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FLORAL EXPRESSION PATTERNS AND
GENETICS OF DAY-NEUTRALITY IN OCTOPLOID FRAGARIA

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FLORAL EXPRESSION PATTERNS AND GENETICS OF DAY-NEUTRALITY IN OCTOPLOID *FRAGARIA*

By

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ABSTRACT

FLORAL EXPRESSION PATTERNS AND GENETICS OF DAY-NEUTRALITY IN OCTOPLOID *FRAGARIA*

By

Sedat Serçe

The temperature and photoperiod interactions of a number of elite genotypes of *F. virginiana*, *F.* ×*ananassa*, and *F. chiloensis* were studied in a series of growth chamber experiments involving: 1) the critical day length of short day (SD) genotypes under 8, 9, 10, and 11 h days at 18 °C; 2) the photoperiod characteristics of day-neutral (DN) and long day (LD) types under 8 and 16 h days at 18 °C, and 3) the effect of temperature on flower bud formation in DN genotypes under 12 h days at 18, 22, 26, and 30 °C. It was found that SD plants were much less sensitive to photoperiod change than previously reported. Two elite native DN genotypes LH 50-4 and RH 30 produced significantly more runners under LD and cool conditions than the DN cultivars now grown, but were not heat tolerant.

Several different greenhouse and field methods were compared to score DN in segregating populations. Scoring DN progeny within 100 days from germination was a poor predictor of field performance. However, greenhouse screens were accurate in predicting field performance, if the flowering behavior of individuals was followed through a whole season. The percentage of DN progeny observed in our second year greenhouse results were highly correlated with the subsequent field evaluations, and the families with the highest

flowering strength in the field also had the highest percentage of DNs in both greenhouse and field screens.

To elucidate the inheritance of day-neutrality in octoploid *Fragaria*, crosses were made between DN x SD and DN x DN types using *Fragaria ×ananassa* cultivars and elite clones of *Fragaria virginiana*. Wide ranges in the percent of day-neutral progeny were found in the various families (30 - 87% in DN x SD and 22 - 93% in DN x DN crosses), suggesting that day-neutrality in octoploid strawberries is a quantitative trait and not regulated by a single, dominant gene as previously suggested. Several other observations supported this conclusion including: 1) Less than half the families produced 1:1 or 3:1 ratios of day-neutrals. 2) DN *F. virginiana* genotypes produced significantly different percentages of DN progeny than DN *F. ×ananassa* cultivars. 3) Two different DN parents crossed to the same short day genotype produced different percentages of DN progeny. 4) None of the DN parents produced 100% DN progeny, which would be expected homozygous dominant DN individuals existed. 5) Some of the day-neutrality sources were more powerful than others in producing of day-neutral progeny (e.g., 'Tribute' > 'Aromas' and RH 23 > Frederick 9). 6) Both general and specific combining abilities for DN were significant.

I dedicate this work to the people of Yellibel Köyü

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ABBREVIATIONS

°C	Degree Celsius
ANOVA	Analysis of variance
CDL	Critical day length
d	Day(s)
DN	Day-neutral or day-neutrality
DN1	100 days to flowering
DN2	1st year field flowering
DN3	2nd year greenhouse flowering
DN4	2nd field flowering
DN5	Runner flowering
FSR	Flowering strength rating
g	Gram
h	Hour(s)
LD	Long day
MSU	Michigan State University
PAR	photosynthetically available radiation
QTL	Quantitative trait locus/loci
SD	short day
SE	Standard error
x	slope of the regression line
y	value observed (in regression equation)

CHAPTER 1

TEMPERATURE AND PHOTOPERIOD REGULATION OF FLOWERING IN OCTOPLOID *FRAGARIA*

Introduction

All three photoperiodic types, short day (SD), long day (LD), and day-neutral (DN) exist in strawberries *Fragaria* sp., although most of the octoploid commercial varieties now grown are either SD or DN (Hancock, 1999). Temperature and photoperiod interactions play a very important role in the flowering of strawberries (Darrow 1936; Durner et al., 1984). DN strawberry cultivars do not flower at high temperature (< 28 °C) (Durner et al., 1984). SD cultivars are facultative, meaning they form flower buds under SD conditions when temperatures are moderate, but they can form flowers under non-inductive LD conditions when temperatures are below 15 °C (Guttridge, 1985; Darrow 1936; Larson 1994). Temperature also significantly affects the number of inductive cycles (days) and the critical day length (CDL) of SD strawberry genotypes (Guttridge, 1985).

Critical day length is associated with how early cultivars begin forming flower buds in the summer and this may affect yield. For example, a cultivar with ~12 h CDL will start initiating flower buds in September in Michigan, while another one with ~11 h CDL would start in October. The cultivar initiating flower buds earliest would have more time to produce flower promordia before the onset of the first severe frost of the fall.

While numerous sources of day-neutrality have been identified in strawberries over the years, the first successful introgression into commercial octoploid strawberries was done by Bringhurst and Voth (1984) at the University of California, Davis. They transferred genes from a native clone of *Fragaria virginiana* ssp. *glauca* from the Wasatch Mountains of Utah and were able to generate commercially useful genotypes within a few generations of backcrossing into *Fragaria ×ananassa*. Currently, DN cultivars are grown on about 60% of the California production area, but remain a minor component of strawberry production in the eastern U.S. (Hancock, 1999).

The same source of day-neutrality has been used to produce new DN cultivars in many North American breeding programs (Sjulin and Dale, 1989; Hancock et al., 1990). However, while the Wasatch source for day-neutrality has proven useful in California and other Mediterranean climates, it has not performed well in other parts of North America (Dale et al., 2002). Cultivars that have the Wasatch source suffer from summer heat in continental climates. In fact, flower bud initiation is completely inhibited at 30/26 °C day/night temperatures (Galletta et al., 1981; Durner et al., 1984; Strick 1985). The DN cultivars have, at best, reduced yields and small, soft fruits in the middle of the summer (Draper et al., 1981). In addition, while early reports indicated that day-neutrality performed as a single dominant gene in the California gene pool (Bringhurst and Voth, 1978; Ahmadi et al., 1988), it does not appear to act in a similar fashion in genetic backgrounds elsewhere, and the DN "gene" varies in expressivity (Barritt et al., 1982; Nicoll and Galletta, 1987; Hancock et al., 2002).

In a number of recent germplasm surveys, several elite clones of *F. virginiana* have been selected (Frederick 9, LH 50-4, and RH 30) that may be DN as they were multiple

cropping in the field and proved useful in breeding multiple cropping progeny (Hancock et al., 2001 a and b; Serçe and Hancock, 2002; Serçe et al., 2002). However, the photoperiod relationships of these elite genotypes have not been tested.

Temperature and photoperiod are the most important environmental factors that regulate the transition from vegetative to floral growth in strawberries (Darrow 1936; Durner et al., 1984). Although the transition from vegetative to floral growth is a continuous process, it can be divided into three stages; induction, initiation, and differentiation (Durner and Poling, 1985). Floral induction takes place in the leaves after the appropriate photoperiod and/or temperatures exposure. The physiological and morphological changes that occur in meristems after the perception of these stimuli in the leaves is called initiation. The development of specific floral organs or of flowers on a single inflorescence is referred to as differentiation (Durner and Poling, 1988). Visual expansion of flowers is called flower development.

Investigators have used a number of different terminologies to describe photoperiod and temperature regulation of flowering sensitivity in strawberries including short day or Junebearing vs. everbearing (Clark, 1937; Powers 1954), as well as perpetual vs. non-perpetual (Richardson, 1917). Strawberries have also been called single, double, or multiple cropping based on how many times they flower each year. In North America and Europe, flower bud initiation takes place in late September or early October in Junebearering and SD types (Goff, 1903; Guttridge, 1958; Jahn and Dana, 1969), regardless of plant age (Hill and Davis, 1929; Schilletter and Richey, 1930; Schilletter and Richey, 1931). In everbearing and perpetual flowering cultivars, flower bud initiation occurs at least twice during the season during long days (Darrow and Waldo,

1934). Day-neutral cultivars were introduced in the late 1970s and they initiate flower buds cyclically all season as long as temperatures are below 26 °C.

Sudds (1927) was the first investigator to suggest that flowering was regulated by photoperiod, when he obtained accelerated floral initiation in plants of 'Howard 17' by maintaining them under 8 h day length. The early studies of Darrow and Waldo (1934) resulted in the classification of strawberries as Junebearer and everbearer. They concluded that the flower bud formation occurs in Junebearer types under short day conditions (less than 14 h), and under long day conditions (more than 12 h) in the everbearer ones. Darrow (1936) also studied the temperature effect on strawberry development and found that high temperatures (21 °C) favored runner formation. Hartmann (1947 a and b) studied photoperiod and temperature effects on flower development in several cultivars ('Blakemore', 'Fairfax', 'Marshall', and 'Missionary'). He followed the response of the cultivars to 10 or 15 h days at 16 and 21 °C temperatures, after acclimating the plants under long days (15 h). He found that at 16 °C all cultivars flowered regardless of day length treatments. He also found that none of the cultivars flowered under 15 h days and 21 °C treatments, while only 'Fairfax' flowered under 10 h days and 21 °C temperature. His results supported the earlier conclusions of Darrow and Waldo (1934) and Darrow (1936).

