Chapter 14

CHRYSANTHEMUM

Dendranthema x grandiflora Tzvelv.

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Abstract: Garden and greenhouse chrysanthemums, *Dendranthema x grandiflora,* have a lengthy association with various world cultures. As a result, flower breeders have created numerous genotypes during the millennia of breeding this crop, which has enabled its establishment in the top ten cuts, potted flowering, and garden crops worldwide. The crop arises from multiple species; its allopolyploid nature (*2n=6x=54)* has complicated progress in crop development due to inbreeding depression, genetic load, and aneuploidy. Consequently, most cultivars are vegetatively propagated, few genes have been characterized, and none have been mapped to chromosomes. Research has focused on seed-propagated hybrids, flowering earliness, winter hardiness, improvement of flower colors/form, plant habit, day neutrality, characterization of self incompatibility, and selection of pseudo-self compatible parents. Future crop ideotypes are proposed to continue transformation of this important crop during the next millennium.

Key words: Allopolyploids, hybrid seed production, inbreeding depression, rapid generation cycling, self incompatibility.

1. INTRODUCTION

The word chrysanthemum is derived from the Greek words ‘chrysos’ (gold) and ‘anthemon’ or ‘anthos’ (flower) (Morton, 1891). Pictorial reliefs from the reign of Thutmose III (ca. 900 B.C.) depict chrysanthemums taken from King Solomon’s royal gardens (Schweinfurth, 1919). “Chu-tzu”, a Chinese book of poetry by Chu Yuan from 300 B.C. mentions chrysanthemum flowers (Ackerson, 1967a). The Chinese cultivated it for pharmaceutical purposes according to Confucius in *Li-Ki,* published in 500 B.C. (Morton, 1891; Bailey, 1914; Herrington, 1917; Arneson,

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1927; Emsweller et al., 1937; Cumming, 1939; Laurie and Poesch, 1939; Woolman, 1953; Randall and Wren 1983). 'Chu' is the Chinese character for chrysanthemum and the Japanese refer to it as 'kiku', although both characters are remarkably similar (Ackerson, 1967a). So popular is this flower in the Orient that the Chinese named a city, Chu-hsien, the Chrysanthemum city (Randall and Wren 1983).

In 385 A.D., Korea bestowed Japan with a gift of chrysanthemum seeds; resultant seedlings had flower colors of blue, red, white, black, and yellow (Ackerson, 1967a). Oddly enough, 'kiku', the character for chrysanthemum existed in the Japanese language before Korea's gift. Cultivation of chrysanthemums became a favored Japanese pastime. Japan instituted several cultural symbols or events in honor of the chrysanthemum, including a festival of happiness (Chrysanthemum Day). A single daisy flower with n=16 petals became the seal and crest of the Chrysanthemum Throne and Emperor of Japan; the imperial Order of the Chrysanthemum became the highest order of chivalry in the country (Emsweller, et al., 1937; Randall and Wren 1983). In addition to their aesthetic value, chrysanthemums are also a food-source, may be fragrant, or used medicinally. Flower petals are eaten in salads or used in teas, whereas new shoots (stems/leaves) are steamed or stir-fried as a vegetable (Woolman, 1953).

The western world was introduced to chrysanthemums via The Netherlands in 1688, where they were cultivated as *Matricaria japonica*, although these apparently died from unknown causes. Chrysanthemums later spread to Great Britain in 1754 and the United States in 1798 (Clark, 1962; Dowrick, 1953; Emsweller, et al., 1937). Indeed the chrysanthemum is an internationally recognized flower and floricultural crop.

While the crop was most likely hybridized in Asia, the first recorded hybridization of chrysanthemums and germination/selection of superior seedlings occurred in 1827 by M. Bernet, although earlier hybridizers undoubtedly crossed chrysanthemums centuries before (Emsweller, et al., 1937). Robert Fortune introduced two 'small-flowered' types (pompon flower types), 'Pompon' and 'Chusan Daisy', to English gardeners in 1843-1846 (Dowrick, 1953; Emsweller, et al., 1937). They were not highly regarded in England, but were sent to France where they were popularized and used widely in hybridization. It is possible that these few genotypes were the progenitors of our modern-day "small-flowered varieties" (Emsweller, et al., 1937). French mum breeders such as Simon Delaux and Auguste Nonin bred many improved cultivars in the late 1800s (Jones, 1958), creating hybrids still popular to this day (Figure 14-1). Some 28 years after being introduced into the United States, Prince's Nursery offered 26 cultivars for sale; in 1835 as many as 50 were listed in Hovey's American Gardener's Magazine and Register (Emsweller, et al., 1937).

American and British National Chrysanthemum Societies have long been recognized for continued popularization of chrysanthemums with gardeners, as well as spawning numerous amateur chrysanthemum breeding programs (Scott, 1957;

Jones, 1958; Clark, 1962). The earliest known chrysanthemum breeder in the U.S. was Robert Kilvington (Philadelphia, Pennsylvania) who exhibited a new cultivar 'William Penn' at the 1841 annual meeting of the Pennsylvania Horticultural Society (Emsweller, et al., 1937). A later meeting of the society in 1846 had a chrysanthemum exhibit, advertising the chrysanthemum as "the coming flower" (Emsweller, et al., 1937). Currently, it is not uncommon to have special chrysanthemum shows in the fall season throughout most of the United Kingdom and North America.

Figure 14-1. Descendants of chrysanthemums derived from the 1800s breeding programs of Monsieurs Simon Delaux and Augusta Nonin displayed at le Tour d'Eiffel, Paris, in 2004.

Noted amateur or private sector breeders of greenhouse chrysanthemums include Charles Totty (Madison, New Jersey, USA), Eugene H. Mitchel (Dreer Co., Philadelphia, Pennsylvania, USA), and Elmer Smith (Adrian, Michigan, USA) (Crook, 1942). Elmer Smith had introduced 445 cultivars by 1928, after 30 years of breeding work (Viehmeyer and Uhlinger, 1955). Garden chrysanthemum breeders in the public and private sectors such as E. M. Byrnes, J.W. Byrnes, F.L. Mulford (U.S. Department of Agriculture), V.R. DePetris (Detroit, Michigan, USA), Alex Cumming Jr. (Bristol, Connecticut, USA), Dr. J.E. Krause (University of Chicago), W.J. Carpenter, T.B. Shackelford (Kansas State University), Dr. L.E. Longley (University of Minnesota), R. Lehman (Mums from Minnesota, Lehman Gardens, Faribault, Minnesota), and Bauer & Steinkemp (Indianapolis, Indiana, USA) bred numerous cultivars (Crook, 1942).

The chrysanthemum craze in the United States is often illustrated by the sale of a large white greenhouse variety to Mr. and Mrs. Alpheus Hardy for US$1,500 in

1888 (Crook, 1942); it was later named 'Mrs. Alpheus Hardy'. By the 1930s as many as 3,000 cultivars existed; most of these were greenhouse chrysanthemums. Numerous publications have recorded the history of this popular flower. Bailey (1914) noted that >100 monographs existed and the number of popular trade articles was exceeded only by that of roses. Crook (1942) noted "chrysanthemum is the flower of the East, as the rose is the flower of the West."

2. CURRENT MARKET STATUS

Chrysanthemums are one of the most important floricultural crops in the cut flower, flowering potted plant, and herbaceous perennial markets of the world. Japan is the leading producing country in the world, with more than two billion stems per year in 1993, primarily for domestic consumption (Horst, 1990). In The Netherlands, chrysanthemums ranked as the second cut flower in Dutch auctions (800 million stems) and fifth in potted plant rankings (Pathfast Publishing, 1994). Other top-producing countries include Columbia (600 million stems), Italy (500 million), and the United States (300 million).

Cut flower, potted plants, and garden chrysanthemums have long ranked in the top ten for sales in the United States. As early as 1939, they were the No. 3 cut flower in the U.S. (Laurie and Poesch, 1939). In 2003, cut pompon chrysanthemums (US$18.181 million wholesale) and flowering potted plant (US$76.093 million wholesale) sales ranked as Nos. 8 and 3, respectively, in the cut flower and potted plant top ten listing (Anderson, 2004; United States Dept. of Agric. National Agricultural Statistics Service, 2004). Garden chrysanthemums are the No. 1 herbaceous perennial in the United States with US$120.424 million (w) in sales (United States Dept. of Agric. National Agricultural Statistics Service, 2004).

3. TAXONOMY AND SPECIES

As many as 200 species were originally contained within the genus *Chrysanthemum*, but the majority have been subdivided into 38 satellite genera of the chrysanthemum complex (Table 14-1; Anderson, 1987). Taxonomic reclassifications of the *Chrysanthemum* complex have happened repeatedly (Heywood, 1976; Humphries, 1976a, b; Kitamura, 1978; Nordenstam, 1976; Tzvelv, et al., 1961). Anderson (1987) theorized that Linnaeus most likely conceived of a *Chrysanthemum* complex (Linnaeus, 1737; 1753). Linnaeus classified species of this complex into five major genera including *Argyranthemum* Webb ex Schultz Bip., *Chrysanthemum sensu stricto* L., *Dendranthema* Des Moul., *Leucanthemum* Miller, and *Tanacetum* L. (Table 1) (Bentham, 1873; Heywood, 1954, 1958, 1976; Hoffman, 1889-1894; Kitamura, 1978; Nordenstam, 1976). Numerous "independent

satellite genera" fill out the remainder of the complex (Table 14-1) (Anderson, 1987; Heywood, 1976a, b; Heywood and Humphries, 1977; Humphries, 1976). Engler and Prantl (1926) originally divided the genus *Chrysanthemum* into eight sections, four sections contained annual species while the remaining sections consisted of perennial species. Cultivated chrysanthemums (*Dendranthema x grandiflora)* and pyrethrum (*Tanacetum cinerariifolium, T. coccineum),* the two most important domesticated species within the genus, were taxonomically classified into Section VI Pyrethrum (Engler and Prantl, 1926). Long stalked capitala with a composite inflorescence is the most common characteristic shared by the 50 species found in this section (Anderson, 1989).

At present, taxonomic classification is based on embryo sac development, cypselar anatomy, plant habit, molecular markers, and phytochemical characteristics (Borgen, 1972; Briquet and Cavillier, 1916-1917; Harling, 1951; Heywood, 1958, 1959; Humphries, 1976a). Cytology is primarily used to characterize species relationships rather than generic ones, since all species in the *Chrysanthemum* complex have a base number of x=9 (Shimotomai and Takemoto, 1940; Tanaka and Watanabe, 1972; Watanabe, 1977a, b). In the strictest sense, therefore, the genus *Chrysanthemum* has two annual species, *C. coronarium* and *C. segetum* (Table 14-1; Anderson, 1987; Boase, et al., 1997). Cultivated greenhouse and garden chrysanthemums, *Dendranthema x grandiflora* Tzvelv. (=*Chrysanthemum x morifolium* Ramat., *C. hortorum*), are members of the Asteraceae Dumort. or formerly the Compositae (Tribe: Anthemideae, Subtribe: Chrysantheminae), the most phylogenetically advanced dicotyledonous family with numerous floricultural crops (Heywood and Humphries, 1977; Anderson, 1987).

Table 14-1. Revised taxonomic designations of genera and commercial species within the chrysanthemum complex (Anderson, 1987; Boase, et al., 1997; Heywood, 1976; Heywood and Humphries, 1977; Humphries, 1976a, b).

