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Tilting at windmills: 20 years of *Hippeastrum* breeding

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ABSTRACT

Hippeastrum Herbert, amaryllis, has yielded popular large-flowered hybrids over a 200-year breeding history, with the Netherlands and South Africa currently dominating the market. The USDA breeding program is now almost ten years old, built upon a ten-year previous history at the University of Florida that yielded three patented triploid varieties. This program has focused heavily on two species, *H. papilio* and *H. brasilianum*, and over 1000 selections of diploid, triploid, and tetraploid progeny have been stockpiled. Attempts to develop a domestic commercial producer have been stymied by economic conditions, and a steady program of patented release is planned. Future directions of the breeding involve induction of tetraploidy in diploid selections, and genomic explorations of genes involved in floral fragrance and pigment expression.

Keywords: plant breeding, flower bulbs, amaryllis, floral fragrance, floral pigments, genetics

INTRODUCTION

Hippeastrum Herbert, amaryllis, has yielded popular large-flowered hybrids over a 200-year breeding history (Read, 2004). Bulbs are produced for indoor forcing and, to a lesser extent, garden use in mild winter areas (USDA Hardiness Zones 7B-11). Amaryllis is much appreciated by gardeners for ease of culture, while amateur plant breeders have found it an easy and rewarding group to hybridize (Meerow, 2000a).

Hippeastrum consists of 50–60 entirely New World species, though one species, *H. reginae* Herbert, appears to have been introduced to Africa. The species are concentrated in two main areas of diversity, one in eastern Brazil, and the other in the central southern Andes of Peru, Bolivia, and Argentina, on the eastern slopes and adjacent foothills (Meerow, 2004). A few species extend north to Mexico and the West Indies. The genus is essentially tropical and subtropical, though some species occur far enough south of the equator and at sufficient elevation to be considered temperate plants. Little of this genetic diversity is represented in modern, commercial amaryllis hybrids. Early hybrids were produced from a relatively small number of species, mainly *H. vittatum* Herbert, *H. reginae* Herbert, *H. puniceum* (Lamarck) Voss, *H. aulicum* Herbert, *H. psittacinum* (Ker Gawler) Herbert, *H. striatum* (Lamarck) H.E. Moore, and *H. reticulatum* Herbert (Traub, 1934a, 1958; Bell, 1973a; Cage, 1978a; Shields, 1979). To date, the only reported intergeneric hybrid involving *Hippeastrum* that appears to be true is one involving *Sprekelia formosissima* (L.) Herbert (Aztec lily), which has been shown to be apomictic (Zonneveld, 2004).

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History of amaryllis breeding

A detailed history of amaryllis breeding and cultivation can be found in Traub (1958) and Read (1999, 2004), and will only be summarized here. *Hippeastrum* × 'Johnsonii', generally acknowledged as the first amaryllis hybrid, was a primary hybrid of *H. vittatum* and *H. reginae* made in England in 1799 (Traub, 1934a). Many additional hybrids were reported during the first 25 years of the 19th century as new species were collected in South America and imported to Europe. The two most significant developments in amaryllis breeding were the development of the Reginae and Leopoldii strains of hybrids (Traub, 1958).

The Reginae strain was developed by Jan de Graaff of Holland and his two sons in the middle of the 19th century by breeding *H. vittatum* and *H. striatum* with *H. psittacinum* and some of the better hybrids available in Europe (Traub, 1958). The introduction of *H. leopoldii* Dombrain and *H. pardinum* (Hook. f.) Lemaire from the Andes by the plant explorer Richard Pearce, in the employ of the British firm Veitch and Sons, would have lasting impact on the future of amaryllis hybridization (Veitch, 1906). Both species are notable for their large, wide-open, and relatively symmetrical flowers. When bred with the best of the Reginae strain of hybrids, a race of large- and very open-flowered progeny was developed, the best of which carried 4–6 flowers on the scape. Veitch and Sons dominated the development of the Leopoldii strain and thus European amaryllis breeding well into the first quarter of the 20th century (Traub, 1958). The best of these Veitch hybrids set the standards that have since largely dominated commercial amaryllis development.

