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# Evaluation of genetic variation in the daylily (*Hemerocallis* spp.) using AFLP markers

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**Abstract** The daylily (*Hemerocallis* spp.) is one of the most economically important ornamental plant species in commerce. Interestingly, it is also one of the most heavily bred crops during the past 60 years. Since the American Hemerocallis Society began acting as the official registry of daylily cultivars in 1947, more than 40 000 registrations have been processed. In order to determine the effects of intensive breeding on cultivar development, and to study relationships among different species, genetic variation in the daylily was estimated using AFLP markers. Nineteen primary genotypes (species and early cultivars) and 100 modern cultivars from different time periods were evaluated using 152 unambiguous bands (average 79% polymorphism rate) derived from three AFLP primer combinations. Overall, pairwise similarity estimates between entries ranged between 0.618 and 0.926 (average=0.800). When comparing cultivar groups from different time periods (1940–1998), genetic similarity was initially increased, compared to the primary diploid genotypes, remained constant from 1940 to 1980, and then steadily increased as breeding efforts intensified and hybridizers began focusing on a limited tetraploid germplasm pool derived by colchicine conversion. Among modern (1991–1998) daylily cultivars, genetic similarity has increased by approximately 10% compared to the primary genotypes. These data were also used to evaluate recent taxonomic classifications among daylily species which, with a few minor exceptions, were generally supported by the AFLP data.

**Keywords** *Hemerocallis* · Daylily · Genetic variation · AFLP

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## Introduction

The daylily is one of the most economically important flowering herbaceous perennial nursery crops in the USA. Ancestral daylily species were originally derived from their center of origin in Asia (Stout 1934). In the early 1900s, the director of the New York Botanical Garden, Arlow B. Stout, began extensive efforts in the collecting, study, and breeding of many daylily species. Other prominent horticulturalists who made a significant contribution to daylily breeding in the early 1900s, using primary germplasm, were Amos Perry and George Yeld in England and Willy Müller in Italy (Stout 1934; Erhardt 1992). Germplasm derived largely from the efforts of these individuals has been exploited extensively by daylily breeders over the past 60 years. Since the American Hemerocallis Society began acting as the official plant registry for daylilies, more than 40 000 registrations have been processed and published in the form of check lists (Stuntz 1957; Monroe 1973 1984 1989 1994; Baxter 1999). Interestingly, continued progress in the development of new variants in flower color and form are still being achieved despite years of extensive breeding, suggesting that the genome of the daylily may be quite diverse.

The breeding of daylilies generally involves the crossing of heterogeneous genotypes followed by the selection of desirable floral and plant growth phenotypes. Selected plants are vegetatively propagated, then the new cultivar is registered with the American Hemerocallis society and introduced into commerce.

While extensive breeding has been undertaken by numerous breeders, very little genetic study has been accomplished in the daylily. Karyotypes of the daylily indi-

cate a basic chromosome number of 11 (Stout 1934; Brennan 1992). The species are usually diploid, with the exception of some triploids which are generally sterile as pod parents. Nearly all of the described species tend to be interfertile (Stout 1934). Thus, there appear to be no secondary or tertiary gene pools according to the breeding-based classification system for plants described by Harlan and deWet (1971). The conversion of numerous diploid cultivars to tetraploids has been accomplished via the use of colchicine treatment. This has resulted in an increase in the size of plant organs in addition to unique patterns of pigment distribution in the flowers. As a result, the modern daylily gene pool is a mix of both diploid and tetraploid germplasm.

Scientific approaches to the conservation and utilization of plant genetic resources require an accurate assessment of the amount and distribution of genetic variation within a gene pool. In recent years, DNA markers have provided the tools needed to accomplish such a task. The multilocus AFLP DNA fingerprinting technique (Vos et al. 1995) has been widely used to study genetic relationships among many different plant species, such as soybean (Maughan et al. 1996), lettuce (Hill et al. 1996), wild bean (Tohme et al. 1996), lentil (Sharma et al. 1996), peanut (He and Prakash 1997) tea (Paul et al. 1997), einkorn wheat (Heun et al. 1997), sunflower (Hongtrakul et al. 1997), potato/tomato (Milbourne et al. 1997; Kardolus et al. 1998), rhododendron (Escaravage et al. 1998), grape (Cervera et al. 1998), geranium (Barcaccia et al. 1999) and olive (Angiolillo et al. 1999). The benefits of this technique include reproducibility, high levels of polymorphism detection, genome-wide distribution of markers, and no required prior knowledge of the genome being studied (Prabhu and Gresshoff 1994; Lu et al. 1996). As a result, the AFLP technique is ideally suited to the study of genetic diversity within gene pools for which little information currently exists.