Genera	Species	Common name	Previous name
Primary Genera			
Argyranthemum Webb ex Schultz Bip.	*frutescens* Schultz-Bip.	Marguerite daisy	*C. frutescens*
Chrysanthemum L.	*coronarium* Schousboe	Rainbow daisy	
	segetum L.		
Dendranthema Des Moulins	*x grandiflora* Tzvelv.	Chrysanthemum	*C. x morifolium*
	indicum L.		*C. indicum*
	japonicum Makino		*C. japonicum*
	weyrichii (Maxim.) Tzvelv.		*C. weyrichii*
Leucanthemum Miller	*x superbum* Berg. ex Kert.	Shasta daisy	*C. x superbum*
	vulgare Lam.	Oxeye daisy	*C. leucanthemum*
Tanacetum L.	*balsamita* L.	Costmary	*C. balsamita*
	cinerariifolium Schultz-Bip.	Pyrethrum	*C. cinerarifolium*

Genera	Species	Common name	Previous name
	coccineum Willd.	Painted daisy	*C. coccineum*
	macrophyllum Scultz-Bip.	Feverfew	*C. macrophyllum*
	ptarmiciflorum Schultz-Bip.	Dusty Miller	*C. ptarmiciflorum*
	parthenium Schultz-Bip.	Feverfew	*C.partheniume*
	vulgare L.	Tansy	*C. vulgare*
Independent Satellite Genera			
Ajania Polj.			
Arctanthemum Tzvelv.			
Balsamita P. Miller	*major* Desf.	Costmary	*C. balsamita*
Coleostephus Cass.	*myconis* (L.) Cass.		*C. myconis*
Glossopappus G. Kunze	*macrotus* (Durieu) Briq.		
Heteranthemis Schott.	*viscidehirta* Schott	Sticky oxeye	
Hymenostemma Kunze ex Willkomm	*pseudoanthemis* (G. Kunze) Willk.		
Ismelia Cass.	*carinatum*	Painted daisy	*C. carinatum*
Leucanthemella Tzvelv.	*serotinum* Tzvelv.	High daisy	*C. serotinum*
Leucanthemopsis (Giroux.) Heywood	*alpina* (L.) Heywood		*C. alpinum*
Nipponanthemum Kitamura	*nipponicum* (Franch. Ex Maxim.) Kitamura		
Phalacrocarpum (DC.) Willkomm	*oppositifolium*		
Pinardia Cass.	*anisocephala* Cass.		
Prolongoa Boiss.	*pectinata* (L.) Boiss		

Chrysanthemums are native to the northern hemisphere, primarily Europe (the Mediterranean region, centered in Algeria and the Canary Islands) and Asia (China, Korea, and Japan) (Dowrick, 1952b; Hemsley, 1889). From these centers of diversity, numerous species are widespread across Eurasia (Figure 14-2). Most New World species are introduced exotics, with the notable exception of twelve species, including seven *Tanacetum spp., Dendranthema arcticum, D. angustifolium, D. camphoratum,* and *D. cespitosum.* Species found in the Mediterranean region are diploid ($2n=2x=18$), with the exception of *Leucanthemum maximum* ($2n=10x=90$). Oriental species have a much greater ploidy range, from diploid to decaploid (Figure 14-2), which led Dowrick (1952b) to theorize that polyploidy was associated with an increase in latitude. Most diploid species occur in the presumed center of origin, the Mediterranean, while only polyploids exist in the far north such as Siberia and the Arctic, e.g. *D. arcticum* ($2n=8x=72$).

All cultivated chrysanthemums are allohexaploid ($2n=6x=54$) with somatic chromosome numbers ranging from $2n=47$-63 both between and within plants (Dowrick, 1953). *Dendranthema x grandiflora* cultivars are complex interspecific

hybrids, whose ancestry includes ten or more primarily hexaploid species, including *Dendranthema erubescens, D. indicum, D. japonense, D. makinoi, D. ornatum, D. sinense,* and *D. zawadskii* var. *latilobum* (Ackerson, 1967b; Dowrick, 1953). Wild populations occur in China and Japan (Crook, 1942). As with most other wild chrysanthemum species, *D. x grandiflora* has a single daisy flower type with flower colors of white, pink, and lavender, to yellow. However, as early as 910 A.D. a double form 'appeared', either as a spontaneous mutation or the result of directed breeding efforts (Crook, 1942).

Several species have been of interest to breeders for genetic improvement and many have been integrated into the *Dendranthema x grandiflora* gene pool (Table 14-2) (Cumming, 1939). Longley (1949, 1950) used an early-flowering parent, 'Deanna Durbin', derived from *D. zawadskii* to improve winter hardiness and stem strength. *Dendranthema coreanum, D. arcticum* 'Astrid', *D. nipponicum, D. rubellum* (now reclassified *D. zawadskii*), and *D. sibiricum* have also been used widely (Cumming, 1939).

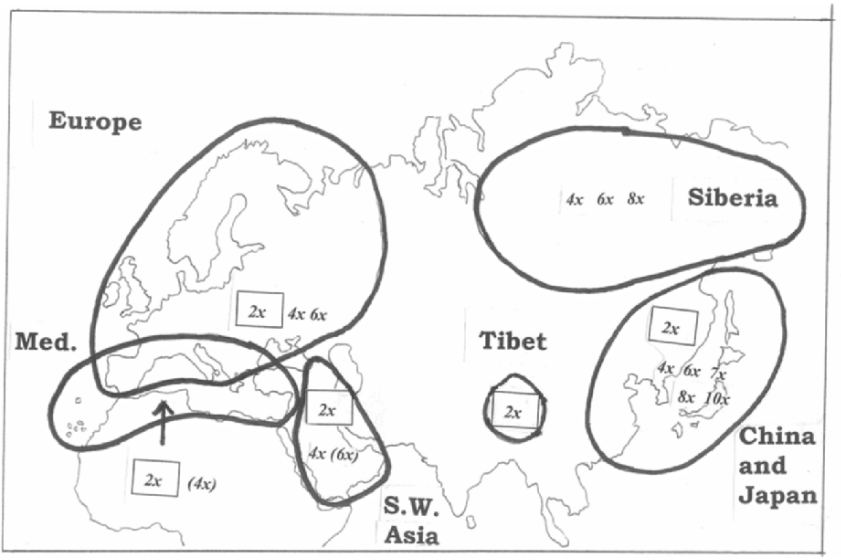

Figure 14-2. Eurasian distribution of chrysanthemum species (Dowrick, 1952b). Most of the diploid species are contained in the center of origin, the Mediterranean, while increasing latitudes are associated with polyploidy.

3.1 Gene Pools

3.1.1 Greenhouse Chrysanthemums

Before 1850, all chrysanthemums were grown out-of-doors (Emsweller, et al., 1937). The hybridization of florist or greenhouse chrysanthemums began after 1850 when culturing mums in greenhouses commenced. Commercial greenhouse chrysanthemum cultivars are 8-14 weeks short day flowering response groups (Crater, 1980). Greenhouse chrysanthemums include both cut flower types and flowering potted plant cultivars, each with specific production protocols (Dole and Wilkins, 2004). Cut and potted mums include the full range of flower colors, flower types, and varying numbers of flowers per flower stalk—ranging from singles (standards) which are disbudded to remove all lateral flower buds (multiple bud removal) to spray types (single bud removal of the terminal flower bud to encourage lateral branching).

Breeding and selection bifurcated greenhouse chrysanthemums from garden types post-1850 (Emsweller, et al., 1937). Selection for both cut flower and potted plant types resulted in numerous series and cultivars which possess longer short day response groups for flower bud development, none to few strap-shaped leaves subtending the terminal floret, stronger but more brittle stems, doubleness (preferred over the singles or semi-doubles to reduce the production of pollen), sterility (a function of Muller's ratchet due to higher frequencies of asexual or clonal propagation to meet high cutting production requirements; and increased post-harvest life (flowering duration) (Anderson and Ascher, 1994; Teynor, et al, 1989b).

Mr. Hosea Waterer largely influenced interest in breeding greenhouse chrysanthemums after he imported ~50 genotypes of cut flowers from Japan into the U.S. (Emsweller, et al., 1937). The expensive cultivar 'Mrs. Alpheus Hardy' appeared shortly thereafter. Other successful breeders during the 1800's in the United States included F. Dorner & Son, E. Fewkes, V.H. Hallock, E.G. Hill, Pitcher & Manda, W.C. Pyfer, and T.H. Spaulding. By 1894 as many as 163 greenhouse cultivars had been bred (Emsweller, et al., 1937). As a result of these breeding efforts and, particularly after the discovery of photoperiodism effects on flower bud initiation and development, numerous public and private sector chrysanthemum breeding programs arose in the early 1900s. Thereafter, greenhouse mums were programmable year-round to flower on specific dates once the response group was characterized and 'artificial' short days were instituted with the use of black cloth.

3.1.2 Garden Chrysanthemums

Garden chrysanthemums are the most popular in North American gardens and, to a lesser extent, in Europe, Japan, and Asia (Crater, 1980). Commercial cultivars

are 6-8 week short day response groups. They are frequently sold in the spring (as small flowering potted plants to be set out-of-doors) and autumn (as mature flowering specimens for containers or direct planting) (Gaston, et al., n.d.; Widmer, 1980). Most early chrysanthemum breeders in Europe and the United States bred both greenhouse and garden types. Some, however, such as Alex Cumming, Jr. (Bristol, Connecticut, USA) focused on garden types during the early 1900s (Emsweller, et al., 1937). Much of this germplasm built the successful private sector breeding program at Yoder Brothers, Inc. (Barberton, Ohio, USA) or resides in plantings at the New York Botanical Garden. The U.S. Department of Agriculture and numerous universities (Minnesota, Nebraska, Kansas, Connecticut) had active chrysanthemum breeding programs during the early 1900s. Now, the only public sector breeding program remaining in North America is at the University of Minnesota (Anderson, 2004).

Hybridization between greenhouse and garden gene pools continues to this day for trait transfer, although as the gene pools diverge hybridizations become increasingly difficult (Viehmeyer and Uhlinger, 1955; Teynor, et al., 1989b). Typical traits for gene pool transfer include flowering earliness (source: garden gene pool), stem strength (source: greenhouse), novel flower colors (striping; source: greenhouse), or flower forms (quilled and carnation-flowered forms were derived from greenhouse germplasm to create garden cultivars, e.g. 'Ak-Sar-Ben', 'Pathfinder', 'Plainsman') (Viehmeyer and Uhlinger, 1955). Crosses in either direction allow for trait transfer but this is usually accompanied by undesirable linked gene(s), necessitating recurrent or congruity backcrosses of the hybrids with inbred or non-inbred parents in the targeted gene pool.

4. FLORAL MORPHOLOGY

The entire flowering shoot or 'spray' of a chrysanthemum is a cyme with multiple inflorescences, the oldest of which terminates the main shoot (Cockshull, 1985). Each inflorescence individually is a raceme with older florets surrounding the outside perimeter. Flowering of the inflorescences subtending the terminal occurs basipetally. Chrysanthemum inflorescences consist of central hermaphrodite disc florets (pistillate + staminate) and/or marginal female ray florets (pistillate) with inferior ovaries (Cockshull, 1985). Collectively, a flower head is known as a capitulum surrounded by an involucre of numerous bracts (Cockshull, 1985). There is a single anatropous ovule per floret for both the disc and ray florets (Anderson, et al., 1990; Cockshull, 1985).

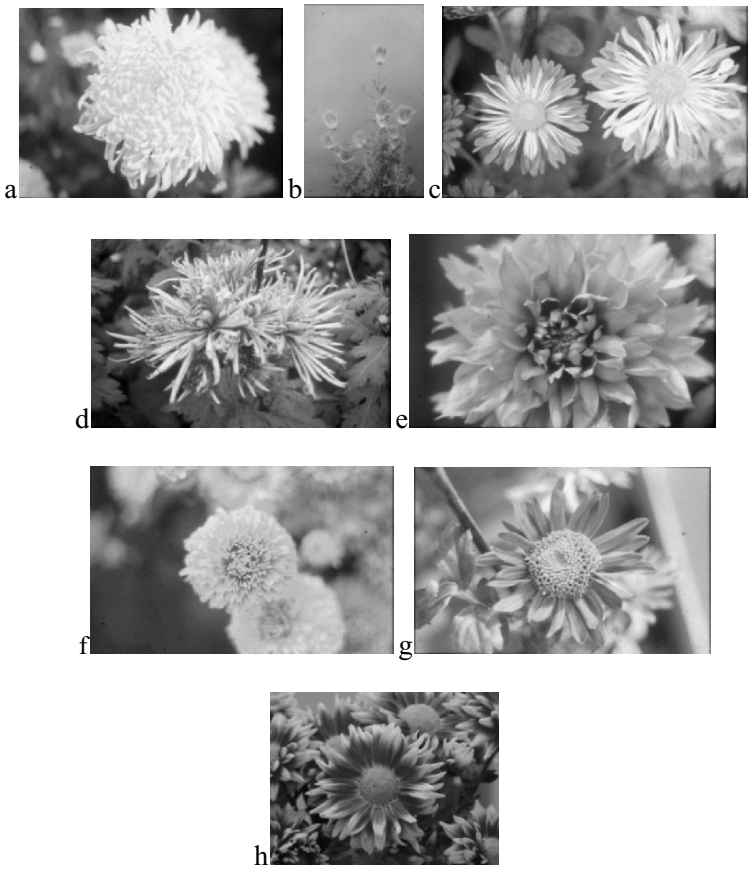

Figure 14-3. Flower types in greenhouse and garden chrysanthemums include modifications to petal number, orientation, and petal types, which produce incurved (a), brush/thistle (b), spoon (c), quill (d), decorative (e), pompon (f), anemone (g), and daisy (h) phenotypes.