Durner *et al.* (1984) studied the interaction between photoperiod x temperature using representatives of Junebearing, everbearing, and day-neutral types. They held plants at 21 °C and examined their flowering response to 9 h SD, 16 h LD, and 9 h SD with a 3 h night interruption. They found that the flowering of DNs is little affected by photoperiod. SD plants had highest inflorescence numbers under SD (SD = 7.5 and LD =

0.8), while LD had highest inflorescence numbers under LD plants had the (SD = 1.5 and LD = 5.0). They also studied the effect of four day/night temperature treatments, the 18/14, 22/18, 26/22, 30/26 °C. Under SD conditions, all types flowered only in 18/14 and 22/18 °C treatments, and not at 26/22, 30/26 °C. The mean inflorescence numbers at 22/18 °C was 0.3, 0.5, and 1.3 for SD, LD, and DN types, respectively. Based on these results they ranked the sensitivity to high temperature as SD > LD > DN.

Reports indicated that from 7-24 days was the minimum number of photo inductive cycles necessary to induce flowering in short day plants, depending on temperature treatments (Hartmann, 1947 a and b; Went, 1957; Guttridge, 1985; Larson, 1994). Ito and Saito (1962) studied the temperature effect on photo inductive cycles and found that longer photo induction periods are required at higher temperatures. Only 10 cycles were needed for floral induction under 8 h photoperiods, at 30 °C more than 20 cycles were necessary. Under 16 h photoperiods, 10 cycles were needed for floral induction at 9 °C, whereas 16 cycles were required at 17 °C.

To express their full potential, SD cultivars often require a chilling period (<7 °C), although the cultivars developed for warm regions do not always need a chilling period (Darrow, 1933). The ability to grow well in North American greenhouses during the short days of October, November and December was used as an indicator of a cultivar's regional adaptation (Arney, 1954). The cultivars well-adapted to cooler regions generally do not grow well during this period and enter a rest period, while those adapted to warmer climates continue to grow (Darrow and Waldo, 1934).

A new type of strawberry was recently developed in Israel for tropical and subtropical environments (Izsak and Izhar, 1984; Izhar and Izsak, 1995). This type of

cultivar, called infra short day, can initiate flower bud under longer light regimes (13.5 to 14 h) and higher temperatures (10 - 26 °C) than traditional short day plants, and they do not have a chilling requirement.

While the distinction between SD, LD, and DN types was elegantly demonstrated by Durner *et al.* (1984), several other important studies which have provided key information on the regulation of flower development in the three photoperiod types (Nicoll and Galletta, 1987; Yanagi and Oda, 1989). These studies have lead investigators to believe that the photoperiod response is continuous rather than discontinuous. Darrow (1966) suggested that strawberry genotypes actually range continuously from obligate short day to facultative short day to complete day-neutrals, and indicated that DN types vary in their flowering expression from weak to strong. The infra short day types are thought to be in the middle of this range (Izsak and Izhar, 1984). Nicoll and Galletta (1987) tested the temperature and photoperiod response of strong, weak, and very weak day-neutrals, as well as older LD and SD types using Durner's maximum temperature regimes. The DN types were ranked based on the proportion of their daughter plants that flowered during the summer. Their results were in agreement with Darrow (1966), as they observed a continuous responses in flowering to photoperiod: all the plants flowered once, but, none of the SD types re-flowered, 27% of very weak day-neutral re-flowered, and 100% of the DNs and LD re-flowered. In addition, there were significant differences among weak and strong DN and LD types in their intensity of flowering, fruit set, fruit number and fruit weights.

Runnering is stimulated by long days and high temperatures in strawberries. Darrow (1936) and Durner *et al.* (1984) demonstrated that if a clone produces any

runners, it will do so under long days and higher temperatures. In a greenhouse study, Darrow tested the runnering ability of several cultivars grown in September with a 16 h day at 21.1 °C, 15.5 °C, or 12.8 °C temperatures. Among these temperature treatments, only 21.1 °C temperature yielded runners. Durner *et al.* (1984) studied the effect of temperature and photoperiod using combination of long day, short day, and night interruptions and 16, 20, 24, and 28 °C temperature treatments. On average, 2.0, 1.2, and 0.4 runners per plant were observed for long day, night interruption, and short day photoperiods, respectively. While there were no runners produced in SD at temperatures below 24 °C, LDs and DNs generated similar numbers of runners under short days regardless of temperature treatments. Under long days, however, runners were observed in all the temperature treatments.

Smeets (1980) studied runner formation in 'Revada' and 'Rabunda' held under combinations of 14, 20, and 26 °C for 8, 16, 24 h days. Although runners were observed in all treatments, 20 and 26 °C treatments generated significantly more runners than 14 °C, while 16 and 24 h days generated significantly more runners than the 8 h day length treatment. Sonsteby (1997) also found higher runner numbers under high temperatures in an experiment where he studied the effect of 9, 15, and 21 °C temperature regimes at 8 and 24 h days using 'Korona', 'Elsanta', 'Bounty', and 'Senga Sengana'.

In this chapter, temperature x photoperiod interactions of a number elite genotypes were studied in a series of growth chamber experiments involving: 1) the CDL of SD genotypes under 8, 9, 10, and 11 h days at 18 °C, 2) the photoperiod characteristics of DN and LD types under 8 and 16 h days at 18 °C, and 3) the effect of temperature on flower bud formation in DN genotypes under 12 h days and 18, 22, 26, and 30 °C.

EXPERIMENT 1

Material and Methods

This experiment was conducted to determine the required CDL and period of induction for a wide range of putative SD genotypes. The representatives and their region of origin were the *F.* ×*ananassa* cultivars 'Allstar' (Mid-Atlantic), 'Chandler' (California), and 'Honeoye' (New York), *F. chiloensis* FRA 0024 (central Chile), FRA 0368 (Alaska), and *F. virginiana* Eagle 14 (Ontario).

Runners from each genotype were gathered from a field planting at the Michigan State University (MSU) Horticultural Research Farm (East Lansing, Mich.) on 8/30/01 and placed under mist for rooting. On 9/10/01, 40 rooted runners were potted into 14 x 12 x 12 cm pots and set in a greenhouse where they were maintained under 13 h day lengths using supplementary lights (~800 μmol s^{-1} m^{-2}). On 10/31/01, 40 plants of each genotype were transferred into four growth chambers at 18 °C for 8, 9, 10, or 11 h days. In each chamber, photosynthetically available radiation (PAR) was initially varied with day length so that the total energy received was equal (~800, 710, 640, and 580 μmol s^{-1} m^{-2} for 8, 9, 10, and 11 days, respectively). After 15 and 30 days (on 11/14/01 and 11/30/01), 5 plants of each genotype from each chamber were placed in a greenhouse held at 13 h day length using supplementary lights (~800 μmol s^{-1} m^{-2}). The number of flowers and runners produced by each plant were recorded on 11/15/01, 11/25/01, and

12/06/01. Total dry weights were also determined for each plant after washing their roots free of soil and holding them at 72 °C for 3 days.

The initial analysis indicated that both 15 and 30 days induced the same number of flowers and runners. As a result, these two treatments could be treated as different blocks in evaluating the effect of the different day lengths. Analysis of Variance (ANOVA) tables, means, and standard errors (SEs) were calculated for all variables using the SAS program (SAS Institute, Cary, N.C.). Regression lines were fitted to the average values of each genotype at each day length to determine the trend of the genotype's performance across day length.

Results and Discussion

Flower number: The number of flowers formed by all genotypes was not significantly different under either the 15 or 30 day induction period (Table 1). The observations indicate that at cool temperatures, such as 18 °C, the number of induction cycles required to form flower buds is less than 15 days for the genotypes used in this experiment. Although CDL has been reported to be between 7-24 depending on temperature (Hartmann, 1947 a and b; Went 1957; Larson, 1994), 7-14 cycles is generally considered adequate under cool temperatures (Guttridge, 1985). Thus, our results are in agreement with the literature.

All genotypes produced one inflorescence, regardless of photoperiod. Varying numbers of flowers were observed depending on photoperiod; however, there was not a consistent trend (Table 2 and Figure 1). The average number of flowers across all

genotypes for 8, 9, 10, and 11h days were 4.2, 3.5, 3.5, and 5.1, respectively (Table 2). Likewise, the individual genotypes produced different numbers of flowers under different photoperiods, but there were few consistent trends (Table 3). Eagle-14 had more flowers under LD than SD and showed a positive increase as day length was increased ($y = -6.8 + 1.3x$), while 'Honeoye' showed an overall decline in flower numbers as day length was increased ($y = 9.9 - 0.7x$) (Table 3). However, all the other genotypes displayed little relationship between flower numbers and day length, with slopes less than $b = 0.2$ (Table X). None of the regression lines for individual genotypes were significant ($P < 0.05$).

The flowering trend of 'Honeoye' was not significant, but it did fit the typical, quantitative SD model reported by others (Darrow 1936; Darrow and Waldo, 1934; Durner et al., 1984). The flat regression lines of 'Allstar', 'Chandler', FRA 0024, and FRA0368 indicates that day length between 8 - 11 h days does not have a significant differential effect on flower bud initiation at 18 °C. Such insensitivity to day length has not been reported previously in SD plants.