Up to now, the variability of daylily germplasm has been described in terms of morphology, growth, and environmental adaptability traits (Stout 1934; Erhardt 1992). No biochemical or molecular analyses aimed at determining diversity or genetic relationships have been reported. In the present study, we have used AFLP markers to estimate genetic variation within the daylily gene pool. This was performed to determine how the genome has evolved in response to over 60 years of intensive breeding and selection. These results provide the basis for determining how the present level of genetic variability might be best classified, managed and utilized by both breeders and geneticists.

## Materials and methods

### Plant material and DNA extraction

A group of 100 modern cultivars, 16 species, and three early cultivars were included in the screening (Tables 1 and 2). In order to best represent the variability within the cultivated germplasm pool, we chose cultivars from all the major time periods of breeding in the 20th century. Since a majority of daylily breeding has occurred in the latter half of the 20th century, most of the cultivars tested have registrations occurring within the past 60 years. Because a rapid increase in breeding efforts occurred in the 1980s and 1990s (Fig. 1), large samples were taken from cultivars having registrations during these time periods. Modern cultivars were either donated and/or purchased from the following commercial nurseries: Hickory Gardens (Worcester, Mass.), Jordans Daylilies (Lugoff, S.C.), and Roycroft Daylily Nursery (Georgetown, S.C.). The species and older cultivars (pre-1970 era) were donated by the Perennial Patch Nursery (Wade, N.C.) where a germplasm collection of ancestral genotypes is maintained. Plant material was cut back to 4 cm above the crown and plants were allowed to re-grow in the greenhouse for harvest of fresh leaf tissue for DNA extraction. Leaf tissue was immediately placed at –80°C for storage until use. Plants were then transferred from the greenhouse to the field and allowed to flower for verification of phenotype.

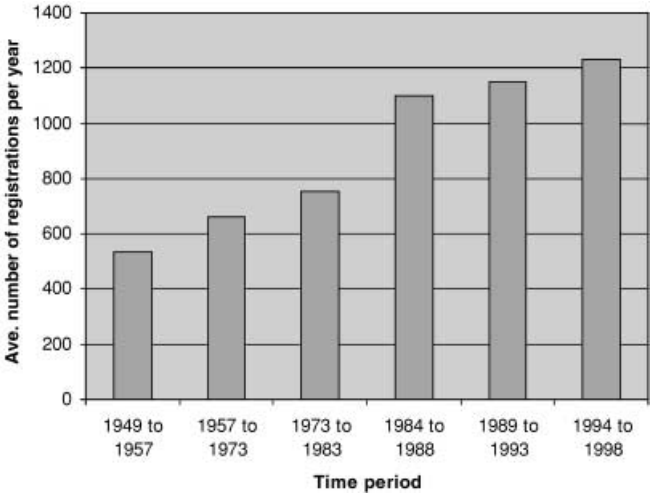


**Fig. 1** Average number of daylily cultivar registrations per year for six different time periods

DNA was extracted using 1 g of frozen leaf tissue. The DNAzol ES guanidine-based genomic DNA extraction system was utilized and protocols followed according to the manufacturer's instructions (Molecular Research Genetics Inc, Cincinnati, Ohio).