Modifications of the basic flower form are common and cultivars are classified by flower type due to ray petal modifications and the relative number of ray petal rows. Most flower forms in existence today were the result of intensive breeding and domestication by the Japanese more than 1,000 years ago (Dowrick, 1953). The most important commercial flower types (Figure 14-3) include incurved (petal tips curved inward), reflexed (opposite of incurved), anemone (center disc floret tubes elongated and colored; also termed 'duet'), pompons (tubular ray florets; no disk florets visible), singles (daisy-type with 1-5 whorls of ray florets; visible disk florets), decoratives (outer ray florets longer than the center ones; disk florets hidden), spiders (ray florets are long and quilled, hooked, and drooping), Fuji (spider-like with shorter ray florets and less drooping), quills (tubular ray florets, not drooping), spoons (quill-like with the ray floret tips flattened like a spoon),

brush/thistle (very fine tubular ray florets), and hairy (hairs on the back of ray florets) (Ackerson, 1957; Bailey, 1914; Everett, 1980; Gortzig and Gillow, 1964; Kofranek, 1980; Langevin, 1992; National Chrysanthemum Society, 1996; Schillinger, 2000; Thistlewaite, 1960). Single versus double flowers is quantitatively inherited (Viehmeyer and Uhlinger, 1955).

Variations in the number of ray versus disc florets are due to the genetic and/or environmental variations, causing considerable differences in seed set potential. Each floret contains a single ovule, producing an achene at the termination of the seed ripening cycle (Anderson, et al., 1990). The variable number of disc and ray florets can significantly impact reproductive studies when seed set data is used to infer self incompatibility status (Anderson, et al., 1988). Researchers must incorporate ovule and seed counts per inflorescence to accurately reflect the impact of the incompatibility system. Excluding ovule counts can lead to inaccurate genetic inferences. Disc and ray florets constitute distinct ovule populations (Anderson, et al., 1988). In diploid *Chrysanthemum spp.,* ray floret numbers are relatively static when the flower type is a single daisy whereas the disc floret numbers are variable (Anderson, et al., 1988). As flower types were domesticated from single daisies to semi-doubles and, ultimately, to complete doubles, the variability shifted from the disc (completely absent in double forms) to ray florets.

5. PLANT HABIT

In addition to taxonomy, floricultural commerce and chrysanthemum societies have additional classifications for chrysanthemums (Boase, et al., 1997; National Chrysanthemum Society, 1996). As many as six plant habits constitute the various commercial greenhouse and garden chrysanthemum products, although some are culturally-derived forms and not under genetic control: specimens, upright (Figure 14-4a; standard, sprays), cushion (Figure 14-4b), charms, lilliputs, large shrubs (Figure 14-4c), and wave (Figure 14-4d). Specimens and most waves are trained to grow in specific forms (Boase, et al., 1997); the former are grown on upright frames for symmetrical growth (Allerton, 1949). Greenhouse chrysanthemums are divided into cut and potted plant types, all with upright (standard/spray) plant habits (Figure 14-4a). Cut flowers are grown upright with either the terminal as the sole flower (all subtending lateral buds removed, known as multiple bud removal) or only the lateral flowers (terminal flower bud in removed, known as single bud removal); standard and spray chrysanthemums are the terms used to denote the former and latter classes, respectively (Dole and Wilkins, 2004). Flowering potted chrysanthemums likewise have standard or spray types with one or multiple plants per container (based on the pot diameter) or may be cushions (Figure 14-4b; potted plants with small flowers), charms (compact plants with very small single flowers), or lilliputs

(dwarf plants with small double flowers) (Jones, 1958; Locke, 1990; Woolman, 1953).

Chrysanthemums grown in hanging baskets for display at public conservatories in the United States and the United Kingdom or for the annual October festivals in honor of 'kiku' (the Japanese character for chrysanthemum or the queen of autumn) in Japan are frequently grown as 'cascade' or 'bonsai' plant habits (Maisano, 1971). Both cascade (Figure 14-4e) and bonsai (Figure 14-4f) chrysanthemums, however, are created using genotypes with an upright plant habit which are 'trained' and manipulated to grow against gravity. First, the upright stems are trained to grow at a 45º angle on wires and then successively bent to create a cascading form during flower bud initiation and development. The cascade and bonsai phenotypes are the result of such cultural manipulations and, thus, have no genetic control for the plant habits.

Until the 1950s, most garden types had an upright plant habit with the flowers at the top of the plant only, due to their derivation from greenhouse types. An upright plant habit out-of-doors required staking to avoid lodging (Anderson, et al., 2001). The result of interspecific hybridizations with related wild species and use of an old cultivar 'Pink Cushion', produced a new garden phenotype in 1955, the 'cushion' plant habit, created by Dr. Richard Widmer at the University of Minnesota chrysanthemum breeding program (Anderson, et al., 2001; Viehmeyer and Whitney, 1955). A cushion habit (Figure 14-4b) infers that the plant is low-growing, forming a symmetrical hemisphere such that, at 100% flowering, the entire outer surface of the plant is covered with flowers and few foliage is visible (Viehmeyer and Whitney, 1955). The first garden chrysanthemum series displaying this new phenotype were the 'Minn' series with all color classes represented in the cultivars 'Minnbronze', 'Minngopher' (U.S. Plant Patent No. 4,327), 'Minnqueen', 'Minnruby', 'Minnwhite', and 'Minnyellow'. Within a few years, both private and public sector breeding programs had incorporated the 'Minn' series cushion habit and were releasing cultivars with this new phenotype. Currently, the cushion habit has the majority market share (Anderson, et al., 2001).

Large shrub chrysanthemums, *D. x hybridum,* with the cushion habit have also been created by interspecific hybridization of *D. weyrichii x* upright *[D. x grandiflora]* (Ascher, et al., 1997). These genotypes reach their maximal plant dimensions in the second year, frequently 1 m high x 1-2 m in width, and are extremely winter-hardy (Figure 14-4c). Several cultivars with this plant habit have been released onto the market under the series names Maxi-Mum™ and My Favorite™.

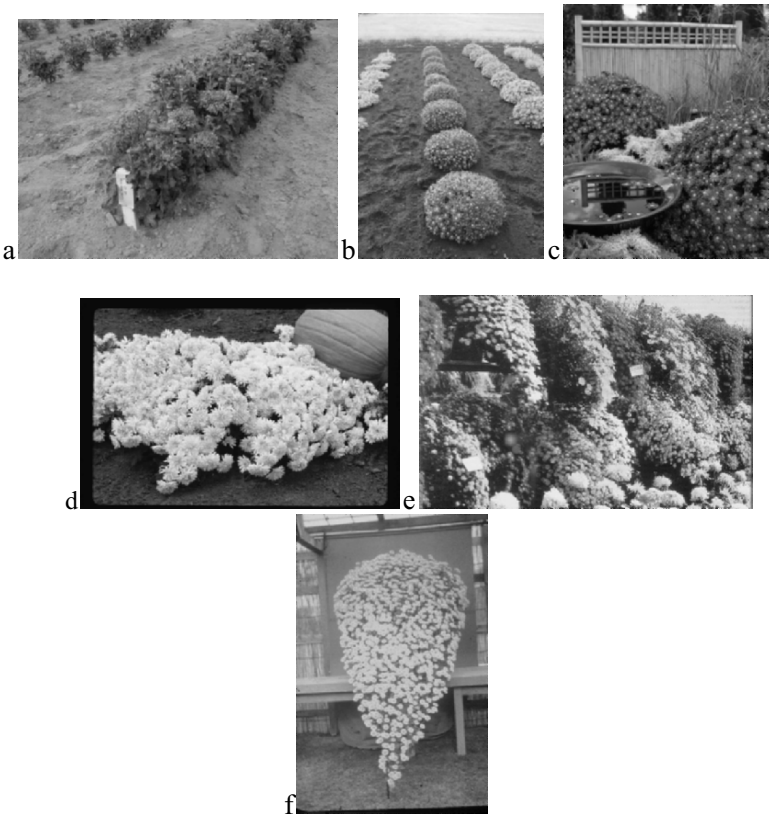

Figure 14-4. Example plant habits bred into commercial product classes of greenhouse and garden chrysanthemums include upright (a), cushion (b), large shrub (c), wave (d), cascade (e) or bonsai (f).

The wave (also known as cascades or prostrate) plant habit is similar in appearance to the cascade, culturally-manipulated phenotype, but differs by being genetically controlled (Chen, et al., 1995). A wave habit is characterized by horizontal growth of the terminal and lateral branches, followed by flowers growing upright (Figure 14-4d). The horizontally-growing branches do not root into the ground, leaving the central crown to overwinter. Interspecific hybrids derived from crossing prostrate *D. weyrichii x* upright *[D. x grandiflora]* produced primarily cushion progeny with large plant diameters and height (Ascher, et al., 1997). Any derived-wave or prostrate progeny often had an obligate vernalization requirement before flowering. Several chrysanthemum breeding programs have this plant habit as a breeding objective (Ascher, et al., 1997; Chen, et al., 1995).

6. FLOWER COLOR

For the majority of flower types, floral pigmentation is located in the ray florets; the notable exception is anemone flowers (Figure 14-3f) with pigmented disc and ray florets. Upper and lower epidermal layers (L1) and the internal cell meristematic layer (L2) are the locations for floral pigments (Langton, 1976; 1980). Two classes of pigments, plastids (carotenoids) and sap-solubles (anthocyanins, anthoxanthins), coupled with cellular pH, copigments, and other compounds, combine to give the pattern and tone of flower colors (Fleming, 1929). Flavonoid pigments, particularly anthocyanins and anthoxanthins, are located only in the cell vacuoles of the L1 layer (Langton, 1980; Stewart and Derman, 1970). Anthocyanins range in color from salmon and scarlet through red and purple to blue. Anthoxanthins (flavones, flavonols) produce shades of pale ivory to deep yellow (Scott-Moncrieff, 1936). Plastid pigments, present in tiny plastid bodies primarily in the L2 cell layers (chromoplasts), range from yellows to oranges due to xanthophylls and carotenoids (carotenes, carotenols) (Kawase and Tsukamoto, 1976). All pigment types may be present as the sole pigment source or produced in concert (Crane and Lawrence, 1952).

High night temperatures can affect pigment production, expression, and the resulting flower coloration (Kosaka, 1932; Rutland, 1968). Studies on 'Fandago' greenhouse chrysanthemum, normally having purple flowers, showed that florets grown at 15 C (nights) were red, at 30 C the color was bright yellow, and pale yellow at 6 C (Stickland, 1974). Particularly in garden chrysanthemums, breeding programs will select against 'pinking' or 'purpling' in seedlings to prevent release of white/cream, yellow, or bronze types which develop anthocyanin pigmentation. An additional trait selected against is fading in bronzes or reds under warm night temperatures ($\geq$30 C). Most anthocyanin compounds may be produced at higher temperatures, but the sugar moieties will not be added on to the molecule as the carbohydrates are required for increased respiration (Post, 1950). Both anthocyanin and carotenoid production are severely hampered or may cease altogether at $\geq$30 C.