The positive relationship between photoperiod and flower production in Eagle-14, while also not significant, is at least suggestive that it is DN or LD rather than SD. In previous studies, Eagle-14 has not flowered in the late summer or fall in the field as would be expected for a LD or DN genotype (Hancock et al., 2001 a), but high summer temperatures may have inhibited its flower production (Durner et al., 1984; Hartmann, 1947 a and b). Occasional flowers have been observed on Eagle-14 during long summer days in our cooled greenhouse (Callow, personal communication), and Eagle-14 has produced a high number of DN progeny in crossing studies (Hancock et al., 2002; Chapter 2).

It is not known why our flowering response patterns were so different from the literature, but all previous studies involved *F. ×ananassa* cultivars that are no longer grown. Perhaps, selection for high yields has altered the response pattern of some of our modern cultivars. A broader comparison of native germplasm might be warranted to uncover the variability available for photoperiod response in strawberry.

Runner number: The day length treatment, genotypes, and their interaction were highly significant for runner number (Table 1). However, 'Allstar' and 'Honeoye' did not runner at all, while 'Chandler' (mean = 0.1) and FRA 0024 (mean = 0.3) produced only negligible runner numbers (Table 2 and Figure 1). Only FRA 0368 and Eagle-14 generated significant numbers of runners in any treatment. The average runner numbers of FRA 0368 were 0.7, 0.6, 0.4, and 2.9 (mean = 0.9), while Eagle-14 had 2.0, 3.0, 2.6, and 3.1 for 8, 9, 10, and 11h days, respectively (mean = 2.7) (Table 2). The slopes of the regression lines (b = 0.3 for Eagle-14 and b = 0.8 for FRA 0368) revealed a progressive trend as day length advanced, although these regression lines were not significant (Table 3).

The positive relationship observed between photoperiod and runner numbers is consistent with the literature, as strawberries has been reported to have more runners under LD (Darrow 1936; Darrow and Waldo 1934; Durner et al., 1984; Larson, 1984). Runner numbers have also been reported to be highest under hot temperatures (Durner et al., 1984; Heide, 1977, Smeets, 1980; Sonsteby, 1997). This may be why many of the genotypes produced few runners overall as our induction temperatures were cool (18 °C).

Dry weights: The main effect of day length treatment was not significant for dry weights (Table 3 and Figure 3) suggesting that most of the differences observed in flower

and runner numbers were not caused by PAR, but photoperiod. 'Allstar' and 'Chandler' did have slightly positive ($y = 12.8 + 1.1 x$) and negative ($y = 16.6 - 0.8 x$) regression lines, but these were not significant.

Table 1. Analysis of variance for flower and runner numbers, and dry weights of strawberry genotypes grown at 18 °C and either 8, 9, 10, or 11 h days.

Source	df	Flower no.	Runner no.	Dry weight (g)
Block	1	32.3	0.2	4.6
Day length (D)	3	34.5*	2.0**	5.2
Whole-plot error	35	8.7	0.4*	5.5
Genotype (G)	5	103.2**	42.2**	569.5**
D*G	15	15.2*	1.3**	15.4**
Error	180	7.8	0.3	6.8

*, ** Significant at 0.05 and 0.01, respectively.

Table 2. Means and standard errors (in parenthesis) for flower and runner numbers, and dry weights of strawberry genotypes grown at 18 °C and either 8, 9, 10, or 11 h days.

	Flower number					Runner number					Dry weight (g)				
Source	8h	9h	10h	11h	Mean	8h	9h	10h	11h	Mean	8h	9h	10h	11h	Mean
'Allstar'	3.8	2.6	3.3	4.2	3.5	0.0	0.0	0.0	0.0	0.0	12.3	10.8	16.0	14.3	13.3
	(0.5)	(0.7)	(0.7)	(0.9)	(0.3)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(1.0)	(0.8)	(1.2)	(1.2)	(0.6)
'Chandler'	1.9	1.7	0.7	2.3	1.7	0.5	0.2	0.0	0.4	0.3	9.9	11.0	8.0	8.5	9.3
	(0.7)	(0.6)	(0.4)	(0.4)	(0.3)	(0.2)	(0.1)	(0.0)	(0.2)	(0.1)	(0.5)	(0.8)	(0.9)	(0.9)	(0.4)
'Honeoye'	5.3	4.2	3.1	3.6	4.1	0.0	0.0	0.0	0.0	0.0	10.5	9.7	10.3	11.1	10.4
	(0.6)	(0.9)	(0.7)	(0.8)	(0.4)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.7)	(0.4)	(0.8)	(1.0)	(0.4)
FRA 0024	6.5	5.0	5.8	7.0	6.1	0.1	0.7	0.2	0.1	0.3	4.5	4.5	4.8	3.2	4.3
	(1.0)	(0.9)	(0.6)	(1.2)	(0.5)	(0.1)	(0.2)	(0.1)	(0.1)	(0.1)	(0.8)	(0.7)	(0.4)	(0.5)	(0.3)
FRA 0368	2.2	4.5	3.3	4.0	3.5	0.7	0.6	0.4	1.9	0.9	7.4	8.5	8.5	8.5	8.2
	(0.6)	(0.6)	(0.7)	(0.9)	(0.4)	(0.3)	(0.2)	(0.2)	(0.3)	(0.2)	(1.1)	(1.0)	(0.6)	(0.8)	(0.5)
Eagle-14	5.6	2.8	4.7	9.3	5.6	2.0	3.0	2.6	3.1	2.7	2.9	3.4	3.8	3.4	3.4
	(1.3)	(1.3)	(1.4)	(1.7)	(0.8)	(0.0)	(0.2)	(0.2)	(0.5)	(0.2)	(0.4)	(0.4)	(0.6)	(0.6)	(0.3)
Mean	4.2	3.5	3.5	5.1	4.1	0.6	0.8	0.5	0.7	0.7	7.9	8.0	8.6	8.1	8.1
	(0.4)	(0.4)	(0.4)	(0.5)	(0.2)	(0.1)	(0.1)	(0.1)	(0.2)	(0.1)	(0.5)	(0.5)	(0.6)	(0.6)	(0.3)

Table 3. Regression lines, R^2, and significance (P) for flower and runner number, and dry weights of strawberry genotypes grown at 18 °C and either 8, 9, 10, or 11 h days.

	Flower number			Runner number			Dry weight (g)		
Genotype	Regression line	R^2	P	Regression line	R^2	P	Regression line	R^2	P
F. virginiana									
Eagle-14	y = - 6.8 + 1.3 x	0.38	0.385	y = - 0.0 + 0.3 x	0.56	0.250	y = 1.6 + 0.2 x	0.44	0.164
FRA 0024	y = 3.9 + 0.2 x	0.12	0.658	y = -2.3 + 0.3 x	0.08	0.775	y = 7.7 - 0.4 x	0.42	0.349
FRA 0368	y = - 0.5 + 0.4 x	0.30	0.456	y = - 0.0 + 0.8 x	0.42	0.352	y = 5.1+ 0.3 x	0.60	0.225
F. ×ananassa									
'Allstar'	y = 1.7 + 0.2 x	0.13	0.644				y = 12.8 + 1.1 x	0.40	0.364
'Chandler'	y = 1.5 + 0.0 x	0.00	0.962				y = 16.6 - 0.8 x	0.28	0.317
'Honeoye'	y = 9.9 - 0.7 x	0.71	0.154				y = 0.3 + 0.2 x	0.46	0.463

Figure 1. Mean flower number of strawberry genotypes grown at 18 °C and either 8, 9, 10, or 11 h days. The bars represent standard errors.