### AFLP analysis

The AFLP technique was carried out as described by Vos et al. (1995) using Gibco BRL (Grand Island, N.Y.) AFLP Analysis System-I kits for plants having large genomes. Genomic DNA (0.5 µg) was digested using both *Eco*RI and *Mse*I enzymes, and adapters were ligated to the resulting fragments. Five microliters of template DNA from a 1:10 dilution were used used for PCR pre-amplification with primers carrying one selective nucleotide. Twenty cycles were performed at 94°C for 30 s, 56°C for 60 s, and 72°C for 60 s in a PTC-200 DNA thermal cycler (MJ Research). The pre-amplification products were diluted 1:10 and used as template for selective amplification. Primers with three selective nucleotides were used (Table 3). *Eco*RI primers were end-labeled with γ-[$^{33}$P]-ATP and the following PCR reactions employed: 94°C for 30 s, 65°C for 30 s, and 72°C for 60 s. The annealing temperature was then reduced every 3 cycles by 1°C, and after 11 cycles it reached the optimal annealing temperature of 56°C. Twenty five additional cycles were done at these temperatures (94°C for 30 s; 56°C for 60 s, 72°C for 60 s) to complete the second amplification. An AFLP DNA ladder ranging in size from 30 to 330 bp was used to determine fragment sizes. The hot amplified products were electrophoresed on 64-lane 6% polyacrylamide denaturing gels in 1× TBE buffer. The dried gels were exposed to X-

**Table 1** *Hemerocallis* cultivars for AFLP analysis listed by year of registration

| Cultivar | Year | Cultivar | Year | Cultivar | Year |
|---|---|---|---|---|---|
| Pink Charm | 1940 | Siloam Bo Peep | 1978 | Pug Yarborough | 1990 |
| Painted Lady | 1942 | Christmas Is | 1979 | Lavender Stardust | 1991 |
| Black Prince | 1942 | Frank Gladney[a] | 1979 | Pirates Patch[a] | 1991 |
| Atlas | 1950 | Midnight Magic[a] | 1979 | Smugglers Gold[a] | 1991 |
| Kindly Light | 1950 | Siloam Virginia Henson | 1979 | Something Wonderful[a] | 1991 |
| Neyron Rose | 1950 | Fairy Tale Pink | 1980 | Daring Dilemma[a] | 1992 |
| Autumn Minaret | 1951 | Kate Carpenter[a] | 1980 | David Kirchoff[a] | 1992 |
| Shooting Star | 1951 | Bette Davis Eyes | 1982 | Dena Marie | 1992 |
| Lady Inara | 1956 | China Lake[a] | 1982 | Dragon King[a] | 1992 |
| Daisy McCarty | 1957 | Matt[a] | 1982 | Good Morning America[a] | 1992 |
| Luxury Lace | 1959 | Seductor[a] | 1983 | Molino Splendor | 1992 |
| Pappy Gates | 1959 | Marys Gold[a] | 1984 | Rhine Maiden[a] | 1992 |
| Carey Quinn | 1960 | Strutters Ball[a] | 1984 | Caribbean Purple Spires[a] | 1993 |
| Fashion Model | 1960 | Barbara Mitchell | 1985 | Chris Salter[a] | 1993 |
| Satin Glass | 1960 | Big Apple | 1986 | Creative Edge[a] | 1993 |
| Suzie Wong | 1962 | Dragons Orb | 1986 | Forsyth Hearts Afire | 1993 |
| Sea Gold | 1963 | Janice Brown | 1986 | Mask of Time[a] | 1993 |
| Green Flutter | 1964 | Always Afternoon[a] | 1987 | Night Dreams | 1993 |
| Little Wart | 1964 | Betty Warren Woods[a] | 1987 | Awash With Color[a] | 1994 |
| Mary Todd[a] | 1967 | Love Those Eyes[a] | 1987 | Banned in Boston | 1994 |
| Small Ways | 1967 | Emperors Dragon[a] | 1988 | Daring Deception[a] | 1994 |
| Tiny Curls | 1967 | Forsyth Hot Lips | 1988 | Ferengi Gold[a] | 1994 |
| No Mistake Plantation | 1968 | Idas Magic[a] | 1988 | Rainbow Eyes[a] | 1994 |
| Little Grapette | 1970 | Jedi Dot Pierce | 1988 | Splendid Touch[a] | 1994 |
| Ed Murray | 1971 | Magic Lace | 1988 | Eyed Fire Dance | 1995 |
| Little Business | 1971 | Beautiful Edgings | 1989 | Curly Pink Ribbons | 1996 |
| Ruffled Apricot[a] | 1972 | Custard Candy[a] | 1989 | Art Gallery Fringe[a] | 1998 |
| Russian Rhapsody[a] | 1973 | Francis Joiner | 1989 | Border Atoll[a] | 1998 |
| Elizabeth Yancy | 1973 | Jason Salter | 1989 | Islelsworth[a] | 1998 |
| Blue Happiness | 1975 | Strawberry candy[a] | 1989 | Jungle Rhythm[a] | 1998 |
| Dance Ballerina Dance[a] | 1976 | Admirals Braid[a] | 1990 | Kea Lihi[a] | 1998 |
| Raspberry Wine | 1976 | Elizabeth Salter[a] | 1990 | Rilly Frilly[a] | 1998 |
| Stella De Oro | 1977 | Fooled Me[a] | 1990 | Wolf Eyes[a] | 1998 |
| Sebastion | 1978 | Jedi Tequila Sunrise | 1990 | | |