The late 19th century to the early 20th saw a modicum of amaryllis breeding efforts in the United States, primarily in Texas, California, and Florida. Luther Burbank developed a large-flowered strain based on the European Reginae and Leopoldii groups (Traub, 1958). A hybridization program was carried on by USDA from 1910 to 1939, and the Department held annual amaryllis shows from 1912 to 1939 (except 1914 and 1915) (Traub, 1958). However, the greatest American contributions to amaryllis hybridization were those of two Florida breeders, Henry Nehrling and Theodore Mead (Nehrling, 1909; Hayward, 1934; Traub, 1934b; Mead, 1935; Bell, 1973a). The Mead hybrids in particular, originating from Nehrling's germplasm, have contributed to some modern hybrids when crossed with Ludwig or other Dutch stock (Bell, 1973a). Though the Mead hybrids did not match the European strains in flower size and number of scapes produced, they were reliable and vigorous performers under Florida garden conditions (Hayward, 1934; Traub, 1958; Bell, 1973a). As amaryllis production in Florida faded—the results of disease, competition, and failures in quality control—much of this germplasm has been lost.

After the first two decades of the 20th century, amaryllis breeding and production in Europe declined, as much the result of two world wars as for any other reason (Traub, 1958). The exception was in the Netherlands, and today, the primary center of modern amaryllis production and breeding is Holland, with additional Dutch stock grown in Brazil (Ferguson, 2006). The large-flowered Ludwig strains (e.g., 'Apple Blossom', Dazzler', 'Dutch Belle', White Christmas') have rapidly become the dominant genotypes among Dutch amaryllis (Ludwig and Co., 1948). Another important Dutch cultivar group is the Gracilis strain of dwarf, multiflora types ('Firefly' and 'Scarlet Baby', for example). The strain was originated by H. Boegschoten breeding *H. striatum* with large-flowered hybrids and developed commercially by the van Meeuwan and Sons Company (Read, 1999). South Africa has also now become an important breeding center and exporter of amaryllis (Buck, 1961; Goedert, 1961; Barnhoorn, 1976, 1991, 2005) as well, particularly the Hadeco strain (Barnhoorn, 1976, 1991, 2005), which dominates the Christmas market. In India, a breeding program has been ongoing at the National Botanical Research Institute in Lucknow (Narain, 1982, 1983, 1987, 1991). In Japan, a program focusing on smaller-flowered hybrids has been led by Miyake Nursery in Chiba-kon (Read, 2004). Breeding programs are also underway at the Volcani Institute in Israel (Sandler-Ziv et al., 1997) and the Agronomic Institute of Campinas in Brazil (Tombolato et al., 1991). The late Fred Meyer, of Escondido, CA, developed several strains of hybrids with unusual floral morphology (Read, 2004), but those that have been commercialized have apparently not found much market acceptance. Likewise, the late Claude Hope, hybrid flower seed breeder in Costa Rica, bred several dwarf amaryllis that were never commercialized (Meerow, 1998).

The legion of amateur breeders of amaryllis, too numerous to list by name, in America, Europe, Asia, and Australia, should be acknowledged. From time to time, selections from these small-scale breeding programs have been offered commercially or purchased as breeding stock by large commercial endeavors.

IMPORTANT TRAITS AND BREEDING OBJECTIVES

The emphasis in commercial breeding efforts in amaryllis, with exception of the Gracilis strain, has traditionally been on large flower size, traits attributable specifically to genes originating in *H. leopoldii* and *H. pardinum* (Traub, 1958; Bell, 1973a; Shields, 1979;

Read, 2004). Commercial breeding efforts subsequent to the initial flurry of primary hybridization have largely been concentrated among the hybrids themselves, leading to a greater complexity of parentage (much without documentation) and dilution of many of the unique characteristics of the original component species (Bell, 1973a,b; Cage, 1978a; Shields, 1979).

The pursuit of novelty in amaryllis hybrids has largely been the province of amateur breeders and collectors, most of whom have little inclination to commercially exploit their hobby or have failed in their attempts to do so (Cage, 1978b; Cothran, 1979; Wilson, 1981; Doran, 1982). Breeding efforts by amateurs have largely been ignored by European breeders with the exception of attempts to develop a large-flowered yellow hybrid (Blossfeld, 1973; Cothran, 1979, 1980, 1981, 1984, 1985; Goedert, 1982). There has also been some commercial interest in double-flowered varieties (Bell, 1977a).