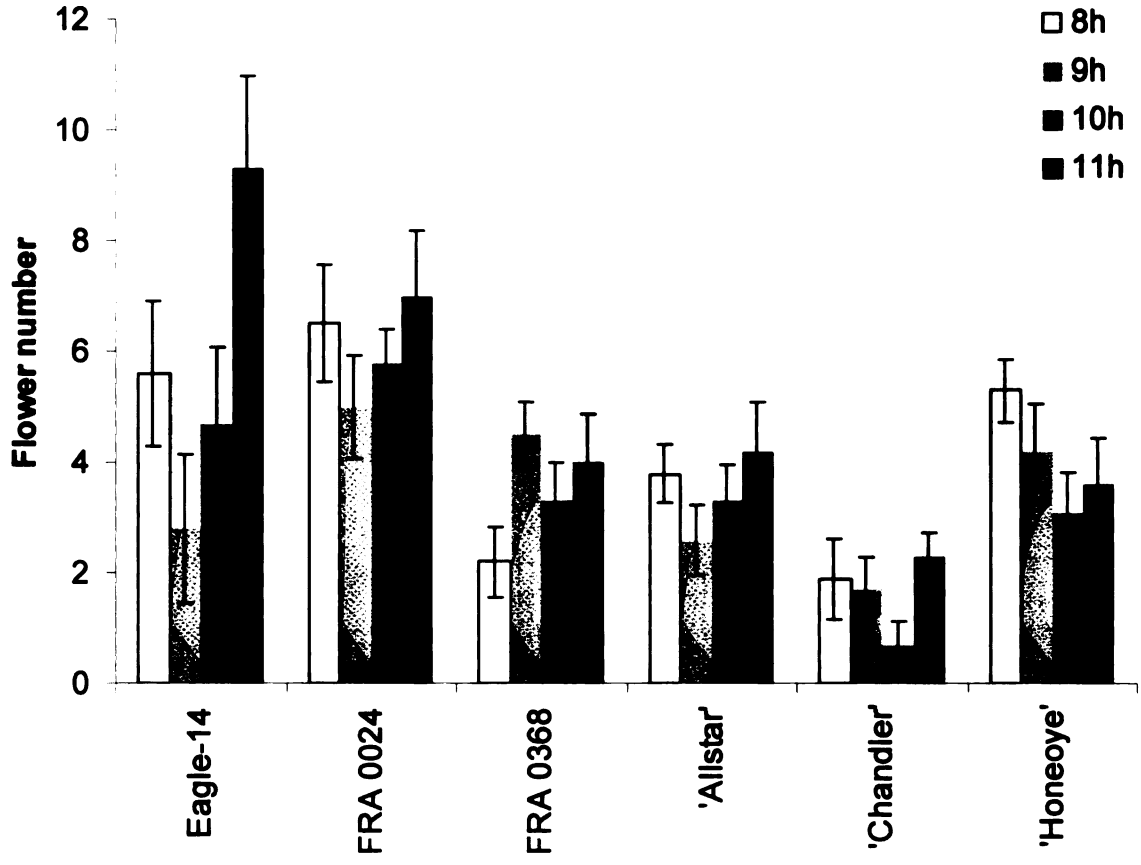


Figure 2. Mean runner number of strawberry genotypes grown at 18 °C and either 8, 9, 10, or 11 h days. The bars represent standard errors.

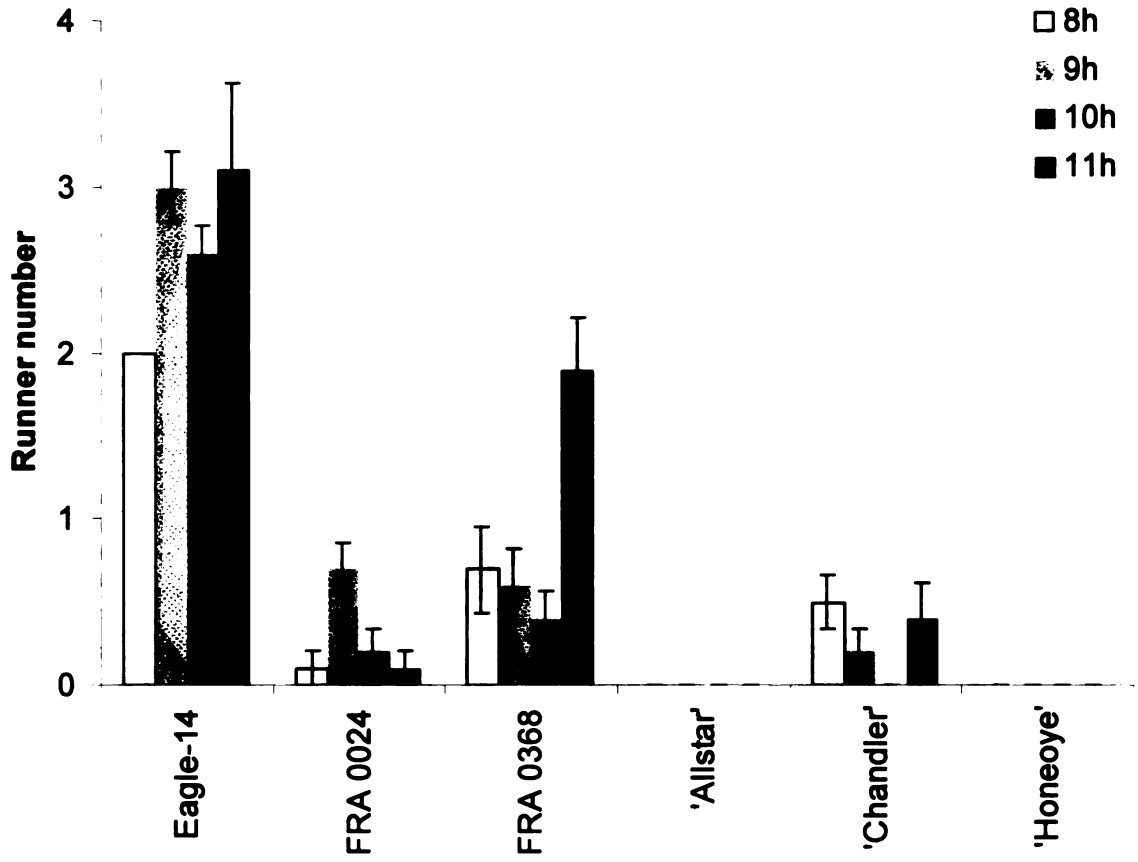
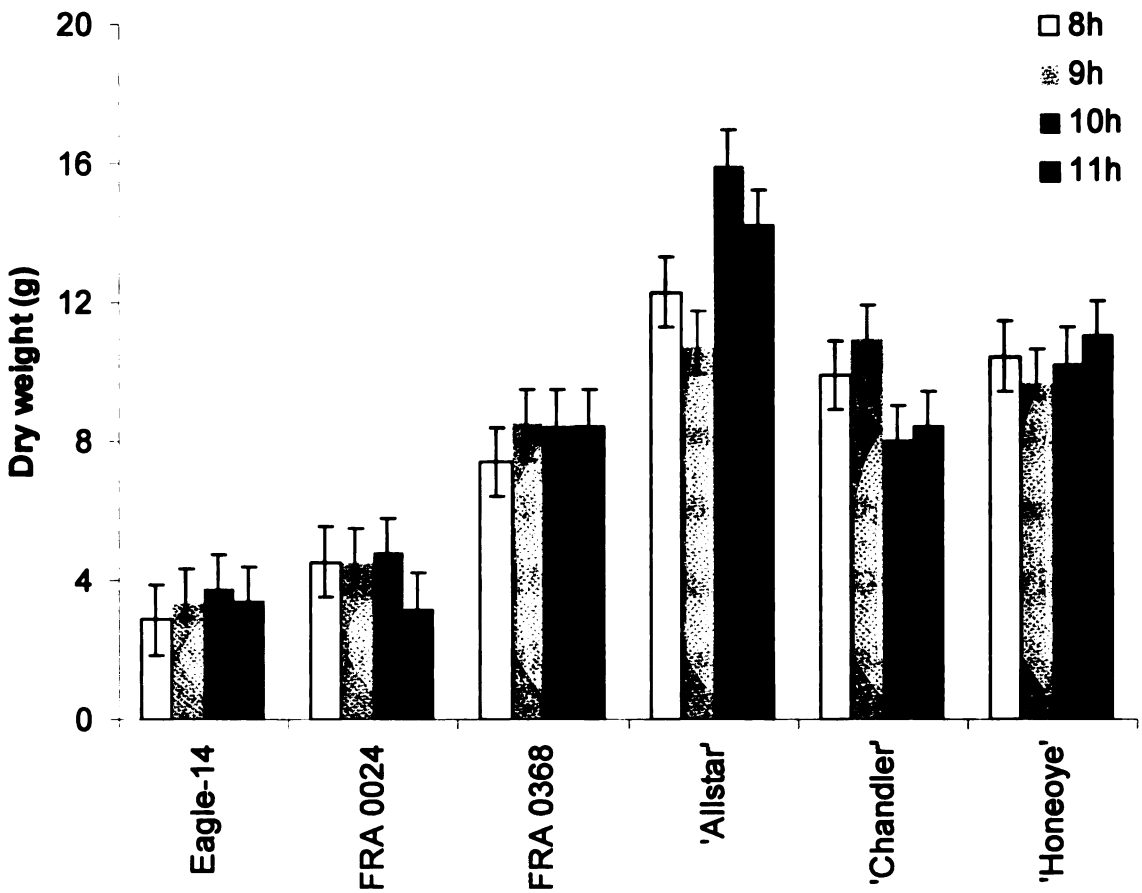


Figure 3. Mean dry weight of strawberry genotypes grown at 18 °C and either 8, 9, 10, or 11 h days. The bars represent standard errors.



EXPERIMENT 2

Material and Methods

This experiment was designed to determine the photoperiod sensitivity of a number of elite wild clones and to verify whether the old "everbearer" strawberry cultivars are DN or LD. Frigo plants were used of the everbearing cultivars 'Fort Laramie' and 'Quinalt'; DN cultivars 'Aromas' and 'Tribute', putative DN *F. virginiana* selections Frederick 9, LH 50-4, RH 30; SD *F. chiloensis* selection FRA 0368. Further information on the elite wild clones is available in Hancock *et al.* (2001 a and b) and www.berrygenetics.com. The cultivars were purchased from Gurney's Seed & Nursery (Yankton, S.D.) and the dormant *F. virginiana* clones were dug from MSU Research Farm, East Lansing, in April 2001.