[a] Tetraploid genotype, all others are diploid

**Table 2** *Hemerocallis* species and early cultivars for AFLP analysis including year of registration, introduction or first description

| Genotype | Year[a] |
|---|---|
| *H. citrina* | 1897 |
| *H. citrina* var. Vespertina | 1941 |
| H. dumortierii | 1830 |
| *H. dumortierii* var. Sieboldii | Unknown |
| *H. fulva* Europa | 1762 |
| *H. fulva* Flore Pleno | 1860 |
| *H. fulva* var Kwanso | 1860 |
| *H. fulva* var Maculata | 1895 |
| *H. fulva* var Rosea | 1924 |
| *H. fulva* var Sempervirens | 1966 |
| H. hakunensis | 1943 |
| H. lilioasphodelus | 1576 |
| *H. middendorffii* | 1860 |
| *H. minor* | 1748 |
| *H. thunbergii* | 1873 |
| Gold Dust | 1906 |
| Orangeman | 1906 |
| Hyperion | 1924 |

[a] Dates for species obtained from Erhardt (1992)

ray film for 2 to 4 days. Reproducibility of AFLP fingerprints was assessed using a control daylily genotype (*Hemerocallis fulva* Europa).

## Data analysis

Unambiguous AFLP bands were manually scored as present (1) or absent (0) from the autoradiographs. Both monomorphic and polymorphic bands were included in the binary data set to provide unbiased estimates of genetic similarity. Estimates of similarity among all genotypes were calculated according to the Nei and Li (1979) definition of similarity: $Sij=2a/(2a+b+c)$, where *Sij* is the similarity between two individuals *i* and *j*, *a* is the number of bands present in both individuals, *b* is the number of bands present in *i* and absent in *j*, and *c* is the number of bands present in *j* and absent in *i*. For estimates of genetic similarity, data sets were organized into the following groups: all genotypes, species and early cultivars ($n$=19) 1940 to 1964 registrations ($n$=22) 1965 to 1980 registrations ($n$=22) 1981 to 1990 registrations ($n$=28) 1991 to 1998 registrations ($n$=31), modern diploids 1980 to 1998 ($n$=22), and modern tetraploids 1980 to 1998 ($n$=22). Principle coordinate analysis was employed on the entire data set using Splus5 for Solaris. The neighbor joining method (Saitou and Nei 1987) was applied to the species and primary cultivars to estimate phylogeny from the distance (1- *Sij*) matrix to obtain an unrooted tree using the NEIGHBOR program from the PHYLIP Package (Felsenstein 1993).

**Table 3** Oligonucleotide primer combinations and polymorhism rates for AFLP analysis in 30 *Hemerocallis* genotypes (9 species, 21cultivars)

| Primer combination | Total number of bands | Polymorhic bands | Polymorhism (%) | Scored bands |
|---|---|---|---|---|
| E-AAG/M-CAA | 126 | 93 | 74 | None |
| E-AAG/M-CAC | 135 | 109 | 81 | None |
| E-ACC/M-CAA | 130 | 109 | 84 | None |
| E-ACC/M-CAC | 103 | 84 | 82 | 61 |
| E-ACC/M-CAG | 87 | 66 | 76 | 36 |
| E-ACT/M-CAT | 136 | 108 | 79 | None |
| E-ACT/M-CTT | 107 | 84 | 78 | 55 |
| E-ACT/M-CTA | 82 | 63 | 77 | None |
| | Total 906 | Total 716 | Mean 79 | Total 152 |