Double-flowered amaryllis

The first reported double amaryllis was found in the wild in Cuba, a form of *H. puniceum* (Traub, 1958). Significant breeding for doubleness was first reported by McCann (1937, 1950). Additional tepal-like structures apparently result from transformations of both stamens and style (male and female reproductive structures). McCann's and other observations (Latapie and Latapie, 1982) on inheritance of the character indicate dominance for doubleness in breeding. Doubles seem to have significant market appeal based on their prominence in catalogs.

Fragrance

The majority of modern amaryllis hybrid cultivars do not have fragrant flowers, nor do the majority of species. Fragrance in the genus is usually associated with white, long-tubed flowers that are probably pollinated in nature by sphingid moths. On the basis of the results of our breeding program in *Hippeastrum*, we can draw two hypotheses: (1) floral fragrance is a recessive character and (2) expression of fragrance is under simple genetic control, perhaps only a single gene.

BREEDING AND PLOIDY LEVEL

The overwhelming majority of *Hippeastrum* species are diploid, with a somatic chromosome number of $2n = 22$ (Naranjo and Andrada, 1975; Flory and Coulthard, 1981; Arroyo, 1982). Virtually all of the complex hybrid material presently in cultivation is tetraploid (Bell, 1973a,b, 1977b; Shields, 1979), a result of both selection for tetraploid progeny (often associated with plant and flower size increases in hybrid amaryllis) and incorporation of a few natural tetraploid species in early hybridization efforts (some forms of *H. striatum*, for example). A few species of *Hippeastrum* have been reported with higher ploidy levels than 4*n* (Traub, 1958), but I am not aware of any breeding efforts with them.

The historical concentration of commercial breeding efforts among the various populations of tetraploids may exist for several reasons: (1) desirable characteristics of flower size, scape number, and plant vigor are already stabilized in the hybrid races; (2) sterile triploid progeny result when diploid species are crossed with tetraploid hybrids (Bell, 1973b, 1977b); (3) many of the diploid species are not readily available; and (4) self-incompatibility, which occurs in most diploid species and diploid hybrids, generally breaks down in the tetraploid hybrids (Bell, 1973a, 1977b; Cage, 1978b; Shields, 1979; Williams, 1980), thereby allowing breeders to obtain a segregating F_2 generation.

Breeding at the diploid level

The advantages of breeding among the diploid species of *Hippeastrum* (which constitute the majority) are (1) novel traits can only be found among the species; (2) diploid species are readily inter-fertile; (3) diploid F_1 hybrids can often be flowered in eighteen months or less from seed; and (4) hybrid vigor is frequently expressed in the F_1 generation in the form of higher scape and bud counts to the scape. The disadvantages are twofold. First, self-incompatibility, which characterizes most wild species, carries over into the hybrids. I have even found that compatibility barriers between siblings of the same cross are more often the rule than the exception, presumably due to homozygosity for S-gene alleles (Takayama and Isogai, 2005). Secondly, the green throat and/or floral tube of most species appears in the hybrids as well. By and large, this is considered an objectionable characteristic in the marketplace. Diploid hybrids will also usually have smaller flowers than the commercial Leopoldii-type cultivars.

Breeding diploids and tetraploids

It is generally considered that diploid and tetraploid amaryllis are difficult to cross. Previous reports have stated that if diploids are used as maternal parents with tetraploid pollen, only sterile triploid progeny will result, while reciprocal crosses (tetraploid as seed parent, diploid as pollen parent) will rarely set any seed at all (Bell, 1973b, 1977b). The ovary may begin to swell with developing seeds but then aborts at a further point in time before full term. In the latter situation, progeny can sometimes be obtained through embryo rescue, in which the developing ovule is excised from the ovary several days after pollination and grown on a sterile medium. However, in my experience, using tetraploid

pollen on diploid seed parents can yield a small number of tetraploid progeny. Interestingly, when I have pollinated some diploid hybrids or species with pollen of many Dutch hybrids, a full and apparently normal seed capsule will develop. Most of the seeds, however, contain no embryo. Approximately 10% of the seed will germinate, and yield a mix of triploids and tetraploids. Unreduced gametes (that is, egg cells with 22, rather than the usual 11 chromosomes) in the diploid parent are the probable source of the few tetraploid progeny that occur in these crosses.