Ten plants of each genotype were potted into 14 x 12 x 12 cm pots with a planting mix purchased from the Michigan Peat Company (Houston, TX). The potted plants were placed in a completely randomized design in a greenhouse at MSU on 4/11/2001 and were held for 3 months under 12 h day length maintained with supplementary light (~800 $\mu mol\ s^{-1}\ m^{-2}$). During this period, all of the genotypes flowered. On 7/11/2001 the plants were randomly placed into two growth chambers at 18 °C, 8 h day length and 800 $\mu mol\ s^{-1}\ m^{-2}$ PAR or 18 °C, 16 h day lengths with 400 $\mu mol\ s^{-1}\ m^{-2}$ PAR. PAR was varied with day length so that the total energy received by the plants in each chamber was equal. The plants were given a 7-week induction period, and then flower and runner numbers were

recorded on 9/4/01, 9/10/01, 9/17/01, and 9/22/01. No data were recorded on the flowers and runners that developed before this time, as they could have been initiated before the plants were placed in the greenhouse. On 9/25/01, each plant was dried at ~72 °C for 3 days and weighed. Means and SEs were calculated for all variables using the SAS program (SAS Institute, Cary, N.C.). Because the growth chamber conditions were not replicated, ANOVA tables could not be constructed; however, SEs could be calculated as the genotype means represented 5 plants within each treatment.

Results and Discussion

Flowering response to LD and SD: Two types of flowering patterns were observed: 1) flowering under both LD and SD conditions ('Aromas', 'Tribute', FRA 0368, Frederick 9, LH 50-4, RH 30) and 2) flowering under LD but not SD conditions ('Fort Laramie' and 'Quinalt') (Table 4 and Figure 4). These patterns represent what would be expected of DN and LD types, respectively. The mean inflorescence number ranged from 1.0 (FRA 0368) to 3.0 (Frederick 9) in the SD types and 0.2 (RH 30) to 3.4 ('Tribute') in the LD types. Frederick 9 had the highest total number of flowers (15.4) under SDs while 'Tribute' was the most productive genotype under LDs (25.6) (Table 4). 'Fort Laramie' produced an average of 2.6 inflorescences and 13.4 total flowers, while 'Quinalt' generated 3.4 inflorescences and 23.0 total flowers (Table 4 and Figure 4).

These results indicate that the multiple cropping behaviors of Frederick 9, LH 50 and RH 30 that were previously observed in the field were due to their being DN and not LD plants. FRA 0368 also appears to be DN, even though it is not typically multiple

cropping in the field. Perhaps, high summer field temperatures have inhibited floral production in FRA 0368, as it is from Alaska where such high temperatures are rare. We did observe some flowers on FRA 0368 in the field in the relatively cool weather of September 2001 (Osborn, personal communication). The fact that DN LH 50-4 and RH 30 produce many runners under long day and cool conditions is exciting, as one of the limitations of modern DN cultivars is their inability to runner (Dale et al., 2002).

Runnering response to LD and SD: 'Aromas', 'Tribute', Frederick 9, and 'Fort Laramie' produced no runners under either LD or SDs (Table 4). FRA 0368 and 'Quinalt' produced runners under just LDs (2.5 and 0.2), while LH 50-4 and RH 30 produced runners under both SD and LD conditions. The mean runner numbers were 2.5 (FRA 0368) and 0.2 ('Quinalt') (Table 4). LH 50-4 generated 1.4 runners under SD and 4.4 under LDs, and RH 30 produced 0.2 under SD and 3.8 under LD (Table 4).

It is not known why some of the genotypes did not runner under LDs, as numerous studies have reported that runnering in strawberries is a LD response (Darrow 1936; Durner et al., 1984; Durner et al., 1984; Smeets, 1980; Sonsteby 1997). Perhaps runnering in 'Aromas', 'Fort Laramie', Frederick 9, and 'Tribute', is more sensitive to cool temperature than the other genotypes. As previously mentioned in the Experiment 1, warm temperatures are generally thought to be stimulatory to runner production than cool but few modern cultivars have been examined. There may also have been differential sensitivities among genotypes to photoperiod, as LH 50-4 and RH 30 produced runners under both long and short days; while the rest of the genotypes produced runners only under LD conditions or none at all. It has been assumed that the genotypes that were photoperiod insensitive for flowering retained LD response of runnering (Durner et al.,

1984; Heide, 1977; Durner et al., 1984; Smeets, 1980; Sonsteby, 1997). LH 50-4 and RH 30 may be photoperiod insensitive for both of these developmental responses.

Response of dry weights to LD and SD: Overall mean dry weights were 23.1 g and 26. 6 g for SD and LD types, respectively (Table 4 and Figure 5). Some differences were also observed among genotypes between the two day length treatments. For example, FRA 0368 had higher mean dry weights (25.3 g) under LD than SD (22.9 g), whereas 'Quinalt' had higher mean dry weights (30.8 g) under SD than LD (22.0 g) (Table 4 and Figure 5). These results indicate that the PAR adjustments made at the beginning of the experiment for day length treatments may not have been maintained throughout the experiment. This imbalance may have influenced relative numbers of flowers in the two treatments, but the main goal of the experiment was to determine whether a genotype flowered under LD or SD conditions. The modest differences in PAR were unlikely to have impeded the overall flowering response of genotypes.

Table 4. Means and standard errors for flower and runner numbers, and dry weights of strawberry genotypes grown at 18 °C and either 8 or 16 h days.

	Inflorescence no.		Total flower no.		Fl. no./inflorescence		Runner no.		Dry weight (g)	
Genotypes	8h	16h	8h	16h	8h	16h	8h	16h	8h	16h
Short day										
FRA 0368	1.0 ± 0.0	0.8 ± 0.2	4.2 ± 0.7	4.6 ± 2.0	4.2 ± 0.7	4.6 ± 2.0	0.0 ± 0.0	2.8 ± 0.4	22.9 ± 0.7	25.3 ± 2.1
Day-neutral										
'Aromas'	1.4 ± 0.2	3.2 ± 0.2	4.0 ± 0.8	14.0 ± 1.3	3.0 ± 0.7	4.4 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	29.8 ± 0.6	33.2 ± 2.3
'Tribute'	1.8 ± 0.2	3.4 ± 0.2	6.0 ± 1.3	25.6 ± 1.1	3.1 ± 0.5	7.6 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	23.1 ± 0.8	30.2 ± 2.7
Frederick 9	3.0 ± 0.0	2.2 ± 0.5	15.4 ± 1.4	16.0 ± 3.8	5.1 ± 0.5	7.2 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	18.4 ± 3.1	15.1 ± 4.0
LH 50-4	2.0 ± 0.5	0.8 ± 0.4	8.8 ± 2.5	4.4 ± 3.9	3.5 ± 0.9	2.4 ± 1.9	1.4 ± 0.4	4.4 ± 0.6	17.1 ± 0.8	20.3 ± 6.6
RH 30	1.2 ± 0.2	0.2 ± 0.2	5.8 ± 1.3	2.4 ± 2.4	4.8 ± 0.8	2.4 ± 2.4	0.2 ± 0.2	3.8 ± 0.5	16.4 ± 2.3	18.5 ± 3.5
Long day										
'Fort Laramie'	0.0 ± 0.0	2.6 ± 0.7	0.0 ± 0.0	13.4 ± 3.5	0.0 ± 0.0	5.2 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	26.3 ± 3.3	36.0 ± 3.7
'Quinalt'	0.0 ± 0.0	3.4 ± 0.4	0.0 ± 0.0	23.0 ± 1.5	0.0 ± 0.0	6.9 ± 0.4	0.0 ± 0.0	0.2 ± 0.2	30.8 ± 2.1	22.0 ± 5.6
Mean	1.2 ± 0.1	1.7 ± 0.2	4.8 ± 0.7	10.3 ± 1.4	2.7 ± 0.3	4.1 ± 0.4	0.4 ± 0.1	1.3 ± 0.3	23.1 ± 1.0	26.6 ± 1.6

Figure 4. Mean total flower number of strawberry genotypes grown at 18 °C and either 8 or 16 h days. The bars represent standard errors.