## Results

The AFLP fingerprinting of an initial 30 daylily genotypes (9 species, 21 cultivars), using eight primer combinations, generated a total of 906 bands ranging in length from 40 to 330 bp (Table 3). The average percentage of polymorphism (number of polymorphic bands/total number of bands) was 79% and the range among primer combinations was 74–84%. Four of the eight primer combinations were too information-rich for reliable manual band calling. The primer combination with the lowest number of bands (E-ACT/M-CTA) contained too many ambiguous bands for reliable band calling and was not used in the analysis. Three of the primer combinations (E-ACC/M-CAC, E-ACC/M-CAG, E-ACT/M-CTT) provided 152 unambiguous bands and were used for AFLP analysis of the remaining 90 genotypes. The presence of a large number of bands with some of the primer combinations indicates a relatively large genome size.

Nei's genetic similarity estimates for the entire data set (all 119 genotypes) ranged from 0.618 to 0.926. In order to estimate the effects of intensive breeding over time, the complete data set was broken into time periods as discussed in Materials and methods. The mean genetic similarities and ranges for each time period are shown in Table 4. The primary gene pool consisting of species and early cultivars showed the least genetic similarity with a mean similarity of 0.762 and the broadest range (0.618–0.926). Assuming that the species and early cultivars formed the basis for subsequent breeding efforts, the first major breeding period (1940–1965) showed an increase in average genetic similarity of 7.2%. Interestingly, there was no apparent increase in genetic similarity in the time period which followed (1965–1980). However, as interest in breeding daylilies greatly intensified in the 1980s (see Fig. 1), genetic similarity began to increase and continued to increase in the 1990s. For the most-recent time period group (1991–1998), there has been a total increase in genetic similarity of 9.7% compared to the primary gene pool.

The modern germplasm pool generally used by breeders is a mix of both diploid and tetraploid culitivars. In order to evaluate genetic variation within each ploidy group, analyses were performed on 44 randomly selected cultivars (22 from each ploidy group) registered between 1980 and 1998 (Table 4). Because tetraploid germplasm is obtained from colchicine conversion of desirable diploid cultivars, the modern tetraploid germplasm pool is only expected to be as diverse as the diploid germplasm pool from which it is derived. As expected, the average genetic similarity for diploids was lower (4.4%) than that detected among modern tetraploids.

**Table 4** Nei's genetic similarity coefficients for the entire AFLP data set and various subgroups. Total change in similarity over time: 9.7%

| Analysis group | Average | Range |
|---|---|---|
| Entire data set | 0.800 | 0.618–0.926 |
| Species and early cultivars | 0.762 | 0.675–0.910 |
| 1940 to 1964 cultivars | 0.800 | 0.722–0.891 |
| 1965 to 1980 cultivars | 0.800 | 0.716–0.869 |
| 1981 to 1990 cultivars | 0.812 | 0.667–0.904 |
| 1991 to 1998 cultivars | 0.836 | 0.749–0.926 |
| 1980 to 1998 diploids | 0.814 | 0.689–0.902 |
| 1980 to 1998 tetraploids | 0.850 | 0.761–0.926 |

Principle component analysis of the AFLP similarity estimates indicated a relatively uniform test population, with only 59% of the variance explained by the first three components (Fig. 2). The modern cultivars generally clustered together. The species and early cultivars, however, were generally more dispersed in the plot and less-tightly grouped with the modern cultivars.