Triploids

The triploid progeny that usually result from 2*n* (diploid) × 4*n* (tetraploid) crosses of amaryllis are usually both self- and inter-sterile (Bell, 1973b). Rarely can they be bred with diploid species or hybrids, though techniques of embryo culture can be used to "rescue" progeny of such crosses, as long as fertilization has taken place and the developing seeds are placed in culture before the ovary begins to abort. Greater success has been reported crossing triploid amaryllis with tetraploids, perhaps due to some degree of random assortment of the third set of chromosomes during gamete formation (Bell, 1973b). Triploid intermediates of diploid species or hybrids may allow introgression of desirable species traits into established tetraploid cultivars without the rapid dilution of those traits that occurs with diploid–tetraploid crosses followed by exclusively tetraploid breeding.

Breeding tetraploids

Self-incompatibility of most diploid amaryllis almost invariably disappears in tetraploid hybrids and most tetraploid species (Bell, 1973b, 1977b; Cage, 1978a; Shields, 1979; Williams, 1980). Most tetraploids can be readily self-pollinated and inter-crossed, with a resulting high percentage of viable seed. This has largely been the source of today's commercial amaryllis cultivars, and is the easiest breeding program to undertake, as much due to the wide availability of tetraploid hybrids as to the absence of compatibility barriers.

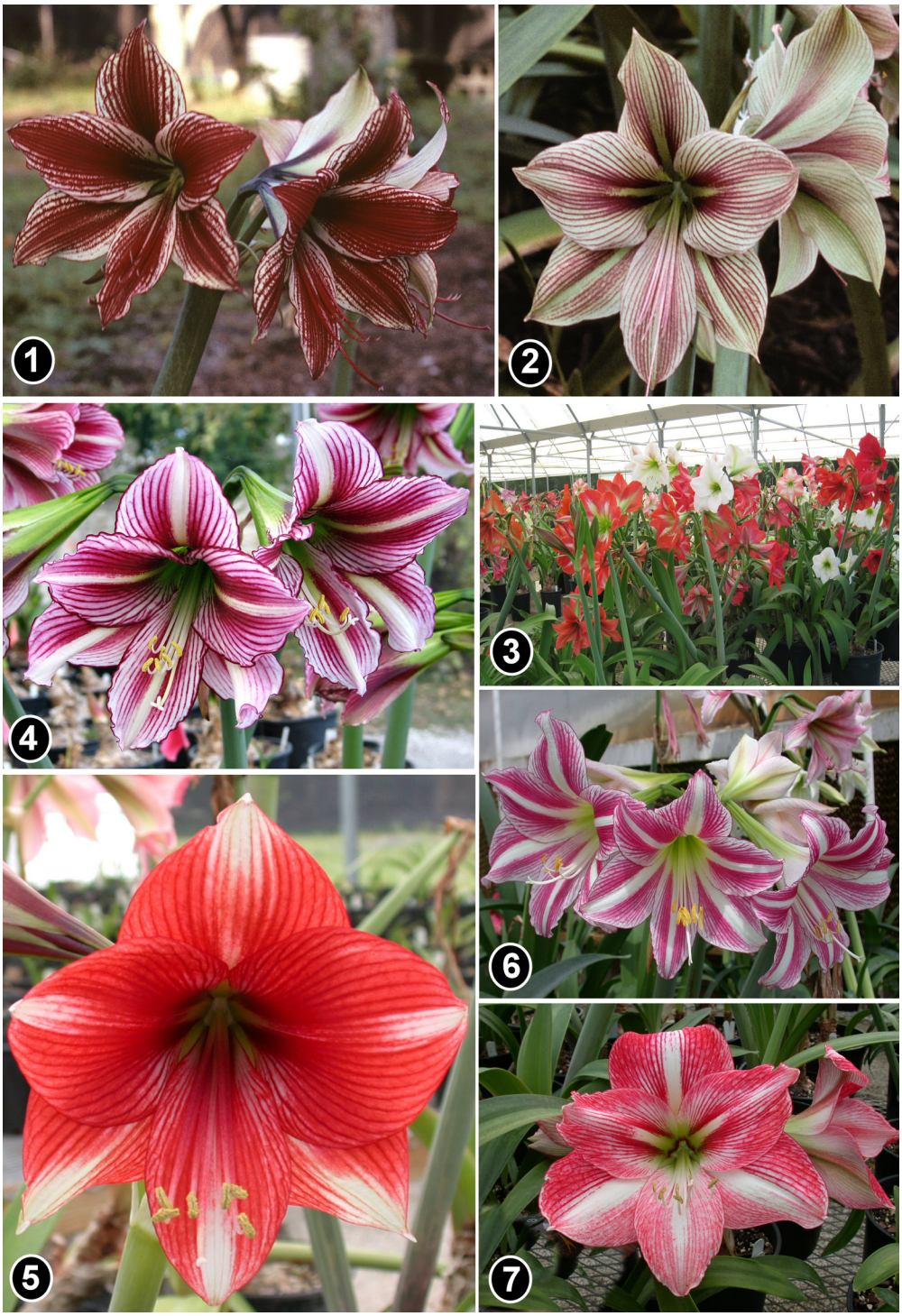
THE PROGRAM AT THE UNIVERSITY OF FLORIDA (1988–1999)