The fundamental flower color classes of chrysanthemum consist of (1) white or cream, (2) lavender to purple, pink, (3) bronze, red, orange, and (4) yellow (Figure 14-5) (Anderson, et al., 1988; Hattori and Futsuhara, 1970; Kawase and Tsukamoto, 1974; Miyake and Imai, 1935). White flowers possess flavonols, but may also have other precursors to anthocyanins, which can develop under cool night conditions. Cream colored flowers typically contain anthoxanthins. Lavender, purple, and pink flowers contain anthocyanins and flavonols, differing either with the pigment base compound, the addition of sugars, and/or cell pH differences. Bronze and red flowers contain predominantly both a background carotenoid or xanthophyll pigment with anthocyanin(s) and flavonols superimposed in the epidermal layers to varying degrees. Yellows usually consist of pure carotenoid or xanthophyll compounds in the L1 apical layer, but not in the L2, but may also have cream-

colored anthoxanthins (Langton, 1980). Yellows are homozygous recessive for both the water soluble and plastid pigment genes which means that they will never sport to a color other than yellow (Teynor, et al., 1989a). In most instances, inbred and non-inbred genotypes within each flower color phenotypic class do not exhibit qualitative pigment differences (Anderson, et al., 1988).

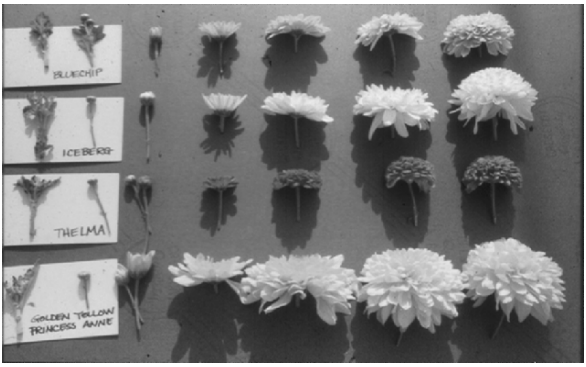

Figure 14-5. The four major flower color classes (white/cream, lavender/purple, red/bronze, and yellow) of cultivated greenhouse and garden chrysanthemums (Anderson, 1985).

Anthocyanin (anthocyanidin) pigments identified in *D. x grandiflora* are chrysanthemin, its derivatives, and cyanin (cyanidin), responsible for pink/purple, orange and red flower colors (Anderson, et al., 1988; Kawase, et al., 1970; Kawase and Tsukamoto, 1976). One flavone and two phenylpropanoids also exist in these color groups (Anderson, et al., 1988). White-flowered cultivars possessed four phenylpropanoids, two of which were caffeic and ferulic acids (Anderson, et al., 1988). Eight carotenoid pigments (including lutein) have been reported in yellow and bronze-red-orange color groups; five of these carotenoids varied quantitatively (Anderson, et al., 1988). Two genes for flower color have been ascribed to *D. sinense, C* and *Y* (Miyake and Imai, 1935). *Chrysanthemum carinatum* has an "anthocyanic-xantheic-xanthin series" in which anthocyanic pigments were found in magentas and crimsons, xanthin in pale yellows, while yellow contained xantheic compounds (Wheldale, 1909). The same four fundamental flower color classes are present in this species as in the cultivated greenhouse and garden *D. x grandiflora*.

Anthocyanin inheritance in orange-flowered 'Vulcan' and its orange progeny may be attributed to a single dominant gene responsible for transposable element expression of red pigments overlaid on carotenoids (Teynor, et al., 1989b). Carotenoid pigment inheritance best fit a disomic inheritance model with one dominant gene, *I-* (inhibitor locus) (Teynor, et al., 1989a). Yellow-flowered genotypes with carotenoid pigments are denoted as *ii*. One family exhibited an excess of carotenoid progeny (deviating from the expected 1:1 ratio) in three

different environments. Segregating progeny from one family deviated from the 1:1 expected ratio with an excess of white (non-carotenoid) genotypes when grown under glasshouse conditions. The opposite (an excess of carotenoid-expressing genotypes) occurred with the identical cloned genotypes under field conditions; this also has been reported with anthocyanin inheritance studies (Teynor, et al., 1989b). Anderson et al. (1988) and Anderson (1985) also reported similar environmental variation in pigmentation for clones. Segregating progenies need to be classified in more than one environment (Teynor, et al., 1989a).

Recent data in the University of Minnesota breeding program have reconfirmed the existence of specific hybrid progeny with an excess of *ii* or *I-* classes, possibly due to a lethal gene tightly-linked to *ii* (Anderson, unpublished data). Teynor et al. (1989a) proposed that such a lethal would convert the expected 1:1 to a 2:1 ratio. Clonal cultivars which have been asexually propagated for numerous generations, e.g. 'Puritan' (Teynor, et al., 1989a), may accumulate recessive alleles at the lethality locus(-i) linked with *ii* on the chromosomes.

7. REPRODUCTIVE BIOLOGY

Chrysanthemums reproduce sexually as outcrossing species and asexually via rhizomes (emergent, non-emergent) in perennial species in the wild. In commercial production they are asexually propagated as terminal stem cuttings (*in vitro, ex vitro*), emergent/non-emergent rhizomes, and division (Anderson, 2004; Dole and Wilkins, 2004; Kofranek, 1980). Self incompatibility (SI) enforces outcrossing and was first reported in cultivated chrysanthemums in 1931 (Niwa, 1931; Mulford, 1937b; Crook, 1942). Self incompatibility occurs at all ploidy levels in species of the chrysanthemum complex: diploid (*Dendranthema boreale*), hexaploid (*D. japonense*), octaploid (*D. ornatum*), and decaploid (*D. shiwogiku*) all possess an active SI system (Table 14-2; Tanaka, 1952). Pyrethrum, *Tanacetum cinerariifolium*, an economically important species (Tables 14-1, 14-2), is also SI (Thorpe, 1940). Genetic analysis of cultivated chrysanthemums revealed the lack of pollen (trinucleate) germination or stigmatic inhibition of pollen tubes and reciprocal crossing differences, indicating the existence of a sporophytic SI system (Drewlow, et al., 1973). There are at least three epistatic *S* loci controlling SI expression (Stephens, et al., 1984; Zagorski, et al., 1983).

Table 14-2. Morphological traits, ploidy levels (2*n*=2*x*=18 to 2*n*=22*x*=198), and crossability of important chrysanthemum species useful for genetic improvement of cultivated *Dendranthema x grandiflora*.

Species	Morphological traits	Ploidy	Crossability with *D. x grandiflora*	References
Chrysanthemum coronarium		*2x*		Gaiser, 1930
C. segetum		*2x*		Gaiser, 1930
Dendranthema alpinum		*4x*		Gaiser, 1930
D. arcticum	Dwarf, winter hardiness	*10x*	+ but no success in transferring hardiness	Gaiser, 1930
D. boreale		*2x*	SI	Watanabe, et al., 1972
D. decaisneanum		*8x*		Shimotomai, 1931
D. x hybridum	large shrub plant habits	*6x*	+ SI or PSC	Ascher, et al., 1997
D. indicum	mini yellow flowers, sprays, early flowering	*4x, 6x*	+	Gaiser, 1930; Taniguchi, 1987
D. japonense		*6x*		Shimotomai, 1934b
D. j. var. octoploid		*8x*	SI	Watanabe, et al., 1972
D. j. var. crassum		*_10x*		Watanabe, et al., 1972
D. japonicum		*4x*		Gaiser, 1930
D. koreanum (=D. coreanum)			+	Cumming, 1939
D. marginatum		*10x*	+ highly fertile hybrids	Emsweller, et al., 1937; Shimotomai, 1931, 1932a
D. makinoi		*2x*	+	Shimotomai, 1934a
D. nipponicum	shrub-like, woody stems, oblong lvs.	*2x*	+ crosses readily	Gaiser, 1930
D. ornatum		*8x*	SI	Shimotomai, 1934b
D. pacificum	dwarf, hairy leaves, apetalous, transposable elements	*10x*		Shimotomai, 1934b; Shimizu, et al., 1998
D. x rubellum 'Clara Curtis'		*8x+1=73*	+	Dowrick, 1952b, 1953
D. shiwogiku		*8x, 10x*	SI	Kawata and Toyoda, 1982
D. sibiricum		*6x*		Shimotomai, 1934b
D. uliginosum	giant daisy,	*2x*		Cumming, 1939

Species	Morphological traits	Ploidy	Crossability with *D. x grandiflora*	References
	plants to 2m tall			
D. wakasaense		*4x*		Dowrick, 1952b, 1953
D. weyrichii	winter hardiness, prostrate plant habit	*6x, 8x*	+ used to create shrub forms	Shimotomai, 1932b
D. yezoense		*10x*		Dowrick, 1952b, 1953
D. yoshinaganthum		*4x*		Kawata and Toyoda, 1982
D. zawadskii		*4x, 6x*		Lee, 1967, 1975
Leucanthemum lacustre	perennial	*22x*		Dowrick, 1952b
L. maximum	large white flowers, waxy leaves	*8x*	~ little; use as male parent	Shimotomai 1934b
Tanacetum cinerariifolium	'painted' daisy flowers, insect resistance	*2x*	+ as male; recessive cytoplasm	Cumming, 1939
T. coccineum	'painted' daisy	*8x*		Magulaev, 1992

Both garden and greenhouse chrysanthemums are highly SI, rarely producing seeds after selfing or outcrossing between genotypes sharing *S* alleles (Anderson and Ascher, 2000; Ronald and Ascher, 1975a). Lack of seed set has plagued chrysanthemum breeders as more species were incorporated into the *D. x grandiflora* genome (Smith, 1913). Emsweller et al. (1937) lamented that one of the greatest challenges to chrysanthemum breeders is the lack of seed set, either due to lack of fertility, inbreeding depression, or SI. Random outcross pollinations among unrelated, noninbred genotypes produce low seed set, ranging from 36% to 71% in one study (Ronald, 1974) and even lower in another—24.5% - 38.5% (Anderson and Ascher, 2000); most seed set is <50%. The primary reason for such low seed set is SI, although inbreeding depression and genetic load also operate in this polyploid crop (Anderson, et al., 1992a, b).

Teynor et al. (1989a, b) found that greenhouse cultivars, which are frequently double (gynoecious florets only) and propagated for multiple asexual generations, were highly sterile. Parental selection for high general combining ability is important to maximize seed set (Mulford, 1937a). It is possible to select for inbred parents with high fertility levels (Anderson and Ascher, 2000).

Pseudo-self compatibility (PSC) exists in garden chrysanthemums, although at a very low frequency (Ronald and Ascher, 1975a). However, end-of-season PSC does not exist (Anderson and Ascher, 1996), although heat treatment can increase self seed set (Ronald and Ascher, 1975a). SI expression and the related temperatures to overcome it are genotype-specific (Ronald and Ascher, 1975a). Drewlow, et al.

(1973) reported that inbreeding decreased the number of heterozygous *S* loci. Zagorski, et al. (1983) found some inbred parents that set higher self than outcross seed set. Greenhouse chrysanthemums rarely express PSC, although F_1 hybrid progenies from crossing greenhouse (SI) x garden (PSC) cultivars segregated with a 1:1 (PSC:SI) ratio (Ronald and Ascher, 1975b), suggesting PSC could be transferred from garden to greenhouse types (Ronald and Ascher, 1975c).