Of particular interest are genetic relationships among species and early cultivars to determine if taxonomic classifications originally performed based on phenotype would be confirmed by molecular relationships obtained in the present study. Therefore, neighbor-joining analysis was carried out on the species and the early cultivars group. The resulting dendrogram is shown in Fig. 3. Taxonomy in the daylily has undergone recent changes and is still somewhat open to conjecture. For our purposes, the AFLP data will be discussed in the context of recent classifications described by Erhardt (1992). Taxonomic classifications were generally supported by the AFLP data. The six *H. fulva* species all clustered together separately from the other species, which formed a separate cluster and were generally grouped according to Erhardt's proposed group classifications for the other species. Within this group fell the three early cultivars which showed close relationships to their respective ancestral species progenitors as described in the 1893 to 1957 *Hemerocallis* checklist (Stuntz et al. 1957). The

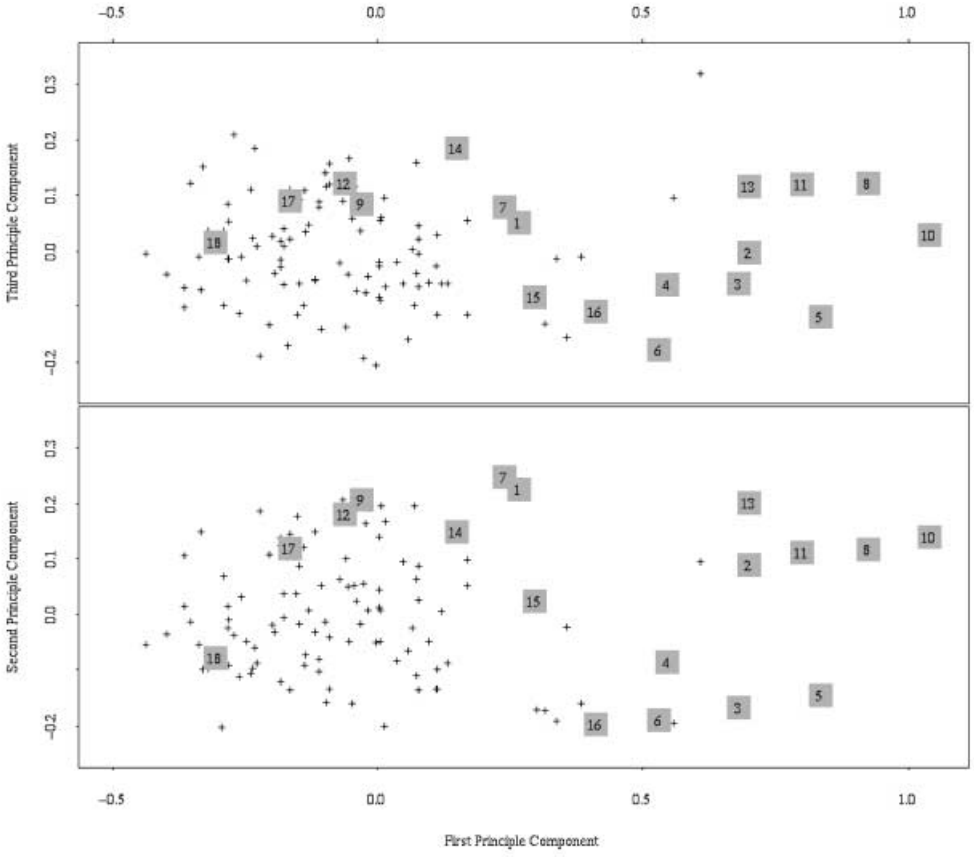


**Fig. 2** Principal coordinate plot of 119 daylily genotypes for three principal components estimated with 152 AFLP markers using the genetic similarity matrix. Species and early cultivars are indicated

Key:

1. *H. citrina*
2. *H. dumortierii*
3. *H. fulva* Europa
4. *H. fulva* flore pleno
5. *H. fulva* var. Maculata
6. *H. fulva* var. Rosea
7. *H. hakunensis*
8. *H. middendorffii*
9. *H. thunbergii*
10. Gold Dust
11. Orangeman
12. Hyperion
13. *H. minor*
14. *H. lilioasphodelus*
15. *H. fulva* var. Sempervirens
16. *H. fulva* var. Kwanso
17. *H. dumortierii* var. Sieboldii
18. *H. citrina* var. Vespertina

only anomalies were two clonal variants of *Hemerocallis citrina* (var. Vespertina) and *Hemerocallis dumortieri* (var. Sieboldii).