From 1988 to 1989, reciprocal F_1 progeny were produced between *H. papilio* Ravenna and, respectively, *H. pardinum*, *H. lapacense* (Cárdenas) J. Van Scheepen, *H. ambiguum* Hook., *H. brasilianum* (Traub and J.L. Doran) Dutilh, and *H. reticulatum* var. *striatifolium*. The choice of *H. papilio* as a primary parent in these initial breeding efforts was made on several bases: the expression of purple coloration in the tepals of many clones, evergreen foliage, and relatively heavy substance (flowers typically last for four or more days). Progeny were established for evaluation in saran-shaded fields in native soil amended with organic matter. The results of these initial crosses were summarized in Meerow et al. (1992). After these initial primary *H. papilio* crosses (Figs. 1 and 2), no further crosses were made with this species because of the dominance of its green background in most of the progeny. In 1991, pollen was received from the late Fred Meyer (Escondido, CA) of some of his more interesting diploid hybrids, and numerous crosses to our F_1s were completed. Many of these progeny were not compatible with Florida conditions and were lost before flowering, but a few survived and have been successfully used in subsequent hybrids. *Hippeastrum brasilianum* was the most important parent in successive hybridization with the F_1 selections and their inter-cross progeny, and 150 crosses were successfully generated. This species produces large, white, trumpet-shaped flowers with intense fragrance, and seems to be resistant to red scorch (*Stagonospora curtisii*). Succeeding years of breeding have focused on (1) inter-hybrid crosses between selected F_1 clones and (2) introgressing Dutch and South African tetraploids to selected diploid clones. Tetraploid progeny resulting from these latter crosses were invariably self-compatible and the more interesting clones were self-pollinated.

In 1997, while I was on sabbatical at the Royal Botanical Gardens, Kew, the evaluation fields were decimated by a newly arrived pest in Florida for which *Hippeastrum* appears to be the primary host (Epsky et al., 2008). The "Amaryllis weevil," a small and as yet undescribed member of the Baradinae, destroyed 80–90% of the field-maintained stocks of the first year F_1 hybrids, and many unevaluated progeny before it was brought under control (imidichloprid has proven effective, but annual and early season application of any broad-spectrum systemic insecticide appears to provide protection). Despite this setback, the years of breeding at the University of Florida ultimately yielded three patented triploid cultivars, 'Bahia', 'Rio', and 'Sampa' (Meerow, 2000b).

THE USDA PROGRAM (1999–PRESENT)

My joining USDA-ARS in 1999 commenced a second period of breeding *Hippeastrum*. The focus shifted from diploids, and, having accumulated a fair number of self-compatible tetraploid selections, a large number of crosses were made between them, as well as self-pollinations. For the first time in many years, a new species was brought into the program, *H. doraniae* Traub, as much to test my hypothesis about fragrance