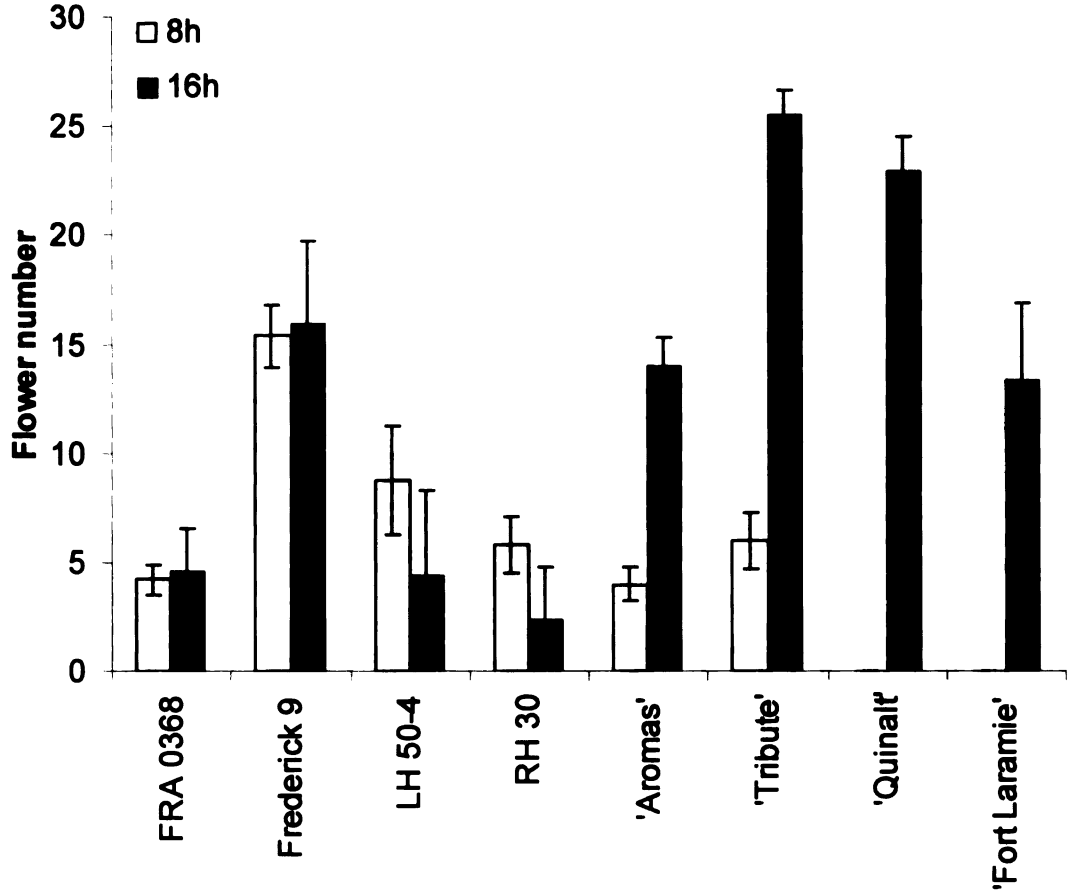
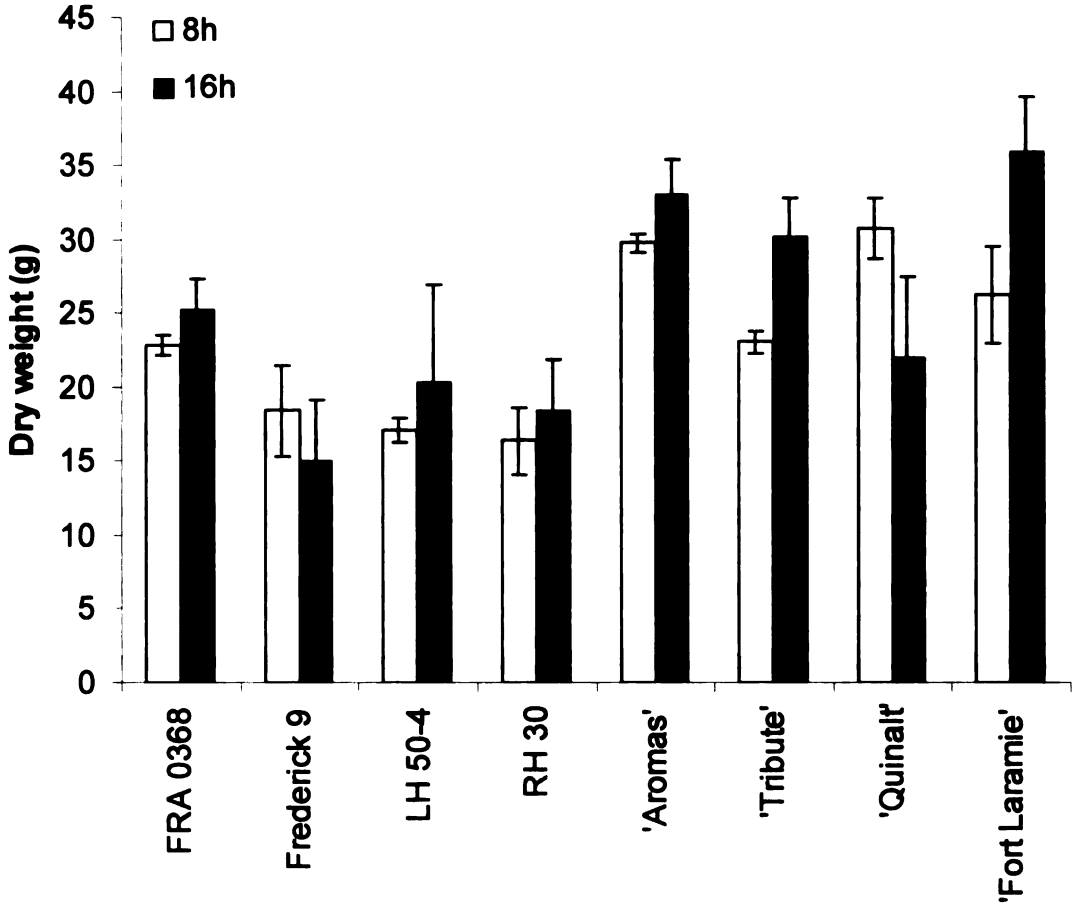


Figure 5. Mean total flower number of strawberry genotypes grown at 18 °C and either 8 or 16 h days. The bars represent standard errors.



EXPERIMENT 3

Material and Methods

This experiment was designed to evaluate the effect of temperature on flowering in a number of old everbearing cultivars developed in North Dakota where very warm summer temperatures occur, and several elite *F. virginiana* genotypes collected from a wide range of environments. The goal was to identify genotypes that can form flower buds under higher temperatures (>26 °C). The genotypes studied were the elite *F. virginiana* genotypes Brighton-3 (Utah), LH 30-4 (Montana), LH 39-15 (Montana), LH 40-4 (Montana), LH 50-4 (Montana), RH 23 (Minnesota), RH 30 (Minnesota), RH 43 (Alaska), RH 45 (Alberta); and *F ×ananassa* 'Aromas', 'Fort Laramie', 'Ogallala', and 'Tribute'. Further information on the native elite genotypes can be found at Sakin *et al.* (1997), Hancock *et al.* (2001 a and b) and www.berrygenetics.com. The cultivars were purchased from Gurney's Seed & Nursery (Yankton, S.D.) and the dormant *F. virginiana* clones were obtained from Michigan State University Horticultural Research Farm in August 1999.

Twenty eight plants of each genotype were potted on 8/5/99 into 14 x 12 x 12 cm pots using planting mix purchased from Michigan Peat Company, Houston, TX, and placed in different growth chambers held at either 18, 22, 26, or 30 °C and 12 h days and ~600 μmol s^{-1} m^{-2} PAR (7 replicates of each genotype in for each). After a seven week induction period, the number of flowers and runners were recorded weekly for a ten-week

period. At the end of this time, the plants were partitioned into root, crown, leaf and runners. The dry weights of the plant parts were determined after holding them at 72 °C for three days.

The same experiment was repeated in the summer of 2002 (from 4/12/02 to 8/17/02); however, some of the genotypes were dropped and replaced by others. The genotypes included in the second trial were: *F.* ×*ananassa* 'Aromas', 'Fort Laramie', 'Quinalt', 'Tribute', and elite '*F. virginiana* genotypes Frederick 9, LH 50-4, RH 30. *Fragaria virginiana* clones Brighton-3, LH 30-4, LH 39-15, LH 40-4, RH 23, RH 43, RH 45 were dropped as they showed little evidence of being heat tolerant in the first trial. Also, the second trial had 6 replicates for each treatment for each genotype. The number of flowers, and runners were recorded weekly for 4 weeks after a 7-week induction period. At the end of the experiment, the total dry weight of each plant was determined by drying them at 72 °C for three days.

The mean values for each genotype in the two trials are presented separately, as different genotypes were included in each. However, the common genotypes in each trial were considered as blocks to calculate Analysis of Variance tables. ANOVAs, means, and SEs were calculated using the SAS program (SAS Institute, Cary, N.C.). The regression lines were fitted on average values for each genotype plotted on day length treatments to demonstrate the trend of the genotype's performance over day length treatments.

Results and Discussion

There was a significant difference in the two trials for plant weight; however, number of crowns, inflorescence, flowers and runners were similar in both trials (data not shown). Plants were 37% (means = 8.5 vs. 6.2 g) heavier in the second trial.

Temperature had a significant effect on flower number (Table 5), but not crown and inflorescence numbers (data not shown). The overall mean flower number increased progressively as temperature decreased. The mean values were 1.6, 3.1, 3.9, and 6.8 at 30, 26, 22, and 18 °C (Table 6). *Fragaria ×ananassa* cultivars had higher mean flower numbers than *F. virginiana* genotypes (5.7 vs. 2.8 overall and 8.4 vs. 5.3, 5.5 vs. 2.5, 4.2 vs. 2.1, and 3.3 vs. 0.4 for 18, 22, 26, and 30 °C, respectively) (Table 6).