## Discussion

At the present time, little is knowm about the daylily genome, but the AFLP technique is ideally suited for examining unexplored genomes. Polymorphism rates were high, indicating a substantial amount of molecular variation and potential genetic diversity. Likewise, daylily cytological studies have demonstrated significant variation in chromosome morphology between species and within clones of a single species (Krikorian, et al. 1981; Noguchi 1986). Furthermore, four of the eight primer combinations were too information-rich to be useful for manual band calling, thus indicating a relatively large genome size. It is known that the genomes of certain monocots can be very large (Arumuganathan and Earl 1991). Recent estimates using flow-cytometry analysis of nuclei from two different daylily cultivars, indicates that the size of the genome is approximately 4522 Mbp/1C (Tomkins and Arumuganathan, unpublished data). Comparably, this would be slightly smaller than the barley genome which has been measured at 4783 Mbp/1C (Arumuganathan and Earl 1991).

One of our major objectives was to determine the effects of intensive breeding over time. By breaking the full data set into subsets based on breeding periods, trends in genetic variation were evaluated over the past 60 years. An initial increase in genetic similarity was ob-

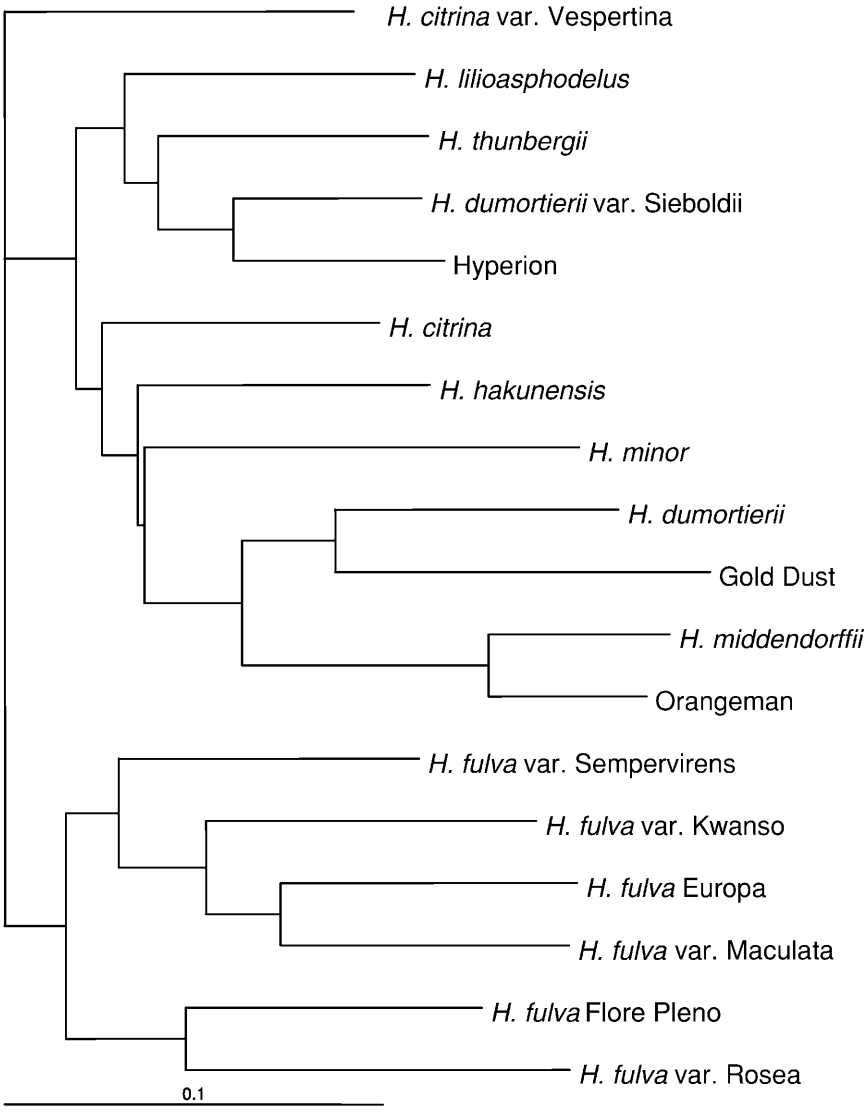


**Fig. 3** Dendrogram for neighbor-joining analysis of daylily species and early cultivars

served in the first major breeding period (1940–1964) compared to the levels observed among the species and early cultivars. Essentially, when the first few generations of improved cultivars became available, breeding with primitive genotypes was largely abandoned. Surprisingly, the next time period (1965–1980) saw virtually no change in genetic similarity. This is most likely due to the fact that the early cultivar germplasm pool was sufficiently diverse to allow continued breeding through this time period without any major change in genetic similarity. However, the following time periods (1981–1990 and 1990–1998), characterized by a large increase in breeding efforts, were also marked by a steady increase in genetic similarity. One of the factors which may haverage contributed to this trend was the increased use of tetraploids in breeding efforts. Because the breeding of tetraploids is based on converted diploids, a smaller genetic base is inherently limiting. The conversion of diploids requires some skill and is not a common practice among most breeders. When we tested the genetic variation among modern (1980–1998) diploids and tetraploids we found, as expected, increased levels of genetic similarity among the tetraploids. Thus, if tetraploid breeders are to regain lost genetic variability, efforts in converting diploid genotypes will need to be pursued.