PSC garden chrysanthemums produced progenies with both SI and PSC individuals following self pollination or when crossed with SI plants (Ronald and Ascher, 1975a). Anderson and Ascher (1996) found that inbred parents had a wide range in %PSC (calculated as [mean self seed set / mean outcross seed set] x 100), ranging from 0 to 68.8%, while recombinant inbreds had wider variation (0.2-99.7%), compared with non-inbreds (0.6-25.7%) which had not been previously selected for PSC. Most parents had low PSC expression, with high PSC levels being the least common (Anderson and Ascher, 1996). PSC distributions within inbred populations were primarily continuous in distribution and quantitatively inherited. High PSC levels were not highly heritable; realized heritability (H_R) ranged from H_R =0.05% to H_R =10.19% (Anderson and Ascher, 1996). Since all low PSC x low PSC crosses and self pollinations of low PSC parents produced 43-50% of the inbred progeny with higher PSC levels, many low PSC parents possess unexpressed PSC genes. A PSC threshold with additive gene action operates when low – mid PSC selection occurs, but as soon as high PSC levels are obtained, non-additive gene action is operating (Anderson and Ascher, 1996).

8. CYTOLOGY

Dendranthema indicum, one of the oldest wild species native to Japan, exhibits karyomorphological diversity in morphology and geographical variance for the number of satellites and C-band variation (Taniguchi, 1987). Shimotomai (1934) found that Japanese species populations with higher ploidy levels were typically distributed near coastal areas whereas those with lower ploidy were inland.

Wolff and Peters-van Rijn (1993) examined genetic variation of 15 genotypes in 13 chrysanthemum species using six RAPD primers. They found high levels of genetic variation among species. Several RAPD primers produced different banding patterns in each species, with some RAPD bands being diagnostic for a species. The mean similarity among species was S=0.49, significantly lower than cultivar similarity within cultivated chrysanthemums (Wolff and Peters-van Rijn, 1993). They did not find any relationship between ploidy levels and the corresponding number of RAPD fragments generated.

Bleier (1934) reported that, in general, interspecific hybrids predominantly formed bivalents, although univalents have been observed. In extreme cases, where the parental ploidy levels differed widely, trivalent and quadrivalent formation also

occurred. Aneuploidy and euploidy causes irregular inheritance patterns and segregation ratios differing widely from expectations (Sansome and Philp, 1932). The occurrence of aneuploidy and euploidy in interspecific crosses between chrysanthemum species indicates that hexaploid *D. x grandiflora* would be expected to segregate, at least occasionally, from expected diploid ratios.

In most polyploid chrysanthemum species (ranging from 2*x* to 22*x,* Table 14-2), preferential pairing or a "5B type gene system" (preventing homoeologous pairing in hexaploid wheat) are the strategies whereby a stable, essentially diploid meiotic process occurs for the establishment of a sexually self-maintaining polyploid (Dowrick and El-Bayoumi, 1969; Watanabe, 1977a, b). Multivalent formation may be prevented in chrysanthemums by restriction of pairing initiation to a single site per chromosome. This multivalent suppressor system may have evolved through a gradual reduction in the number of zygomeres and the differentiation in their homology recognition and regulation systems. Genetic stabilization of diploid-like meiosis occurs in all polyploid chrysanthemums (Watanabe, 1977a). Bivalent formation is the norm and multivalents are rare.

Interspecific hybridization between wild species with differing ploidy levels, as a general rule, produce true-breeding (non-segregating) hybrids (Table 14-2). For example, *D. japonense (2n=6x=54) x D. pacificum (2n=10x=90)* produces octaploid hybrids (*2n=8x=72*); hybrids from the cross *D. makinoi* (*2n=2x=18) x D. decaisneanum (2n=8x=72)* were also all octaploid, while *D. makinoi* (*2n=2x=18) x D. japonense (2n=6x=54)* hybrids were septaploid (*2n=7x=63)* (Shimotomai, 1934).

Greenhouse and garden chrysanthemums, *D. x grandiflora,* have somatic chromosome numbers which vary from the expected *2n=6x=54* for this allohexaploid. As many as n=125 aneuploid genotypes have been documented to range from *2n=6x=47-63* (Dowrick, 1953; Shimotomai, 1934). There is a direct relationship between increasing chromosome number and inflorescence diameter (Dowrick, 1953). Fertile hybrids have also been obtained by crossing wild species with *D. x grandiflora*: *D. marginatum (2n=10x=90) x [D. x grandiflora] (2n=6x=54)* created fertile hybrids (*2n=16x=144*) (Table 14-2; Emsweller et al., 1937).

8.1 Sports

At least one third of the commercial cultivars on the market are 'sports' arising from mutation (Wasscher, 1956). Many sports arise from spontaneous mutations, due to background irradiation, while others are artificially induced with X rays (1000r total; 120 KV, 5 mA at a dose rate of 120r/min.) and γ rays (1 K rad total from Co^{60} at a dose rate of 350 rad/min.) exposure, as well as chemical mutagens (Dowrick and El-Bayoumi, 1966). Higher irradiation dosage rates from either

ionizing radiation source are lethal to all tissues. Mitotic or meiotic division errors account for numerous sports which commonly arise in any type of asexual propagule (cuttings, division, tissue culture) (Dowrick and El-Bayoumi, 1966). Sports may be due to point mutations, inversions, deletions, or the gain/loss of chromosomes. Several researchers noted that mutations affecting morphology (particularly flower coloration) coincided with cytological differences; higher chromosome numbers also convey larger flower sizes (Dowrick and El-Bayoumi, 1966; Sampson, et al., 1958). Earlier reports found a similar correlation (Morton, 1891).

Bud sports (chimeras) are common sources of new mutations; often a subtending lateral branch will mutate into a flower color that differs from the original clone (Dowrick and El-Bayoumi, 1966). Mitotic cell division errors (chromosome non-disjunction, lagging and sticky chromosomes ends at anaphase) are highly likely to produce mericlinal chimeras; lateral branches (the result of many cell layers) arising within a mericlinal chimera may have one (mericlinal) or both (periclinal) cell types (Dowrick and El-Bayoumi, 1966). Most chrysanthemum mutants are periclinal chimeras. Sectorial chimeras, mutant flower petals occurring in pie-shaped wedges within a single flower cannot be directly propagated asexually via cuttings but must first be tissue cultured. Root tip chromosome counts do not accurately reflect the chimera cytology. Tissue culturing mutated ray florets can be accomplished with Murashige and Skoog (MS) basal medium plus 0.5 mg/L 1-naphthaleneacetic acid (NAA) and 1 mg/L 6-benzylaminopurine (BAP) (Datta, et al., 2001). Shoot organogenesis begins within 2 wks followed by transfer to rooting medium and transfer out of culture for evaluation.

As early as 1918, ~400 cultivars had originated by sporting (Shamel, 1918). Another noted effect of mutation included striping (‘Queen of England’ sported from red to striped) (Crook, 1942). Emsweller et al. (1937) observed that mutation breeding was an important tool in the development of new cultivars. Crook (1942) postulated that mutation rates were higher in some genotypes than others. Selected mutants must be tested for stability through asexual propagation and production cycles, prior to market release, to ensure that the mutant does not revert to the original clone (Yoder Brothers, Inc., 1967). If they are stable sports, each new mutant genotype can be forced under the exact production protocols for the original clone. Thus, a mutant family or series arises with each new mutant possessing the name of the original clone with a color added on and may differ in chromosome counts, e.g. the Fred Shoesmith Family consists of numerous mutants derived from ‘Fred Shoesmith’ ($2n$=54-58) such as ‘Apricot Fred Shoesmith’ ($2n$=54-58), ‘Yellow Fred Shoesmith’ ($2n$=57-58), or ‘Golden Fred Shoesmith’ ($2n$= 56-58) (Dowrick and El-Bayoumi, 1966). Clones within a mutation family often have a wide range in ploidy (Dowrick, 1953, 1958).

Mitotic division errors (spontaneous mutations) in clonally maintained cultivars were found to be inducible by high (23 C) or low (3.5 C) temperatures, causing a 0-2.3% abnormality at anaphase due to nondisjunction, lagging, or stickiness of

chromosomes (Dowrick, 1953, 1958; Walker, 1955; Dowrick and El-Bayoumi, 1966). These chromosome abnormalities occur primarily in clonal cultivars several asexual generations removed from sexual cycles. In addition, meiotic errors causing unreduced gametes have been reported in a closely related species, *D. atratum* (Dowrick, 1952a).

Yellow flower color, being recessive for both anthocyanin and carotenoid pigment genes, will not produce any new flower color sports. All other flower colors (red, bronze, purple, white, and cream) will produce varying types of new color mutants. Typically any breeding program will endeavor to create and own the rights to as many sports as possible, prior to the release of a new cultivar onto the market. Virtually all commercial, asexually-propagated sports or new seedlings are protected by plant patents (United States) or plant breeder rights (PBR; in Canada, Europe, Africa, Japan, Australia, and New Zealand), prior to release into the commercial market (Vandenberg, 2004).

Molecular markers are useful for cultivar identification (genetic fingerprinting in patent or PBR infringement litigation), as well as identifying genetic variation within a sport family. Wolff and Peters-van Rijn (1993) used random amplified polymorphic DNAs (RAPDs) in a sport family (n=13 genotypes using n=27 primers) and found that the sport family derived from a single cultivar all had identical fragment patterns. In another study, Wolff, et al. (1995) reported that a sport family had the same DNA fragment patterns with RAPDs, ISSRs (inter-simple sequence repeats), or RFLPs (restriction fragment length polymorphisms) techniques. However, when DNA extractions of various cells layers (L1 from epidermal peels, L1 & L2 from florets, L1– L3 from leaves) were analyzed, polymorphisms between cultivars within a sport family could be found (Wolff, 1996).

9. GENETICS & BREEDING

Several gene designations have been published for the species, although none have been mapped to chromosomes. *Y* (yellow plastids) and *C* (colored anthocyanins) were the first genes proposed (Miyake and Imai, 1935). White (*ccY-*), magenta (*C-Y-*), orange-red (*C-yy*), and yellow (*ccyy*) flowers result from interactions between these two genes. *F-* (anthocyanin factor) was introduced as a component of several partially allelic anthocyanin genes, including *C* (Reimann-Philipp and Jordan, 1978). Later, Jordan and Reimann-Philipp (1983) proposed a dominant gene *I-* (inhibitor) for elimination of carotenoid production in either the L_1 or L_2 cell layers of chrysanthemum flowers. *I,* in conjunction with an anthocyanin production gene, *A,* would account for flower color. The literature is unclear whether the first- and latter-named flower color genes are, in fact, different. It also remains controversial whether the inheritance of flower color and other traits is

under disomic, tetrasomic, or hexasomic control (Reimann-Philipp and Jordan, 1978; Teynor, et al., 1989a).

Genes controlling traits other than floral characteristics include *S* for strong growth habit (Reimann-Philipp and Jordan, 1978) and *Ph* for resistance to *Puccinia horiana* (de Jong and Rademaker, 1986). It should be noted, however, that *S* is also the universal gene designation for the self incompatibility locus, which exist in chrysanthemums (Ascher, 1976). Diploid plants taller than 50 cm possess the dominant allelic form for strong growth habit (*S*-), while those <50 cm are *ss.* The *Ph* gene acts similarly, with *Ph*- genotypes being resistant and *phph* diploids being susceptible to *P. horiana.* Numerous genes have been proposed for flower size, type, and petal orientation and are discussed subsequently (see Floral Traits section; Crook, 1942). The lack of genetic information for other economically important traits provides an open field for future research.

The existence of transposable elements has been reported in two hexaploid species of chrysanthemums, *Dendranthema x grandiflora* and *D. pacificum* (Anderson and Ascher, 2004; Shimizu, et al., 1998; Teynor, et al., 1989b). Embryo-rescued seedling cotyledons expressed transposable elements with epidermal cells producing anthocyanin pigment(s) (Anderson and Ascher, 2004). Polymerase chain reactions (PCRs) demonstrated that wild *D. pacificum* genotypes possessed conserved sequences of the reverse transcriptase domain of *Ty-1-copia* group with the 'AFLNG' motif derived from *copia* of *Drosophila* (Shimizu, et al., 1998). None of the retrotransposon sequences in three clones were identical; one clone closely resembled retrotransposons in rice.