Both the main effect of genotype and the genotype x temperature interaction were highly significant (Table 5). The overall flower number ranged from 0.7 (Frederick 9) to 10.6 (RH43) in *F. virginiana* genotypes and 2.9 ('Fort Laramie') to 9.1 ('Tribute') in the *F. ×ananassa* cultivars (Table 6). All of the *F. virginiana* genotypes, except RH 43, had their lowest flower numbers at the high temperatures (26 and 30 °C) and showed a non-significant, negative relationship; the rate of decline was only significant for RH 43 (Table 6 and 7). 'Aromas', and 'Tribute' also had their lowest flower number at 30 °C (1.7 and 2.6, respectively) (Table 6 and Figure 6) and showed a non-significant negative trend (Table 7). Floral production in LD 'Fort Laramie' however, was higher at 30 °C than 18 °C and it displayed a non-significant positive relationship (Tables 6 and 7).

Temperature did not have a significant effect on runner number, although the main effect of genotypes and the genotype x temperature interaction were highly significant (Table 5). LH 40-4, RH 23, and RH 45 did not runner in any of the temperature treatments, while LH 50-4 produced almost equal runner numbers at all

temperatures (overall mean = 2.5) (Table 6). All of the *F.* ×*ananassa* cultivars had very low runner numbers regardless of temperature (Table 6 and Figure 7).

For dry weights, the main effect genotype and the genotype x temperature interaction were highly significant, while the main effect of temperature was not significant (Table 5). *Fragaria* ×*ananassa* cultivars had higher dry weights (overall mean = 9.4) than the *F. virginiana* genotypes (overall mean = 4.5) (Table 6). Overall, the mean dry weights of both species were significantly reduced by high temperatures (12.4, 9.9, 9.0, and 6.7 for *F.* ×*ananassa* and 7.5, 3.7, 3.2, and 3.2 for *F. virginiana* for 18, 22, 26, and 30 °C, respectively) (Table 6 and Figure 8). The smaller plants of *F. virginiana* had flat, non-significant regression lines, while most of the other genotypes showed non-significant negative trends as temperature was increased. Only 'Aromas' and 'Tribute' had negative trends that were significant (Table 7).

Unfortunately, all of the DN genotypes screened were negatively affected by high temperature. The trends were often non-significant but in all cases flower numbers were substantially higher at 18 °C than 26 °C and 20 °C. Several other studies have shown growth rates, flowering and CO_2 assimilation rates to be reduced in strawberry by high temperatures and this relationship is probably reflected here in biomass (Hellmann and Travis, 1998; Serçe et al., 2002).

Because the genotypes in the experiment came from a wide range of environments, there is a diminishing hope of finding a DN genotype that flowers well under high temperatures. This suggests that in hot northern climates, it may be a better breeding strategy to concentrate on LD double cropping parents rather than DN multiple cropping ones. LD genotypes flower in the relatively cool days of spring and late

summer, and thus avoid the hot, mid-summer temperatures. In fact, the old everbearing cultivars 'Fort Laramie' is LD and appears to have some resistance to heat. The fruits of 'Fort Laramie' do not meet current commercial standards, but these cultivars could prove to be a useful parent.

Table 5. Analysis of variance for flower and runner numbers, and dry weights of strawberry genotypes grown in growth chambers at 12 h days and 18, 22, 26, and 30 °C.

Source	df	Flower no.	Runner no.	Dry weight (g)
Block	1	55.1	1.0	79.1**
Temperature (T)	3	158.1**	1.2	9.5
Whole-plot error	7	13.7	0.7	28.7**
Genotype (G)	14	236.9**	15.1**	220.5**
G*T	40	56.9**	1.3**	20.0**
Error	340	26.1	0.7	8.0

*, ** Significant at 0.05 and 0.01, respectively.

Table 6. Means and standard errors (in parenthesis) for flower and runner numbers, and dry weights of strawberry genotypes grown in growth chambers at 12 h days and 18, 22, 26, and 30 °C.

	Flower number					Runner number					Dry weight (g)				
Source	18 °C	22 °C	26 °C	30 °C	Mean	18 °C	22 °C	26 °C	30 °C	Mean	18 °C	22 °C	26 °C	30 °C	Mean
F. virginiana	5.3	2.5	2.1	0.4	2.8	0.8	1.1	1.0	1.2	1.0	7.5	3.7	3.2	3.2	4.5
	(1.2)	(0.5)	(0.4)	(0.2)	(0.4)	(0.2)	(0.2)	(0.2)	(0.3)	(0.1)	(0.7)	(0.3)	(0.2)	(0.3)	(0.2)
BT3	0.4	0.0	0.0	0.0	0.1	0.0	0.3	0.3	2.9	0.9	11.4	4.8	3.8	4.8	6.2
	(0.2)	(0.0)	(0.0)	(0.0)	(0.1)	(0.0)	(0.3)	(0.2)	(0.7)	(0.3)	(1.3)	(1.2)	(0.3)	(0.8)	(0.7)
Frederick 9	2.2	0.0	0.0	---	0.7	0.3	1.8	0.8	---	1.0	3.9	2.5	2.1	---	2.8
	(0.7)	(0.0)	(0.0)	---	(0.3)	(0.3)	(0.6)	(0.3)	---	(0.3)	(0.7)	(0.3)	(0.6)	---	(0.4)
LH 30-4	2.0	0.0	3.0	0.0	1.3	0.7	1.0	0.7	2.0	1.1	2.8	2.4	1.7	2.3	2.3
	(1.5	(0.0)	(0.6)	(0.0)	(0.5)	(0.7)	(0.6)	(0.3)	(1.0)	(0.3)	(0.3)	(0.3)	(0.4)	(0.7)	(0.2)
LH 39	2.9	0.0	0.3	0.0	0.8	0.9	1.0	0.3	0.6	0.7	14.7	3.3	1.9	2.1	5.5
	(1.9	(0.0)	(0.2)	(0.0)	(0.5)	(0.5)	(0.4)	(0.2)	(0.3)	(0.2)	(1.8)	(0.3)	(0.3)	(0.6)	(1.1)
LH 40-4	5.0	1.0	2.3	0.0	2.3	0.0	0.0	0.0	0.0	0.0	1.0	2.0	3.6	1.7	2.1
	(2.5	(1.0)	(1.9)	(0.0)	(1.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.4)	(0.3)	(1.0)	(0.3)	(0.4)
LH 50-4	3.9	3.0	1.0	1.0	2.4	2.2	2.7	2.5	2.4	2.5	9.1	5.0	3.9	3.1	5.6
	(1.0	(0.7)	(0.5)	(1.0)	(0.4)	(0.4)	(0.4)	(0.5)	(0.7)	(0.2)	(1.3	(0.6)	(0.3)	(0.5)	(0.5)
RH 23	30.0	2.0	6.3	0.0	10.5	0.0	0.0	0.0	0.0	0.0	4.4	2.2	2.6	2.3	2.9
	(17.0)	(2.0)	(1.2)	(0.0)	(5.6)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.1)	(0.3)	(0.4)	(0.6)	(0.3)
RH 30	5.4	4.9	3.4	0.4	3.9	0.8	0.6	1.1	0.1	0.7	5.5	3.4	3.9	3.7	4.2
	(1.5)	(1.2)	(1.0	(0.4)	(0.6)	(0.2)	(0.3)	(0.2)	(0.1)	(0.1)	(0.7)	(0.4)	(0.6)	(0.8)	(0.3)
RH 43	19.0	11.7	9.7	2.0	10.6	0.0	0.0	0.0	0.0	0.0	1.3	2.1	1.7	1.8	1.8
	(2.0)	(6.2)	(3.0)	(1.0)	(2.6)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.2)	(0.9)	(0.5)	(0.8)	(0.3)
RH 45	4.0	2.3	2.7	0.7	2.4	0.0	0.0	0.0	0.0	0.0	8.8	7.2	3.3	3.9	5.8
	(2.0)	(1.2)	(2.7)	(0.7)	(0.8)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(3.5)	(1.7)	(0.3)	(1.4)	(1.1)
F. ×ananassa	8.4	5.5	4.3	3.3	5.7	0.0	0.1	0.1	0.3	0.1	12.4	9.9	9.0	6.7	9.9
	(1.3)	(0.7)	(0.8)	(0.6)	(0.5)	(0.0)	(0.1)	(0.0)	(0.2)	(0.0)	(0.7)	(0.5)	(0.5)	(0.4)	(0.3)
'Aromas'	6.3	6.1	2.2	1.7	4.4	0.0	0.3	0.1	0.6	0.2	13.0	10.8	8.9	8.0	10.4
	(1.5)	(1.4)	(1.2)	(0.9)	(0.7)	(0.0	(0.2)	(0.1)	(0.4)	(0.1)	(1.3)	(0.8)	(0.7)	(0.9)	(0.6)

