | |
| Lycopene | 446, 473, 505 | 3.5 | 106.3 | | — | — | |

| Yellowish carotenoids | 63.8 | 1938.2 | 91.8 | 1409.3 | |
| Reddish carotenoids | 33.3 | 1011.6 | 1.7 | 26.1 | |
| Total carotenoids (µg·g⁻¹ FW) | 3037.9 | 1535.2 | |

* Percentage of peak area in the HPLC chromatogram at 450 nm.
* Lutein equivalent.
* Range of main absorption maxima from 401 to 445 nm.
* Range of main absorption maxima from 446 to 473 nm.
petals of more carotenoids than yellow-flowered cultivars; orange as much anthocyanins as carotenoids. The petals of these species appear orange when they contain 0.8 to 10 times anthocyanins and yellow carotenoids. The orange petal color was due to a mixture of red anthocyanin contents than yellow-flowered cultivars. Orange-flowered cultivars had remarkably higher differences in carotenoid contents and components between orange- and yellow-flowered cultivars, but differences in anthocyanin contents and carotenoid components formed in this way. These species showed slight concentration of carotenoids caused the orange color (Table 3).

| Carotenoid          | Absorption maxima (nm) | Orange cultivar | Yellow cultivar |
|---------------------|-------------------------|----------------|----------------|
|                     |                         | % of total carotenoid | Carotenoid content | % of total carotenoid | Carotenoid content |
|                     |                         | (µg·g⁻¹ FW)         | (µg·g⁻¹ FW)       | (µg·g⁻¹ FW)         | (µg·g⁻¹ FW)       |
| Lutein              | 444, 473                | 6.3              | 22.7            | 19.1              | 46.8             |
| β-carotene          | 452, 479                | 6.6              | 23.8            | 10.0              | 24.5             |
| Unknown carotenoid 2| 443, 469                | 15.6             | 56.3            | 11.6              | 28.4             |
| (5Z,9Z,5Z)-Carotene | 463, 493                | 7.6              | 27.4            | 6.4               | 15.7             |
| (5Z,9Z,5Z)-Lycopene | 442, 467, 497           | 12.1             | 43.7            | 9.9               | 24.2             |
| Lycopene            | 446, 473, 505           | 46.9             | 169.2           | 37.2              | 91.1             |
| Yellowish carotenoids | 6.3                 | 22.7            | 19.1            | 46.8             |
| Reddish carotenoids | 88.9                  | 320.8           | 75.1            | 183.8            |
| Total carotenoids (µg·g⁻¹ FW) | 360.8              |                  |                  | 244.8            |

Discussion

We have shown that petals of yellow- and orange-flowered cultivars of 9 Compositae species contain yellowish carotenoids, such as lutein, zeaxanthin, and flavoxanthin, in common. We have revealed three different ways for orange-flowered cultivars to add redness to the yellow base in order to form orange petals.

The first is to accumulate more anthocyanins than yellow-flowered cultivars; the orange petal color of *C. morifolium*, *G. jamesonii*, and *Z. elegans* was mainly formed in this way. These species showed slight differences in carotenoid contents and components between orange- and yellow-flowered cultivars, but orange-flowered cultivars had remarkably higher anthocyanin contents than yellow-flowered cultivars. The orange petal color was due to a mixture of red anthocyanins and yellow carotenoids. The petals of these species appear orange when they contain 0.8 to 10 times as much anthocyanins as carotenoids.

The second way to display orange is to accumulate more carotenoids than yellow-flowered cultivars; orange petals of *H. annuus*, *T. erecta*, and *T. petula* were formed in this way. They had a brilliant color, showing high values of chroma (Table 1). These species had only slight differences in anthocyanin contents and carotenoid components between orange- and yellow-flowered cultivars, but significantly higher carotenoid contents in orange-flowered cultivars than in yellow-flowered cultivars. The a* value of the carotenoid solution was proportionate to the carotenoid content; that is, a high concentration of carotenoids caused the orange color (Table 3).

The third way to display orange is to accumulate more reddish carotenoids than yellow-flowered cultivars; orange petals of *C. officinalis*, *Gazania* and *O. ecklonis* were mainly formed in this way. Orange-flowered petals of those species had a brilliant color and showed relatively high values of chroma (Table 1). We previously reported that reddish carotenoids, defined as those whose main absorption maximum was longer than 450 nm, were found only in the petals of orange-flowered cultivars of *C. officinalis*, and most of these had a 5-cis configuration (Kishimoto et al., 2005). Here, we found that an orange-flowered cultivar of *Gazania* also has unique carotenoids with a 5-cis configuration, including (5Z)-rubixanthin and (5Z,9Z,5Z)-lycopene. *O. ecklonis* also contained 5-cis components, and their ratio to total carotenoids in the orange-flowered cultivar was higher than that in the yellow-flowered cultivar. We assume that the 5-cis configuration is formed enzymatically, and 5-cis isomerization activity may exist in orange petals of *C. officinalis* and *Gazania* and in both orange and yellow petals of *O. ecklonis*.

The orange petal colors of *C. morifolium*, *G. jamesonii*, and *Z. elegans* appeared dark and dull, and their lightness and color saturation were generally lower than those of the other 6 species (Table 1); therefore, the accumulation of anthocyanins decreases the lightness and color saturation of petals. In contrast, orange-flowered petals of *C. officinalis*, *Gazania*, *H. annuus*, *T. erecta*, and *T. petula*, whose petal color was formed only with carotenoids, had a brilliant color. The accumulation of carotenoids had little effect on lightness and color saturation.

In conclusion, it is clear that petals of the 9 Compositae species accumulated yellowish carotenoids, and that orange-flowered cultivars also added red by one of three different ways: accumulation of anthocyanins, carotenoids, or reddish carotenoids. These three ways could
be combined through inbreeding to produce orange flowers that vary in color tones through variations in lightness, color saturation, and redness.

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