Figs. 1 and 2. *Hippeastrum* primary interspecific hybrids that were used extensively in subsequent breeding efforts by the author. 1. *H. papilio* × *H. lapacense*. 2. *H. papilio* × *H. pardinum*. Fig. 3. *Hippeastrum* hybrid progeny trials at USDA, Miami, FL. Fig. 4. F_1 hybrid of *H. papilio* and *H. brasilianum*. Note the purple pigment expression in the flower. The progeny of this cross are also being used to explore genetics of floral fragrance and pigment expression. Fig. 5. Complex diploid *Hippeastrum* hybrid with novel peloric floral pattern: (*H. ambiguum* × *H. papilio*) ×[(*H. papilio* ×*H. pardinum*) ×(*H. papilio* ×*H. fragrantissium*)]. Fig. 6. Complex diploid *Hippeastrum* hybrid with trumpet-shaped perianth: {(*H. papilio* ×*H. pardinum*) ×[(*H. brasilianum* ×*H. ambiguum*) ×*H. fragrantissium*]} ×*H. brasilianum*. Fig. 7. Complex triploid *Hippeastrum* hybrid: (*H. ambiguum* × *H. papilio*) × *H.* 'Kalahari'.

in *Hippeastrum*, as to generate novel phenotypes. All of our *Hippeastrum* are grown in containers, either in a glasshouse or polycarbonate-roofed Quonset house with saran-screen side walls.

The pursuit of yellow

Despite the release in recent years of cultivars such as 'Yellow Pioneer', 'Lemon Lime', and 'Germa', a Leopoldii-type clear, rich yellow amaryllis has remained elusive. Three species produce y000.ellow flowers, *H. evansiae* (Traub and Nelson) H. E. Moore (Bolivia), *H. parodii* Hunziker and Coccuci (Argentina), and *H. algaiae* (Castellanos) Hunziker and Coccuci (Argentina), and breeding efforts for yellow have concentrated on these. Of the three, *H. evansiae* is best adapted for growing in hot, humid climates. Cothran (1979, 1980, 1981, 1984, 1985) has detailed his efforts (and trials) toward a large, yellow hybrid amaryllis. We have expended considerable effort to try to improve the expression of yellow in amaryllis hybrids, using the aforementioned species, the current commercial "yellow" hybrids, and, most recently, a diverse introgression program with orange-flowered species and hybrids, followed by self-pollination of self-compatible progeny. The rationale was the fact that the best yellow expression observed on both with *H. evansiae* and 'Yellow Pioneer' seemed to be accompanied by orange suffusions or light striations in the perianth. Both colors are produced via the carotenoid biosynthetic pathway (Grotewald, 2006). Our results have been disappointing, largely producing yellows no better than available cultivars. This difficulty is interesting, considering that rich, "buttery" yellow color expression occurs in two related genera, *Rhodophiala* and *Zephyranthes*, as well in two genera of Eurasian Amaryllidaceae (sister group to the American genera, Meerow et al., 1999), *Narcissus* and *Sternbergia*, and suggests that genetic transformation may be the only the way to develop a high-quality yellow *Hippeastrum*.

Oryzalin induced tetraploidy

Efforts to induce chromosome doubling in plants date to 1940 when colchicine was found to be an effective agent for inducing polyploidy angiosperm cells via its potent anti-microtubular activity (Hancock, 1997). Forty years later, over 150 plant species were documented as having had chromosomes successfully doubled by colchicine (Dewey, 1979). Unfortunately, colchicine is highly toxic, thus interest developed in compounds such as phosphorothioamidates and dinitroanilines as potential polyploidy inducers (Sree Ramulu et al., 1991; Tosca et al., 1995; Zhao and Simmonds, 1995; Chalak and Legave, 1996; Salon and Earle, 1998). While they share the anti-microtubular activity of colchicine, they are usually less toxic because of their more specific binding affinity to plant tubulins and efficacy at low concentrations (Morejohn and Fosket, 1991). The dinitroanilines oryzalin and trifluralin have successfully induced autoploid plants in a number of ornamental genera including *Rosa* (Zlesak et al., 2005), *Euphoriba pulcherrima* (Pickens et al., 2006), *Gentiana* (Morgan et al., 2003), *Gaura* (Pietsch and Anderson, 2006), *Spathiphyllum* (Eeckhaut et al., 2004), *Miscanthus* (Petersen et al., 2003), *Hypericum* (Olsen et al., 2006a) × *Chitalpa* (Olsen et al., 2006b), and *Rhododendron* (Vainola, 2000; Contreras et al., 2007). Of particular interest to us is the success of oryzalin as a polyploidy inducing agent in Amaryllidaceae and Liliaceae (van Tuyl et al., 1992). Having recently moved into a new research building with a tissue culture lab, we will begin a long-deferred program of polyploidy induction in our best diploid selections.

