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Dark Disk Color in the Flower of *Gerbera hybrida* is Determined by a Dominant Gene, *Dc*

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***Abstract*. The cultivated gerbera daisy [*Gerbera hybrida* (*G. jamesonii* Bolus ex Adlam × *G. viridifolia* Schultz-Bip)] produces flowers that have either a dark (shades of dark brown, brown-black, black-purple, or black) or light (shades of green-yellow, yellow-green, or light yellow) central disk. The dark-centered varieties have increased in popularity over the past 20 years and provided an exciting color contrast, especially in white, yellow, and various pastel-colored flowers. The objective of this investigation was to determine the mode of inheritance of disk color in gerberas. A series of crosses were made to produce P_A, P_B, F_1, F_2, BC_{1A}, and BC_{1B} progeny to complete the Mendelian genetic analysis. Phenotypic segregation ratios indicated that dark disk color was determined by a single dominant gene, designated *Dc*, and the light disk color by a recessive gene, *dc*. Dominance appeared to be complete in that the disk color was similar in both homozygous and heterozygous *Dc* plants.**

Cultivated gerbera daisies are diploids resulting from crosses of *G. jamesonii* × *G. viridifolia* and are given the provisional designation *Gerbera hybrida* (Asteraceae) (Bremer, 1994; Hansen, 1999). They are widely used by florists and nurserymen throughout the world. Much of the breeding and cultivar development has taken place in Europe, Israel, Japan, and the United States (Rogers and Tjia, 1990). Several investigators have examined various gerbera flower traits using quantitative genetic approaches (Drennan et al., 1986; Harding et al., 1990; Huang and Harding, 1998) and a Mendelian genetic analysis (Kloos et al., 2004; Martens and Forkmann, 2001; Tyrach and Horn, 1997). Kotilainen and coworkers have determined several of the molecular mechanisms controlling organ identity and shape and anthocyanin pigmentation during gerbera flower development (Elomaa et al., 1998; Helariutta et al., 1995; Kotilainen et al., 1999; Yu et al., 1999).

Some of the most attractive gerberas marketed have dark-centered flowers. The dark brown, brown-black, black-purple, or black disk in the center of the capitulum provides an exciting color contrast that is most noticeable in white, yellow, and various pastel-colored flowers. The dark disk pigmentation is due to the accumulation of anthocyanins in the pappus bristles found at the base of florets (Helariutta et al., 1995). The pappi of gerberas and other Asteraceae are considered to be true sepals that are homologous to the sepals of other angiosperms (Albert et al., 1998; Yu et al., 1999). The disk of wild-type flowers is usually green-yellow, yellow-green, or light yellow in color (Lynch, 1905; Vilmorin, 1909; Watson, 1889). In these flowers, the pappi do not accumulate significant amounts of anthocyanins.

Lynch (1906, 1909) apparently was the first to hybridize *G. jamesonii* and *G. viridifolia*. While making a series of species crosses and backcrosses, he obtained perfectly fertile hybrids exhibiting a variety of floret and disk color combinations, including the unusual dark-centered type. Dark-centered gerberas have increased in popularity, especially over the past twenty years. Although the inheritance of gerbera flower disk color had not been thoroughly investigated, Elomaa et al. (1998), while examining the effect of the *trans*-acting regulator GMYC1 in the regulation of the dihydroflavonol-4-reductase (*dfr*) gene, a late gene of the anthocyanin pathway, incidentally observed that in crosses of gerbera cultivars Regina (light disk) × Nero (dark disk) pappus pigmentation segregated in a ratio of 1:1 (data not presented). These results are consistent with what would be expected for a monogenic trait for disk color. The expression of the *gmyc1* gene in different cultivars suggested that it regulates *dfr* activity in the corolla and carpel, but not in the pappus and stamen.

Dark central disks are present in the flowers of several other members of the family Asteraceae (Armitage, 1997). Chrysanthemum *Dendranthema* x*grandiflorum* (Ramat.) Kitam. cultivars have yellow or yellow-green disks; however, the recently introduced cultivar 'Vyron' is unique in that it has a dark brown disk. The smooth oxeye *Heliopsis helianthoides ssp. scabra* (Dunal) Fernald 'Ballerina' has a brown disk and 'Goldgreenheart' and 'Summer Sun' have a greenish disk. Coneflowers *Rudbeckia maxima*, *R. triloba*, *R. fulgida*, and *R. hirta* L. usually have dark brown, black, or black-purple disks, whereas *R. nitida* and *R. laciniata* L. have green disks. The *R. hirta* mutant cultivar 'Green Eyes' has a green disk. Sunflowers *Helianthus angustifolius* and *H. decapetalus* L. have dark brown, black, or black-purple disks, whereas *H. atrorubens* L. has a dark red disk and *H. giganteus* L. and *H. decapetalus var. multiflorus* L. (*H. annus* × *H. decapetalus*) have yellowish disks (Armitage, 1997; Gill et al., 2003). The annual sunflower *H. annus* L. is represented by both dark-centered (anthocyanin-pigmented) and light-centered cultivars (Stoenescu, 1974; Fick, 1976). Disks lacking anthocyanins are usually yellow-green, yellow, or gold.