Due to recent escalations in heating costs for greenhouse production, breeding programs have been striving to decrease the short day response period. Effort has also been devoted to the development of seed-propagated cultivars to minimize the costs unique to vegetative propagation, i.e. stock plant maintenance of disease-free material, etc. (Strefeler, et al., 1996; Meynet, 1978). In the past decade, the University of Minnesota garden chrysanthemum breeding program has been pursuing studies on the reproductive biology and creation of acceptable, uniform seed-propagated cultivars by developing inbred lines homozygous for important phenotypic traits (Anderson, et al., 1995; Anderson, 2004). Whether seed-propagated hybrids will replace a portion or all of the vegetative market has not been determined.

9.1 Rapid Generation Cycling

Most hybridizing in private and public sector breeding programs has been conducted in greenhouse conditions. Viehmeyer and Uhlinger (1955) developed a 'water culture' method to accommodate situations of limited greenhouse space. This technique was accidentally discovered after cut flower stems for use as pollen sources were allowed to senesce in a vase and produced seed. In 1951, the water

culture method was re-tested with cut stems placed in jars of water, placed in lab conditions (ambient temperatures and light conditions), and proved successful in seed production. Subsequent trials demonstrated that seed could be ripened in darkness, seed viability and % seed set were equal to *in situ* ripening, and seed maturation occurred 2-4 wks. earlier (Viehmeyer and Uhlinger, 1955).

Inbred line development has been compounded by SI and a long generation time of 6-8 months (minimum). Embryo rescue is a technique that has improved seed germination, prevented the loss of embryos resulting from wide intra- and inter-specific crosses, and reduced generation times (Anderson, et al., 1990; Watanabe, 1977b), since *in situ* seed-ripening procedures are 1-2 months in duration (Scott, 1957). Before tissue culture media components had been developed for embryo rescue, attempts at rescuing crosses of cultivated chrysanthemums frequently were unsuccessful (Fan, 1965). Recently, however, intra- and inter-specific crosses have been embryo rescued with success (Kaneko, 1957; Watanabe, 1977a).

Figure 14-6. Laboratory seed ripening of pollinated inflorescences suspended (floating) in a floral preservative solution to facilitate ease of pollination, maximize seed set, and hasten embryogenesis (Anderson, et al., 1990).

Cut flower preservatives may be used as nutritive and bactericide sources in ripening seeds *ex situ*. Anderson, et al. (1990) successfully used 200 ppm 8-HQC (8-hydroxy-quinoline citrate) plus 1% sucrose at 150 μmol m^{-2} sec^{-1} (16 hr photoperiod supplied by cool white fluorescent lamps) and 29°C (Figure 14-6) to open chrysanthemum flower buds 50-60 mm dia. in size (Marousky, 1971) and ripen seed, a technique termed laboratory seed ripening. Since 8-HQC is no longer commercially available, Anderson's lab uses commercial floral preservatives (prepared at the recommended rate) as substitutes. This, coupled with embryo rescue at the heart stage as early as 2 d. post-pollination, allowed for an average generation time of ~100 d (compared with 200-550 d *in situ*). Embryogenesis occurred significantly faster with laboratory seed ripening than *in situ* ripening (Figure 14-7). Embryo rescue proceeds with seed coat removal and placement of embryos on MS medium with 1% sucrose and no plant growth regulators

(Anderson, et al., 1990). Culture vessels are placed in the dark for germination, followed by a 16 hr photoperiod (92 μmol m^{-2} sec^{-1}) at room temperature (29°C constant). The combination of laboratory seed ripening and embryo rescue techniques are both used in rapid generation cycling to enhance seed production by reducing source/sink interactions, promote rapid embryo germination, and significantly decrease generation times.

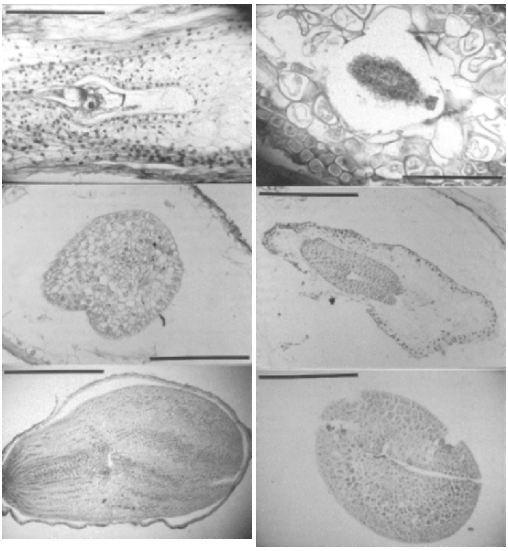

Figure 14 -7. Embryogenesis stages of embryo-rescued seedlings using the rapid generation cycling technique (Anderson, et al., 1990). Embryo sac (top left) with an unfertilized egg cell (arow) at day 0; (top right) globular embryo at day 1 (arrow denotes hypophysis); (center left) heart stage at day 2; (center right) torpedo on day 4; (bottom left) cotyledonary at day 6 with an apical dome defined and vascular traces appearing in the cotyledons, and (bottom right) a mature seed occurring at day 25. Bars = 0.1 μm.

9.2 Inbreeding, Inbreeding Depression, & Genetic Load

Marshall (1973) proposed that, for cultivated allohexaploid chrysanthemums, inbreeding may be relatively ineffective due to fixed heterozygosity. If this were the case, chrysanthemums would follow the pattern of other polyploid species (with disomic inheritance) that have fixed heterozygosity in the multiple genomes, e.g. homosporous ferns (Haufler and Soltis, 1986), *Equisetum* (Soltis, 1986), *Triticum, Avena, Nicotiana,* and *Gossypium* (Bingham, 1979). Inferences about the possible occurrence of inbreeding depression in polyploid chrysanthemum species appear in experiments designed to analyze the inheritance of other traits. In the first report to

document SI, wild and cultivated chrysanthemum species were selfed to produce n=1-3 inbred generations in five years (Niwa, 1931). Selfing resulted in decreased height, seed set, germination, and flowering, when compared to outcross pollinations. This led to the initial conclusion that these species were SI and that inbreeding depression could exist. Subsequent studies (Mulford, 1937b; Tsukamoto, et al., 1964; Kawase and Tsukamoto, 1966) also compared the performance of F_1 and I_1 (first inbred generation) progeny for fertility or flower characters. While there was a general reduction in the average I_1 performance, the standard errors were large enough to encompass the ranges observed for the F_1.

The use of rapid generation cycling techniques (Figure 6) accelerated the rate of progress in chrysanthemum breeding programs and inbreeding by decreasing the generation time (Anderson, et al., 1990). In one report, it was used to further inbred line development with eight generations of inbreeding (Anderson and Ascher, 2000). Use of these techniques allowed for the creation and evaluation of three inbred generations in <1 year using multiple plant descent. Sixty-six non-inbred or inbred parents selected for PSC were used to create 1-3 inbred generations, depending on the level(s) of inbreeding depression. By the end of the second inbred generation, all noninbred parent-derived populations were extinct due to SI or inbreeding depression (Anderson, et al., 1992a, b). Inbreeding level (generation) on seed germination and survivorship (yield potential) were negatively correlated and highly significant. Seed germination and yield potential were highly correlated, which suggests that lethality due to inbreeding is not independent between life cycle stages. High levels of inbreeding (F=0.995) did not eliminate the expression of inbreeding depression, which was attributable to both dominance and epistasis (Anderson, et al., 1992a). Fertility (seed set, pollen stainability) was also negatively affected by continued inbreeding (Anderson and Ascher, 2000). Anderson, et al. (1993) demonstrated that the best means of circumventing significant inbreeding depression in early and/or advanced inbred generations was by using recombinant inbreeding, whereby inbreeding and outcrossing is juxtaposed every 2-3 generations (Bailey, 1971; Campbell, 1988).

9.3 Hybrid Seed Production

Public and private sector breeding programs have historically emphasized continued development and release of asexually propagated cultivars. The reasons for this are varied, including the ease of obtaining natural or irradiation-induced sport families which are readily adaptable to current production protocols, long generation time, SI, sterilities associated with clonal cultivars, source/sink interactions in herbaceous perennials, and polyploidy (Anderson, et al., 1988). Thus, less attention has been given to investigating the potential alternative of hybrid seed production. While asexual propagation provides homogeneity, it has the distinct disadvantages of virus buildup in stock plants and the high cost of plant

material (production, shipping, etc.), particularly if it is certified virus-free (Langton, 1987). Hybrid seed is predominantly virus-free and comparatively cheaper than cuttings to produce.

Hybrid seed cultivars are not new to the floricultural crop arena. They have been primarily applied to annuals (e.g. *Ageratum, Impatiens, Petunia, Tagetes*) and less commonly to biennials or perennials (e.g. *Begonia x tuberhybrida, Cyclamen, Freesia, Streptocarpus)* (Wellensiek, 1959; Sparnaaij, 1968; Schmidt and Erickson, 1981; Reimann-Philipp, 1983). In recent decades, renewed interest has arisen among flower breeders to develop hybrids in crops that have been asexually propagated, e.g. *Pelargonium x hortorum* (Craig, 1976; White and Quatchak, 1985), *P. peltatum* (Langton, 1987), and *Gerbera jamesonii* (Meynet, 1978). Such has also been the case for chrysanthemum (Satory, 1986). This trend is likely to continue, as releases of seed-propagated flower crops escalates (Anderson, 2004; Anderson, et al., 1995).

Breeding programs aimed at hybrid seed production can face formidable problems: SI, new incompatibility specificities, PSC, ploidy, and severe inbreeding depression (Frankel and Galun, 1977; Langton, 1987). While chrysanthemums possess all of these attributes, such barriers have not been insurmountable in other polyploid crops commercially available as hybrids. In other species, the problems are not as severe and it has been postulated that *Kalanchoe* may also join the ranks as a as seed-propagated floriculture crop (Royle, 1982).

Both the private (T. Sakata Seed Company, Yokohama, Japan; Bodgers Seeds., Gilroy, California, USA) and public (Hiroshima and Kyoto Universities, Japan; University of Minnesota, USA; Federal Research Center, Ahrensburg, Germany) sector breeding programs (past and present) have initiated the research and development of hybrid chrysanthemum seed production (Anderson and Ascher, 1996, 2000). Initial studies concentrated on characterizing the SI system and creation of homozygous inbred lines. Use of techniques, such as rapid generation cycling, enabled faster derivation of nearly homozygous inbreds.

The private sector has released several F_1 and F_2 cultivars, e.g. 'Autumn Glory', 'Petit Point', 'Korean Eriso', 'Super Jet', 'Korean Jewel', 'Golden Dream', and 'Fanfare' (Anderson, et al., 1988, 1995; Park Seed Co., 1985), although none are grown extensively or on a commercial basis. Standard comparison trials of these hybrids by the University of Minnesota breeding program (45ºN lat.) demonstrated that these seed lines lacked uniformity for important phenotypic traits, particularly flower color, quality, and shape; plant habit; flowering time (Figure 14-8a; Anderson, et al., 1988, 1995). These hybrids, presumably produced from crossing inbred parents, lack uniformity for phenotypically important traits (flower color and type, flowering earliness, and plant habit) and environmental stability (flowering time).

Realizing the potential contribution F_1 hybrid seed-propagated cultivars could have for commercial production, the University of Minnesota breeding program has

devoted 15-20 years on this objective (Anderson, et al., 1995). Genetic information and inheritance studies are needed before such hybrids may be commercialized. However, significant progress has been realized with the use of inbred parents to create hybrids, which are uniform for flower color, flowering time, and plant habit (Figure 14-8b). Such seed-propagated hybrids, however, will most likely never reach the level of clonal uniformity in asexually-propagated cultivars.