Current state of the program and the prospects of commercialization

After many years of breeding and selection, we have over a thousand clones that can be roughly characterized into several phenotypic categories: (1) diploid complex hybrids with novel floral pigmentation patterns or colors (Figs. 4 and 5), which await attempted tetraploidization; (2) triploids (Fig. 7), with many of the market characteristics associated with current commercial varieties (short to medium scapes, a minimum of 4 buds per scape, a minimum of 2 scapes produced per bulb), and with at least one novel phenotypic character; (3) long-stemmed trumpet-shaped flowers (Fig. 6), both fragrant and unscented, with potential as cut flowers (no post-harvest research has been conducted); and (4) tetraploid selections representing self progeny of tetraploid hybrids as well as complex hybrids among tetraploid selections.

After a long search among domestic bulb growers, we succeeded in finding a grower well-positioned to partner with us in the commercial development of our finer selections. A five-year Cooperative Research and Development Agreement was set up to facilitate this process. Unfortunately, the grower was forced to withdraw after one year due to the economic downturn. At present, our strategy is to seek patents for five or six cultivars and widely publicize their availability for licensing.

GENOMIC APPROACHES TO *HIPPEASTRUM* MPROVEMENT

Isolation and identification of floral fragrance genes in *Hippeastrum* via a subtraction cDNA library

Flower fragrance is a complex character involving a combination of molecules synthesized by plants. Hun-

dreds of constituent chemicals have been isolated and characterized (Knudsen et al., 1993), and the majority can be assigned to three major biosynthetic pathways: phenylpropanoids, fatty acid derivatives, and terpenoids (Croteau and Karp, 1991). Although the complete pathways leading to the final products have not been characterized, common modifications such as hydroxylation, acetylation, and methylation have been described (Dudareva, 2002).

As yet, there is no model system for the elucidation of the genetics of floral fragrance synthesis or expression in plants (Guterman et al., 2002; Pichersky and Dudareva, 2007). Floral fragrance is not a character that can be observed visually, and is rarely consistent biochemically (and thus, by inference, genetically as well). The flowers of *Arabidopsis*, a model for so many plant genetic pathways, do not produce a large amount of volatile compounds (Vainstein et al., 2001). Consequently, genetic characterization of flower scent is still in its infancy (Dudareva and Pichersky, 2000; Dudareva, 2002; Dudareva and Negre, 2005). A few floral fragrance genes have been identified from plants with very fragrant flowers (e.g., *Clarkia breweri* and *Antirrhinum majus*; Dudareva et al., 1996; Vainstein et al., 2001). Recently, Verdonk et al. (2005) isolated a transcription factor that regulates floral scent biosynthesis in *Petunia*.

Petals appear to be the major organ of origin for fragrance compounds in most angiosperms, with developmental control of production and release (Guterman et al., 2002). More specifically, the cells of the petal epidermis have been implicated as sites for fragrance production and emission on the basis of expression analyses of fragrance related genes (Dudareva and Pichersky, 2000; Kolosova et al., 2001; Vainstein et al., 2001), particularly at the advanced stages of flower development during cell expansion (Guterman et al., 2002).

Expressed Sequences Tags (ESTs) are DNAs representing tissue-specific genes with diverse levels of transcription (e.g., Ohlrogge and Benning, 2000). EST projects are extremely time- and labor-intensive without the application of subtraction and equalization, as the sequencing of many thousands of cDNA clones isolated from the specific tissues or after inducing of a particular physiological response. A smaller scale project focusing on the isolation of rare specific transcripts in the cDNA can efficiently target a specific response or tissue of interest with much less cost in time and resources. Several different approaches isolating rare genes in cDNA libraries based on subtraction of cDNAs have been reported (Harper, 1997; Hubank and Schatz, 1999). Others incorporate equalization of the representation of transcripts (Patanjali et al., 1991; Kohchi et al., 1995; Gurskaya et al., 1996). The more sophisticated methods involve both normalization and subtraction of the cDNA libraries (Diatchenko et al., 1999; Carninci et al., 2000). Our general strategy to identify genes involved in fragrance would be to look at the up-regulated genes in the fragrant individuals, identify by BLAST search any that are related to biosynthetic pathways involved in volatile production, and conduct real-time PCR time course analysis of those candidate genes to determine if their expression is correlated with fragrance production in the fragrant individuals. Our target family for the analysis is *H. papilio*, *H. brasilianum*, their F_1 hybrid (Fig. 3), and various backcross full sibs between the F_1s and *H. brasilianum* (Fig. 1).