The objective of this study was to determine the mode of inheritance of the dark disk color in the flower of *Gerbera hybrida*. We used a Mendelian genetic analysis designed to identify the major gene(s) controlling this trait (Griffiths et al., 1999; Kloos et al., 2004).

Materials and Methods

Plant material. *Gerbera hybrida* used in experimental crosses were part of the North Carolina State Univ. (NCSU) gerbera collection. The origin and composition of the collection has been described previously (Kloos et al., 2004). The collection contained 507 breeding plants derived from 24 different cultivar lines. These and an additional 42 of their progeny were used as parents for inheritance studies. The parental stock included 421 plants with dark disks and 128 with light (wild-type) disks.

Genetic analysis. The genotype of each gerbera parent was determined by testcrosses (parent × wild type). Based on the parental genotypes, a series of crosses were made to produce P_A, P_B, F_1, F_2, BC_{1A}, and BC_{1B} progeny. Crosses were performed between 1997 and 2004. For each plant, the disk color phenotype of the first two to five mature capitula was classified using the new Royal Horticultural Society (RHS) Colour Chart (Royal Horticultural Society, 1995). Flowers usually appeared when the plant was 5 to 8 months of age. Chi-square tests were used to interpret phenotypic ratios and quantify the various deviations expected by chance. Pollination and seed storage methods have been described previously (Kloos et al., 2004).

Cultivation. Cultural practices for seed germination and the growing of breeding and progeny gerbera plants have been described previously (Kloos et al., 2004). Progeny from the above crosses were grown for at least 6 to 8 months in NCSU greenhouses during 1997–2005 and in fields located in Angier, Cary, and Sanford during 1997–99.

Results and Discussion

Disk color phenotypes. The mature capitula of selected gerberas representing the two disk color classes are shown in Fig. 1. The dark-centered gerbera parents and progeny (Fig. 1A

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Fig. 1. Mature capitula of representative gerberas having a dark or light central disk. Allelic symbols: *Dc* = dark disk color; *dc* = light (wild-type) disk color. (**A**) 12-5 (*Dcdc*), (**B**) 470-1 (*dcdc*), (**C**) 493-15 (*DcDc*), and (**D**) 467-11 (*dcdc*); (×0.5).

and C) had flower disks that varied from dark brown (RHS chart no. 200B), brown-black (RHS chart no. 200A) to black (RHS chart no. 202A). Less frequently, progeny flower disks exhibited a black-purple color (RHS chart no. 187A). Different progeny of a single cross often exhibited two or more of the above hues, and as the capitulum matured the disk color often faded to lighter brownish hues. The pappus bristles of individual florets and the achene (following fertilization) appeared brownish-purple or purple in color. We found that the dark disk phenotype could, in most instances (>98%), be predicted at the earliest bud developmental stage [stage 1, according to Helariutta et al. (1993)] by the presence of a purplish anthocyanin pigment lining the edge of bracts. The intensity of the bract pigment diminished as the bracts and corresponding flower capitulum matured. This pigment was not observed on the bracts of stage 1 through 7 buds or at the base of the mature capitulum of flowers with light-colored disks or on the bracts of buds of <2% of crosses producing progeny with the dark disk phenotype. The light–centered (wild type) gerberas (Fig. 1B and D) had green-yellow (RHS chart no. 1A or 1C), yellow-green (RHS chart no. 144A, 144B, 144D, 150B, 151B, 152C, 153A, 153D, or 154A), or light yellow (RHS chart no. 12B or 12C) disks. In several cultivars of our collection and some of their progeny, the center of immature light disks developed a light brownish pigmentation that eventually disappeared as the capitulum matured, eliminating any confusion with the dark disk phenotype. The pappus bristles of wild-type gerberas did not exhibit anthocyanin-like pigments. Subtle differences in shades or hues within dark and light disk colors were not examined biochemically or genetically. Since a specific shade or pattern of shades may be characteristic of the disks of progeny from certain crosses, one might expect that modifying genes play a role in disk color shade variation.