Another major objective in the present study was to evaluate taxonomic classifications among species. The first extensive efforts at taxonomy within the daylily were attempted by A.B. Stout (1934), in which two major classifications were proposed: those having branched scapes (Euhemera) and those without branched scapes (Dihemera). Scapes are the stalks which arise from the crown region and bear the flowers. Stout's classification, however, is now not generally well accepted. A more recent classification of daylily species into five major groups is presented by Erhardt (1992), and generally supported by the AFLP data in the present study. Erhardt's classification of the five groups comprises (1) *fulva*, (2) *citrina*, (3) *middendorffii*, (4) *nana*, and (5) *multiflora*. In the present study, we tested species from groups 1, 2 and 3. We attempted to test *Hemerocallis multiflora* from group 5, but were unable to extract

good-quality DNA even after repeated attempts. It should be noted that the primary goal of the present study was to test species largely representing the ancestral foundation of the modern germplasm pool. Recent germplasm introductions and species not generally utilized as breeding stock, due to poor horticultural merit, were not included.

Utilizing neighbor-joining analysis, the six *H. fulvas* were distinctly separated from the other species. Clustering within the *fulvas* also supported some fine-scale taxonomic classifications. For example, the distinction described by Erhardt between the two fulva double-flowered genotypes 'Kwanso' and 'Flore Pleno' is reflected in the molecular data. Within the *middendorffii* group, *H. dumortierri*, *Hemerocallis middendorffii* and *Hemerocallis hakunensis* all grouped together as proposed by Erhardt. However, the distinction between the *citrina* group and the *middendorffii* group was not well defined and contained some overlap. *H. citrina* and *Hemerocallis minor* were grouped together as proposed by Erhardt, but were also grouped with members of the *middendorffii* group. Erhardt had proposed a close relationship between two other members of the citrina group, *Hemerocallis lilioasphodelus* and *Hemerocallis thunbergii*, which was well supported by our data, but they did not closely group with the other *citrina* members. In fact, our data suggest that the *middendorffii* group and the *citrina* group should be merged into one large taxonomic group.

As mentioned previously, the three early cultivars (Hyperion, Orangeman, Gold Dust) were closely grouped with their respective ancestral species progenitors as described by pedigrees in the 1893 to 1957 *Hemerocallis* check list (Stuntz et al. 1957).

The only major anomalies among the species analysis were supposed clonal variants of *H. citrina* (var. Vespertina) and *Hemerocallis dumortierii* (var. Sieboldii). While both did cluster within the *middendorffii-citrina* group, they did not closely group with their respective parental clones from which they were supposedly derived. Traditionally, there have been a number of variants of *H. dumortierrii* in commerce (Schabell 1992). Thus, the var. Sieboldii may or may not include the traditional species *H. dumortierrii* as a direct ancestor even though there are phenotypic similarities. *H. citrina* is self-incompatible (Stout 1930; Hu 1968) and thus any variant arising from it would have to be obtained from an outcross. Hence, these genotypes may either have arisen via cross-pollination or may represent distinctly different genotypes.

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