Reaching for the sky

Flower color is a complex trait that is determined by more than the presence of a given pigment. Flavonoid-based color is determined by anthocyanin, co-pigment, vacuolar pH, and metal ions (Grotewald, 2006). The anthocyanins are the primary pigments responsible in plants for red through blue colors (Grotewald, 2006). By understanding the biochemical basis of flower color within a given species, and the genetics behind it, one can develop a genomic strategy for creating new colors (Tanaka and Ohmiya, 2008). Considerable information is available on the biochemistry and genetics of these pigments (Griesbach, 2005).

Blue flower coloration is rare in the Amaryllidaceae sensu stricto, but is not uncommon in the two closest families, Agapanthaceae and Alliaceae. In the Amaryllidaceae, expression of a true blue is observed only in *Lycoris sprengeri*, where it occurs in the apical zone of the tepals, while lilac-purple floral coloration is characteristic of the tribe Griffineae (Meerow and Snijman, 1998). The blue pigments in *Agapanthus* have been well characterized as delphinidins (Bloor and Falshaw, 2000), thus this genus presents the best target for determining the genes responsible for expression. The lack of violet to blue flowers in roses, carnations, chrysanthemums, and lilies has been linked to the apparent absence of the gene for flavonoid 3′,5′-hydroxylase (*F3′5′H*), which mediates the transformation of dihydroquercetin to dihydromyrciten, a precursor of delphinidin (Okinawa et al., 2003; Chandler and Tanaka, 2007; Katsumoto et al., 2007; Tanaka and Ohmiya, 2008). Our plan is thus to first examine *Agapanthus*, *Griffinia*, *Lycoris sprengeri* Comes ex Baker, and our purple F_1 hybrids of *Hippeastrum papilio* and *H. brasilianum* to see if *F3′5′H* is differentially present and/or expressed. We also plan to create cDNA libraries from tepal tissue of these same taxa at three different stages of pigment expression.

A major gap in our knowledge concerns the genetic regulation of anthocyanin biosynthesis. While there is

some information available on the identity of the regulatory genes affecting biosynthesis, there is little or no information on how these regulatory genes control tissue-specific expression. What is known is that anthocyanin structural gene transcription requires the expression of at least one of each of three distinct transcription factor families: MYC, MYB, and WD40 (Hartman et al., 2005; Ramsay and Glover, 2005; Baudry et al., 2006). The three transcription factors form a complex in which MYB binds to the structural gene promoter's MYB recognition element (MRE), and MYC binds to the promoter's E-box. Structural gene regulation is defined by the diversity among the *Myc* and *Myb* alleles, each of which regulates expression in a different manner. For example in petunia, the combination Myc_{An1}/Myb_{An2} induces anthocyanin pigmentation in the flower, whereas the Myc_{An1}/Myb_{Ph4} combination induces vacuolar acidification (Quattrocchio et al., 2006).

If we are able to determine the genetics behind blue flowers in *Agapanthus*, it may not be far-fetched to imagine being able to engineer a blue *Hippeastrum*. Likewise, investigating expression of yellow floral coloration in species such as *Zephyranthes citrina* Baker and *Rhodophiala bagnoldii* (Herb.) Traub may open the door to truly yellow amaryllis as well.

CONCLUSIONS

While our hope of developing domestic production of our *Hippeastrum* hybrids has been a victim of the economic downtown, we are still optimistic that many of our selections have great market potential. In the years to come, we expect to begin a steady program of cultivar release, as well as bring the tools of genomics to bear on further improvement of *Hippeastrum* varieties. While I believe that there is great opportunity for domestic production of *Hippeastrum* bulbs in the United States, overcoming grower resistance to compete with market leaders has so far proven daunting.

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