Genetic analysis of disk color. Testcrosses between dark-centered and wild-type gerberas produced progeny that were either dark-centered in a 1:0 ratio or dark-centered and light-centered in a 1:1 ratio ($\chi^2 = 0.15$ to 0.35, $P \geq 0.5$). These results are consistent with what would be expected if the dark disk color were under the control of a single dominant gene. This gene was designated *Dc* (dark disk color). The recessive wild-type allele was designated *dc* (light disk color). The total parent collection contained 123 homozygote and 298 heterozygote dark-centered plants and 128 homozygote wild-type plants. Homozygote *DcDc* plants could not be distinguished phenotypically from heterozygote *Dcdc* plants.

The pooled results of crosses between dark-centered and wild-type gerberas representing each genotypic class are given in Table 1. Large numbers of progeny were produced and classified as a consequence of concurrent genetic studies of other gerbera traits. Wild-type parents (P_A) when crossed to one another produced only wild-type progeny. *Dc* homozygotes (P_B) when crossed to one another produced only dark-centered progeny. F_1 progeny resulting from all crosses between *Dc* homozygotes and wild-type (*dcdc*) plants exhibited the dark disk trait. The F_2 generation, produced by crossing *Dcdc* heterozygotes, segregated into the ratio 3 dark-centered: 1 wild type as would be expected if the dark disk trait was determined by a single gene (*Dc*) that was dominant to the gene (*dc*) determining the wild-type phenotype. Dominance appeared to be complete. The mean number of F_2 progeny was 15/cross and the range was 6 to 61. Three chi-squares testing the same null hypothesis indicated that the various samples were taken from a common population with the above ratio. These included the sum of 168 chi-squares ($\chi^2 = 117.94$, $P = 0.99$), pooled chi-square ($\chi^2 = 0.74$, $P = 0.39$) (Table 1), and the heterogeneity chi-square ($\chi^2 = 117.2$, $P = 0.99$), the latter indicating a high consistency in the sample ratios. The BC_{1A} generation segregated as expected in a ratio of 1 dark-centered: 1 wild type. The mean number of BC_{1A} progeny was 14/cross and the range was 6 to 92. Chi-squares testing the null hypothesis included the sum of 126 chi-squares

Table 1. Phenotypic segregation ratios resulting from crosses between gerbera plants with different combinations of *Dc* alleles.

Cross[z]	Crosses (no.)	Progeny phenotype, Disk color shade[y] Dark	Light	Total	Expected ratio (dark/light)	χ^2	*P*
F_1: Dark disk (*DcDc*) × light disk (*dcdc*)	67	843	0	843	1:0	0	1
F_2: Dark disk (*Dcdc*) × dark disk (*Dcdc*)	168	1875	650	2525	3:1	0.74	0.39
BC_{1A}: Dark disk (*Dcdc*) × light disk (*dcdc*)	126	860	838	1698	1:1	0.28	0.59
BC_{1B}: Dark disk (*Dcdc*) × dark disk (*DcDc*)	130	2002	0	2002	1:0	0	1
P_B: Dark disk (*DcDc*) × dark disk (*DcDc*)	47	807	0	807	1:0	0	1
P_A: Light disk (*dcdc*) × light disk (*dcdc*)	36	0	437	437	0:1	0	1

[z]Allelic symbols: *Dc* = dark disk color; *dc* = light (wild-type) disk color.

[y]Phenotypes: descriptions are for capitula developed to the commercial stage where at least two whorls of disk florets were mature. Dark disk color: dark brown, brown-black, black-purple, or black. Light disk color: green-yellow, yellow-green, or light yellow.

(χ^2= 114.51, P= 0.76), pooled chi-square (χ^2= 0.28, P= 0.59) (Table 1), and the heterogeneity chi-square (χ^2= 114.22, P= 0.75). Collectively, they indicated that the various samples were taken from a common population with the above ratio. Crosses producing five or less F_2 or BC_{1A} progeny (primarily from full- and large-crested parents) were not included in the chi-square calculations or pooled data in Table 1 (Snedecor, 1956). All of the BC_{1B} progeny exhibited the dark disk trait.

A single dominant gene also determines the dark color of the central disk of *H. annus* (Fick, 1976; Mosjidis, 1982; Stoenescu, 1974). However, the phenotype in *H. annus* is not identical to *G. hybrida* in that the dark color of the disk of *H. annus* is primarily due to the presence of anthocyanin pigments in the corolla of disk florets (Fick, 1976); whereas the dark color of the disk of *G. hybrida* is due to the presence of anthocyanin pigments in the pappus bristles of florets (Helariutta et al., 1995).

This study examined the inheritance of the dark disk phenotype observed in flowers of certain cultivated gerberas. The single, major gene (*Dc*) controlling this phenotype was identified by a Mendelian genetic analysis. Since a dominant gene determines the dark disk trait, it can be easily monitored following distribution to various cultivar lines. Future investigations may be aimed at determining the linkage relationship of the *Dc* gene to other known gerbera genes (Kloos et al., 2004, 2005).

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