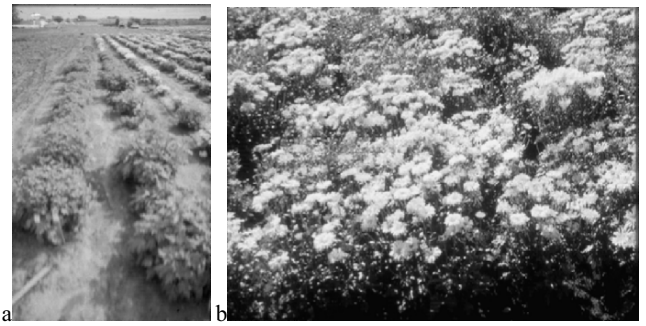

Figure 14-8. Commercial F_1 or F_2 hybrid seed-propagated commercial cultivars (a) which do not flower at 45ºN. lat. (St. Paul, Minnesota, U.S.A.) and flowering, uniform hybrids (b) selected for northern latitudes.

9.4 Floral Traits—Size, Type, Petal Orientation

Several research programs have reported on the inheritance of various floral traits, from flower size to color and form. Typical problems noted throughout many experiments are accurate classification of phenotypes (many appear to be more quantitative than qualitative in nature) and small progeny sizes (Crook, 1942), which can limit the reliability of goodness of fit tests (Chi-square, χ^2) for genetic classes.

Flower size was hypothesized to be controlled by four unnamed genes (Crook, 1942). Four size classes were identified, ranging from 'baby' (<0.75" dia.) to 'small' (0.75-1.75"), 'medium' (1.75-2.75"), and 'large' (>2.75"). Test crosses between progeny derived from crossing two parents of similar flower size, 'Mrs. Tricker' x 'Crimson Splendor', showed high levels of agreement between observed and expected ratios although the progeny sizes were small (ranging from n=3 – 79).

Flower type is based on the number of ray petal rows (single to fully double) as well as petal types (plain to quill, spoon, etc.) (Figure 14-3). Both categories are not mutually exclusive for most combinations. Additionally, petal orientation is another floral trait, which may range from flat to incurved (petal tips curved towards the center of the flower) or reflexed (petal tips curved away from the floral center). Incurved petalage was first termed "petals which reverse over the eye" (Crook, 1942).

The number of ray petal rows is classified into single (1 row), duplex (2), triplex (3), quadriplex (4), pentaplex (5), and so forth. When the number of ray petal rows

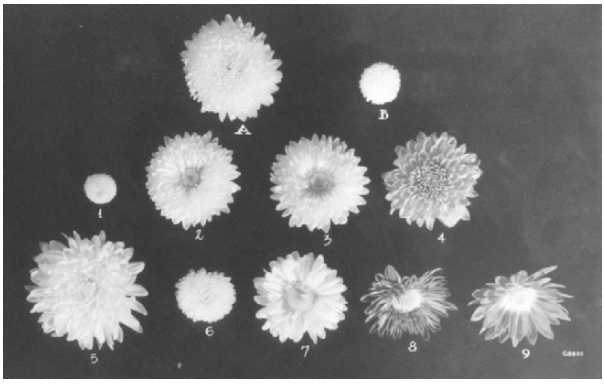

Figure 14-9. Example inheritance of floral forms and petal number in the cross between 'Yellow Doty' (A) x 'Varsity' (B); the F_1 hybrid progeny (1-9) display the parental and additional flower types (Crook, 1942).

is equivalent to replacing 50% of the disc floret rows (ranging from 3-10 rows of ray petals) it is termed 'semi-double'; if the number of ray petal rows reaches 100%, the fully double or 'super double' state is achieved (with >10 rows of ray petals and zero rows of disc florets). Crook (1942) proposed that four dominant genes (all designated as *T* with superscript numerical designators to distinguish between varying genes for this trait) conferred single flowers (T^1- T^2- T^3- T^4-), while any three sets of genes were dominant led to semi-doubleness and the presence of one or no dominants led to super-doubleness. Crosses between unrelated parents which differ in floral morphology create F_1 hybrid progeny with parental types and other flower forms (Figure 14-9).

Incurved flower petals were hypothesized to be controlled by two dominant genes, designated as *O* and *P* (Crook, 1942). Genotypes must have at least one dominant allele at each loci to exhibit the 'incurved' phenotype. Quilled petal inheritance studies are incomplete, since inadequate numbers of progeny were routine (Crook, 1942). However, positive assortative mating of parents is required to obtain a majority of progeny with quilled petals.

Disc floret morphology may also vary, ranging from essentially colorless upon pollen dehiscence (wild type in most chrysanthemum species) to colored florets with morphological modifications. For instance, the 'anemone' flower type possesses such modifications. One species, *Leucanthemum frutescens,* has anemone flowers as a diagnostic key (Heywood, 1976; Heywood and Humphries, 1977).

Promiscuous ray petals or 'petals in the eye' occurring in the disc florets are problematic. The U.S. national flower judging standards manual (Pi Alpha Xi, 1998) notes this to be a significant grower-related fault in commercial cut chrysanthemums and, to a lesser extent, in flowering potted types. Genotypes with promiscuous rays can be created via mutation breeding; the expression of this trait

may be stable or unstable when the genotype is grown in varying environmental conditions (Crook, 1942). Two dominant genes, *R* and *S,* were postulated to control this trait (Crook, 1942).

9.5 Flowering Requirements—Earliness, Day Neutrality, Heat Delay Insensitivity

Flower initiation and development are controlled by short day photoperiods that naturally occur by shortening day lengths in late summer and early fall (Post, 1949). Both garden and greenhouse chrysanthemums are short day (SD) plants for flowering (Cathey and Borthwick, 1957, 1961, 1964). Flower bud development (FBD) of SD chrysanthemums is reversibly controlled by red (~660 nm) and far red (730 nm) light. Continuous or intermittent exposure to red light, with the use of either incandescent, high pressure sodium, or fluorescent light sources in the middle of a long dark period (night), inhibits flower bud initiation (FBI) and development (FBD) (Borthwick and Cathey, 1962).

Garden and greenhouse chrysanthemums are categorized into SD response groups, the number of weeks from the start of the SD treatment to anthesis (Anderson and Ascher, 2001). Vegetative growth has a critical photoperiod of $\geq$13.5 hr while reproductive development (flowering) requires $\leq$12 hr (Cockshull, 1985). Genotypes are categorized into response groups of early, mid, and late depending on the number of weeks of SD required for FBI and FBD. Garden chrysanthemum response groups range from six to eight weeks while greenhouse types are 6.5-11 wks for flowering potted plants and 8-15 wks for cut flowers (van Zanten, North America, 1999; Yoder Brothers, Inc., 2000). Early flowering types (6-8 wk response group) are facultative SD for FBI but qualitative (obligate) SD plants for FBD (Cockshull and Kofranek, 1992). Later flowering response groups (>8 wks SD) are obligate SD plants for both FBI and FBD.

Early, mid, and late flowering response groups will initiate flower buds under non-inductive long day conditions (morphologically termed ‘crown buds’ with subtending strap-shaped leaves), but only early response groups will also undergo FBD (Langton, 1977). Crown buds have a flattened appearance due to arrested floret development, as well as the lack of subtending axillary meristems (Anderson and Ascher, 2001).

Long day leaf number (LDLN), defined as the mean number of leaves initiated by the terminal meristem prior to commencing FBI under a long day photoperiod, is used as a quantitative measurement of vegetative growth and juvenility in seedlings (Cockshull, 1976; Cockshull and Kofranek, 1985). FBI, but not short day response group (based on FBD), is linked with LDLN (Anderson and Ascher, 2001; Langton, 1981). Broad sense heritability for LDLN ranges from $h^2 = 0.79$, with a 95% C.I. of 0.76-0.82 (Anderson and Ascher, 2001), to $h^2 = 0.83$ (Langton and Dixon, 1984).

Mean stem lengths of the terminal shoot are significantly longer with SD genotypes, in comparison with day neutrals (Anderson and Ascher, 2001). Stem length has a broad sense heritability of $h^2 = 0.91$, with a 95% C.I. of 0.90-0.92 (Anderson and Ascher, 2001). The number of nodes with axillary branching is unrelated to photoperiodic response and has a broad sense heritability of $h^2 = 0.75$ (95% C.I. of 0.74-0.76) (Anderson and Ascher, 2001).

Chrysanthemum breeders in the U.S. during the early 1900s focused on early flowering genotypes—particularly those reaching anthesis prior to the first frost date (Mulford 1935). Since a number of environmental factors can influence occurrence of FBI and the rate of FBD extensive testing over years and locations is necessary to ensure stability of cultivars prior to their release. Mulford (1938) found three exceptional garden genotypes that did not vary in flowering time from year-to-year. Decades of selection for early flowering garden chrysanthemum genotypes resulted in FBI and FBD occurring under natural long day photoperiods of the summer. When these genotypes were tested in the critical photoperiod experiments, many were day neutral (Anderson and Ascher, 2001).

True day neutral chrysanthemums have been selected which will undergo both FBI and FBD under any combination of photoperiods within the normal temperature range (10-12C nights) for commercial production of the crop (Kawata and Toyoda, 1982; Anderson and Ascher, 2001). Day neutral genotypes will undergo autonomous FBI and FBD for all flower buds in the inflorescence (primaries, secondaries, tertiaries, etc.) under any photoperiod or combination of light quality (Cathey and Borthwick, 1970; Kawata and Toyoda, 1982; Langton, 1977; Schwabe, 1953). It is not necessary to test for day neutrality under all combination of photoperiods. Rather, a continuous (24 hr) far-red + red light treatment during FBI and FBD may be used to select day neutral progeny in which all of the first six flower buds (ranging from the first or terminal flower bud to the fifth subtending lateral) reach anthesis (Anderson and Ascher, 2001). This environment may be supplemented with high night temperatures to simultaneously select for heat delay insensitivity.

A variety of day neutral genotypes have been selected including ‘Dr. Longley’ and Mn. Sel’n. 83-267-3 (Figure 14-10) from the University of Minnesota breeding program (Anderson and Ascher, 2001; Seeley, 1966)—both of which flower under any photoperiod ranging from 8 – 24 hrs in duration; ‘Jeongwoon’ and ‘Mezame’ from Japanese breeding programs (Kim and Lee, 1998; Langton, 1978), and numerous others (Harada and Nitsch, 1959; Okada, 1957; Satory, 1986; Seeley, 1966). Mn. Sel’n. 83-267-3 is both day neutral and heat delay insensitive (Anderson, et al., 1989). An ideotype for selecting day neutral, heat-delay insensitive genotypes was proposed by Anderson and Ascher (2001) and subsequently tested (Anderson and Ascher, 2004).

Day neutrality offers commercial greenhouse growers advantages when producing garden chrysanthemum for spring sales (flowering in pots), potted and cut

greenhouse types. For example, the institution of black cloth for SD photoperiods during periods of the year when natural photoperiods exceed the requirement for FBI and FBD in SD genotypes would be eliminated (Anderson, 1991; Anderson and Ascher, 2001; Ascher, 1986). Day neutral cultivars, as well as those with short juvenility periods, require exogenous applications of ethylene using Ethephon (Florel, Union Carbide Co., Research Triangle Park, North Carolina, USA) to inhibit FBI and/or FBD in stock plants (Cockshull, et al., 1979; Strefeler, et al., 1996; Stuart, et al., 1988). Home gardeners could also benefit from growing day-neutral cultivars as this trait would offer the opportunity for continuous flowering during the growing season.

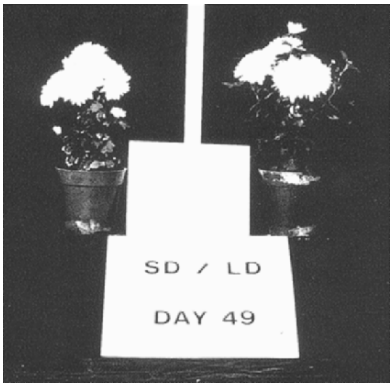

Figure 14-10. Flowering of day-neutral garden chrysanthemum Mn. Sel'n. 83-267-3 under short days and long days (red light) photoperiods (Anderson and Ascher, 2001).

High night temperatures (≥22C) during FBI and/or FBD, termed heat delay, can delay flowering and produce abnormal inflorescence development (Cathey, 1954; Cockshull, 1979; Crater, 1980; Whealey, et al., 1987). Likewise, prior to FBI, high night temperatures may also increase LDLN (Cockshull, 1979). Heat delay most frequently occurs during greenhouse production when black cloth is pulled over the crop for FBI and FBD in SD cultivars. Example cultivars sensitive to heat delay are: 'Delano', 'Yellow Mandalay', and 'Sunny Mandalay' (Yoder Brothers, Inc., 2000). Heat delay insensitivity has been bred into numerous greenhouse cultivars and may also be accompanied by a corollary low temperature (10 C nights) delay (de Jong, 1978).

9.6 Winter Hardiness

Winter hardiness is an essential trait for herbaceous perennials in northern temperate regions circumboreally (Still, et al., 1988; Griesbach and Berberich, 1995). Early cultivated forms were not winter hardy in northern temperate regions, as noted by Morrison (1923): "chrysanthemums are of little value as hardy plants in

the extreme North...". Winter hardiness or cold tolerance continues to be an important trait for perennial garden chrysanthemums (Anderson and Gesick 2004) that are frequently sold as "hardy" mums (Holley, 1945; Wulster and Lacey, 1985). This is a frequent misnomer as many cultivars are not perennial (winter hardy) north of the 40th parallel, necessitating repurchase each year as herbaceous annuals (Anderson and Gesick, 2004; Holley, 1945; Wulster and Lacey, 1985).

In the early 1900s, garden chrysanthemum breeding programs commenced with breeding for increased winter hardiness (Hieke, 1976; Askew and Chaput, 1987; Griesbach and Berberich, 1995; Widmer, 1958; Wildung, 1979). Dr. A.C. Hildreth (Cheyenne Horticulture Field Station, USA) planted n=2,000 cultivars of chrysanthemums at the field station in 1932 and selected n=20 hardy genotypes that survived the winter (Viehmeyer and Uhlinger, 1955). These became the parental gene pool of winter hardiness in U.S. breeding programs (North Platte Station, Nebraska; University of Minnesota, USA) and included subsequent releases ('Red Chief'). Crosses between 'hardy' and 'tender' parents demonstrated that hardiness is not dominant (Viehmeyer and Uhlinger, 1955). F_1 hybrids were primarily in the midparent range, although a few genotypes approach that of the hardy parent. Viehmeyer and Uhlinger (1955) theorized that genotypes with shallow rhizomes were more likely to receive winter damage than deep ones although genetic variation existed in shallow rhizome genotypes to occasionally survive through the winter.

The U.S. Department of Agriculture's program breeding program released n=12 genotypes in 1937 with promising hardiness (Mulford, 1937b, 1938). Other public sector breeding programs—particularly the University of Minnesota followed with continued release of winter hardy cultivars beginning with 'Duluth' in 1939 (Widmer, 1997). Holley (1945) found that ~-12.2C killed chrysanthemum crowns (roots, rhizomes). Controlled freezing studies in the laboratory found that –10 to –15C injured acclimated rhizomes (Widmer, 1958). Yang (1995) also found that free proline content and electrolyte leakages were correlated with winter hardiness.

More recent studies have shown that a breeding program may implement a non-destructive method to select among first-year seedlings by counting the number of emergent rhizomes (Anderson and Gesick, 2004). Non-winter-hardy genotypes had significantly fewer emergent rhizomes (Figure 14-11a) than winter-hardy types (Figure 14-11b) (Anderson and Gesick, 2004). This selection method, however, requires a corollary 'test' winter with adequate cold temperatures and snowfall. The recent resurgence in el Niño throughout central North America has necessitated supplementing selection for high emergent rhizome genotypes with laboratory freezing tests of the entire crown to replace the 'test' winter (Kim and Anderson, 2005).

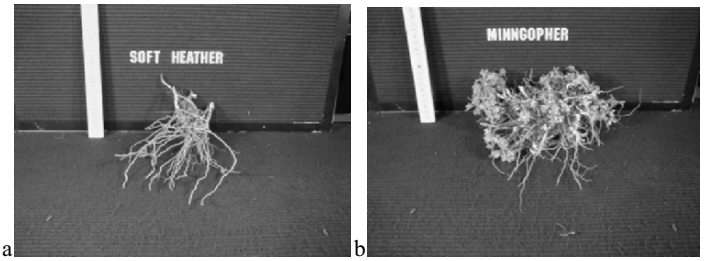

Figure 14-11. Phenotypes of (a) non-winter-hardy 'Soft Heather' and (b) winter-hardy 'Minngopher' garden chrysanthemum crowns recorded at the end of the first growing season (Anderson and Gesick, 2004).

Two different laboratory freezing tests were evaluated for their effectiveness in determining cold tolerance (Kim and Anderson, 2005). Acclimated crowns of hardy and non-hardy garden chrysanthemum genotypes were used in Omega Block (using detached, emergent rhizomes) and chamber (using entire, intact crowns with emergent, non-emergent rhizomes) freezing test methods. Comparative winter survival in the field was monitored over locations and years. Cold tolerance was assessed at 0°C to -12°C with varying ramp and soak time periods. The chamber freezing method was the most powerful to discern LT_{50} values (lethal temperature at which 50% of the samples were killed) (Figure 14-12). Cold tolerant genotypes included 'Duluth' and Mn. Sel'n. 98-89-7 (LT_{50} = -12°C). Three genotypes had intermediate cold tolerance (LT_{50}=-10°C) and one genotype was not cold tolerant (LT_{50} = -6°C). Cold-tolerant genotypes also had significantly higher regrowth ratings for rhizomes at 1cm and 3cm depths (Kim and Anderson, 2005).

10. MOLECULAR BIOLOGY

The highly interspecific nature of cultivated chrysanthemums and the tight nature of SI, coupled with the repeated introduction of new species and germplasm into breeding programs, have resulted in high levels of genetic diversity in the gene pool (Wolff and Peters-van Rijn, 1993). Genetic variation in *D. x grandiflora* is similar to other cultivated crops with similar mating systems and breeding programs (Carlson, et al., 1991; Reiter, et al., 1992; van Heusden and Bachmann, 1992).

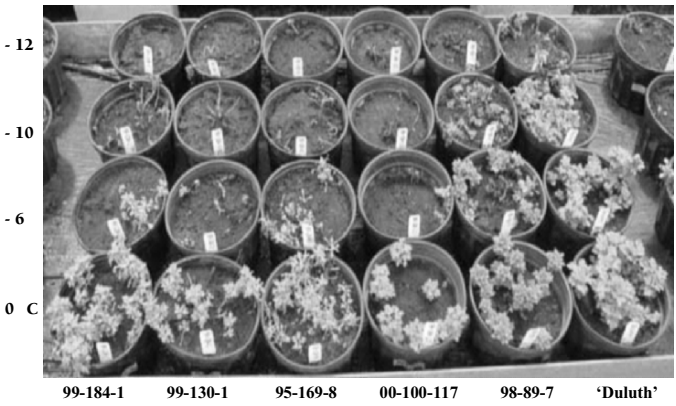

Figure 14-12. A comparison of regrowth results of chrysanthemum crowns, *Dendranthema ×grandiflora*, after exposure to laboratory freezing tests of 0ºC, -6ºC, -10ºC, and -12ºC (rows). Clones of genotypes are depicted in each row (Kim and Anderson, 2005).

Molecular techniques have been used with chrysanthemums for a wide range of purposes from detecting genetic diversity (RAPDs; Wolff and Peters-van Rijn, 1993), RFLP probe and primer development (Wolff, et al., 1993; 1994), sport and chimera characterization (Wolff, 1996), transformation (Young, et al., 1998), to genetic fingerprinting (Wolff, et al., 1995). Wolff and Peters-van Rijn (1993) used RAPDs to study clonal stability in a sport family (n=13 genotypes using n=27 primers), cultivar variation (using n=18 cultivars from three breeding programs and n=8 primers), and species variability (n=13 species, n=15 genotypes, and n=6 primers). Intercultivar variation was high and as few as two primers could be used to distinguish between cultivars. The sport family derived from a single cultivar all had identical fragment patterns. Wolff, et al. (1995) found similar results within sport families when regardless of which molecular fingerprinting method was used, i.e. from RAPDs, inter-SSR PCR (simple sequence repeat polymerase chain reaction), hybridization-based DNA fingerprinting, or RFLPs (restriction fragment length polymorphisms). Thus, due to the high levels of polymorphism and clonal stability in chrysanthemum, RAPDs were used for cultivar identification or fingerprinting.

Regeneration and transformation systems have been developed to successfully transform cultivated chrysanthemums. Young, et al. (1998) transformed two cultivars using *Agrobacterium tumefaciens* LBA4404 with three vectors, pBI121, pCMAsCP121-123, and pTOK233; other researchers have noted cultivar-strain specificity to *A. tumefaciens* (Bush & Pueppke, 1991). *Agrobacterium* readily works with chrysanthemums since the species is also readily infected with crown gall

(Miller, et al., 1975). The most effective shoot regeneration medium consisted of Murashige-Skoog basal salts with 2.0 mg/L NAA and 0.5 mg/L BA (benzyl adenine) (Young, et al., 1998). A 20 mg/L kanamyacin concentration resulted in the highest tissue formation rates. De Jong, et al. (1994) reported stable expression of the GUS reporter gene in chrysanthemums.

While most molecular techniques are useful and regeneration/transformation systems are easily developed, they are limited in value due to the wide range of genetic variability present in this interspecific, polyploid crop. Future research may be useful in focusing on marker-assisted selection and the development of molecular maps, since classic genetic maps are nonexistent (Wolff, et al., 1994).

11. IDEOTYPE BREEDING

Plant ideotypes are predictable plant growth models for a crop in a defined environment (Donald, 1968). In chrysanthemum breeding programs, ideotypes may be used to select plants with the suite of traits for the defined environment and market class. Langton and Cockshull (1976) developed an ideotype for cut spray chrysanthemums. Ascher (1986) also presented an ideotype for F_1 hybrid (seed-propagated) chrysanthemums. More recently, Anderson and Ascher (2001) proposed an ideotype for breeding and selecting day-neutral, heat-delay insensitive genotypes. Other ideotypes can be created, depending on the breeding objectives.

Chrysanthemum breeding programs have a variety of breeding objectives, depending on the product being created. Laurie and Poesch (1939) forwarded traits such as flower color, size, form, doubleness, foliage texture, flowering time, and plant habit to be important characteristics. Cumming (1939) also added winter hardiness, heat tolerance, long flowering periods, insect resistance, frost tolerance, dwarf types (for edging borders and roc gardens), and fragrance. Both garden and greenhouse (cut, potted) types require strong stems to prevent lodging (Smith and Laurie, 1928). Resistance for flower color fading in color groups with anthocyanins (reds, bronzes, purples, lavenders) is also a desirable trait (Crook, 1942). More recently, the lack of purpling in white or cream-colored flowers is a necessary trait for selection.

Historically, breeders have used positive assortative mating to obtain the highest frequency of hybrids with the desired traits (Crook, 1942). For instance, to obtain progeny that flower within a specific short day response group a breeder should use parents that flower within or as close to the desired response group as possible (Smith and Laurie, 1928). Private and public sector chrysanthemum breeding programs should incorporate as many desired traits as possible into their ideotypes for simultaneous selection in the target environments (Anderson and Ascher, 2001).

12. FUTURE DIRECTIONS

Visionary chrysanthemum breeding must focus beyond the immediate product potential of this crop to proactively create a continually expanding array of phenotypes (Anderson, 2004). Breeders should spend sufficient time collecting new germplasm and observing new phenotypes in wild germplasm. Numerous species have not been collected from the wild (particularly in western China) or the genetic variation sufficiently sampled and placed in repositories for future use. The U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) which operates the Ornamental Plant Germplasm Center (OPGC) at Ohio State University, has identified chrysanthemums as a primary genus for preservation (http://opgc.osu.edu/). The USDA-ARS OPGC also has a Chrysanthemum Working Group of public and private sector professionals to direct the directive to collect and preserve chrysanthemum germplasm. Such focused efforts in this area will assure that future generations of chrysanthemum breeders will have access to wild species populations and genetic variation to use in the creation of new flower colors, forms, and plant habits.

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