'Fort Laramie'	3.5	2.5	1.8	4.6	2.9	0.1	0.0	0.0	0.0	0.0	13.0	10.4	11.3	6.5	10.8
	(0.9)	(0.6)	(0.7)	(1.4)	(0.4)	(0.1))	(0.0)	(0.0)	(0.0)	(0.0)	(1.0)	(1.0)	(1.0)	(0.7)	(0.6)
'Ogallala'	9.1	8.1	6.3	4.3	7.0	0.0	0.0	0.0	0.0	0.0	12.0	8.9	5.4	6.8	8.3
	(1.6)	(2.1)	(1.1)	(0.6)	(0.8)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.7)	(1.0)	(0.8)	(0.7)	(0.6)
'Quinalt'	7.0	4.0	4.3	---	5.1	0.0	0.0	0.0	---	0.0	4.7	8.0	6.9	---	6.5
	(2.2)	(1.1)	(1.2)	---	(0.9)	(0.0)	(0.0)	(0.0)	---	(0.0)	(0.4)	(1.1)	(1.2)	---	(0.6)
'Tribute'	15.8	7.1	7.8	2.6	9.1	0.0	0.0	0.2	0.7	0.2	15.0	9.9	9.5	5.7	10.6
	(3.9)	(1.8)	(2.6)	(1.2)	(1.6)	(0.0)	(0.0)	(0.2)	(0.5)	(0.1)	(1.8)	(1.2)	(1.2)	(0.8)	(0.8)
Mean	6.8	3.9	3.1	1.6	4.1	0.5	0.6	0.5	0.8	0.6	9.8	6.6	5.8	4.6	6.9
	(0.9)	(0.5)	(0.5)	(0.3)	(0.3)	(0.1)	(0.1)	(0.1)	(0.2)	(0.1)	(0.5)	(0.4)	(0.4)	(0.3)	(0.2)

Table 7. Regression lines, R^2, and significance (*P*) for flower and runner number, and dry weights of strawberry genotypes grown in growth chambers at 12 h days and 18, 22, 26, and 30 °C.

	Flower number			Runner number			Dry weight (g)		
Genotype	Regression line	R^2	*P*	Regression line	R^2	*P*	Regression line	R^2	*P*
F. virginiana									
BT3	y = 0.8 - 0.0 x	0.60	0.225	y = - 4.3 + 0.2 x	0.11	0.173	y = 18.7 - 0.5 x	0.59	0.232
Frederick 9	y = 1.2 - 0.0 x	0.75	0.333	y = - 0.4 + 0.1 x	0.57	0.788	y = 7.8 - 0.2 x	0.91	0.198
LH 30-4	y = 3.0 - 0.0 x	0.07	0.742	y = - 1.1 + 0.1x	0.43	0.246	y = 3.6 - 0.1 x	0.39	0.375
LH 39	y = 5.8 - 0.2 x	0.59	0.229	y = 1.7 - 0.0 x	0.42	0.347	y = 29.0 - 1.0 x	0.67	0.179
LH 40-4	y = 10.3 - 0.3 x	0.67	0.183				y = - 0.1 + 0.1 x	0.19	0.566
LH 50-4	y = 8.6 - 0.3 x	0.89	0.055	y = 2.3 + 0.0 x	0.06	0.752	y = 16.7 - 0.5 x	0.86	0.075
RH 23	y = 61.0 - 2.1 x	0.64	0.202				y = 6.4 - 0.1 x	0.55	0.261
RH 30	y = 13.4 - 0.4 x	0.90	0.053	y = 1.7 - 0.0 x	0.24	0.509	y = 7.1 - 0.1 x	0.45	0.327
RH 43	y = 42.4 - 1.3 x	0.96	**0.021**[1]				y = 1.1 + 0.0 x	0.19	0.570
RH 45	y = 8.12 - 0.2 x	0.81	0.098				y = 17.0 - 0.5 x	0.83	0.088
Mean	y = 15.8 - 0.5 x	0.16	**0.011**	y = 0.1 = 0.0 x	0.02	0.440	y = 10.5 - 0.3 x	0.19	**0.005**
F. ×ananassa									
'Aromas'	y = 14.7 - 0.4 x	0.86	0.072	y = - 0.8 + 0.0 x	0.61	0.219	y = 20.3 - 0.4 x	0.97	**0.015**
'Fort Laramie'	y = 1.54 + 0.1 x	0.08	0.725	y = 0.2 - 0.0 x	0.60	0.225	y = 21.5 - 0.4 x	0.76	0.128
'Ogallala'	y = 16.7 - 0.4 x	0.98	**0.010**				y = 19.7 - 0.5 x	0.74	0.141
'Quinalt'	y = 12.5 - 0.3 x	0.67	0.391				y = 0.5 + 0.3 x	0.43	0.546
'Tribute'	y = 31.7 - 1.0 x	0.84	0.085	y= - 1.2 + 0.1 x	0.81	0.101	y = 27.0 - 0.7 x	0.92	**0.043**
Mean	y = 15.3 - 0.4 x	0.29	**0.015**	y = - 0.4 + 0.0 x	0.21	**0.046**	y = 18.4 - 0.4 x	0.37	**0.006**
Grand mean	y = 15.7 - 0.5 x	0.17	**0.001**	y = 0.2 + 0.0 x	0.02	0.285	y = 13.3 - 0.3 x	0.14	**0.003**

[1]Significant slopes, at 0.05, are bolded.

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Grand mean	y = 15.7 - 0.5 x	0.17	**0.001**	y = 0.2 + 0.0 x	0.02	0.285	y = 13.3 - 0.3 x	0.14	**0.003**

[1]Significant slopes, at 0.05, are bolded.

Figure 6. Mean flower number of strawberry genotypes grown in growth chambers at 12 h days and 18, 22, 26, and 30 °C. The bars represent standard errors.

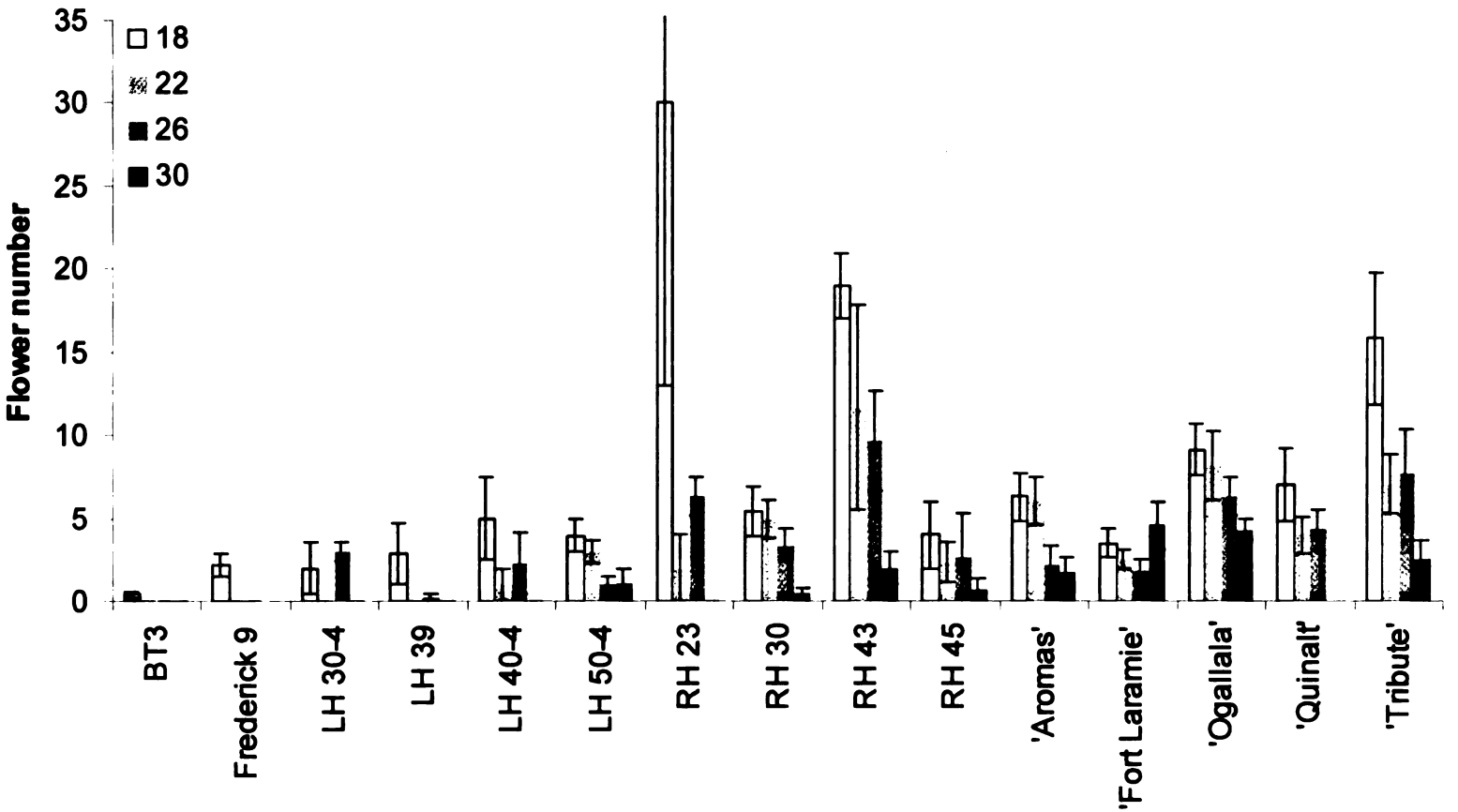
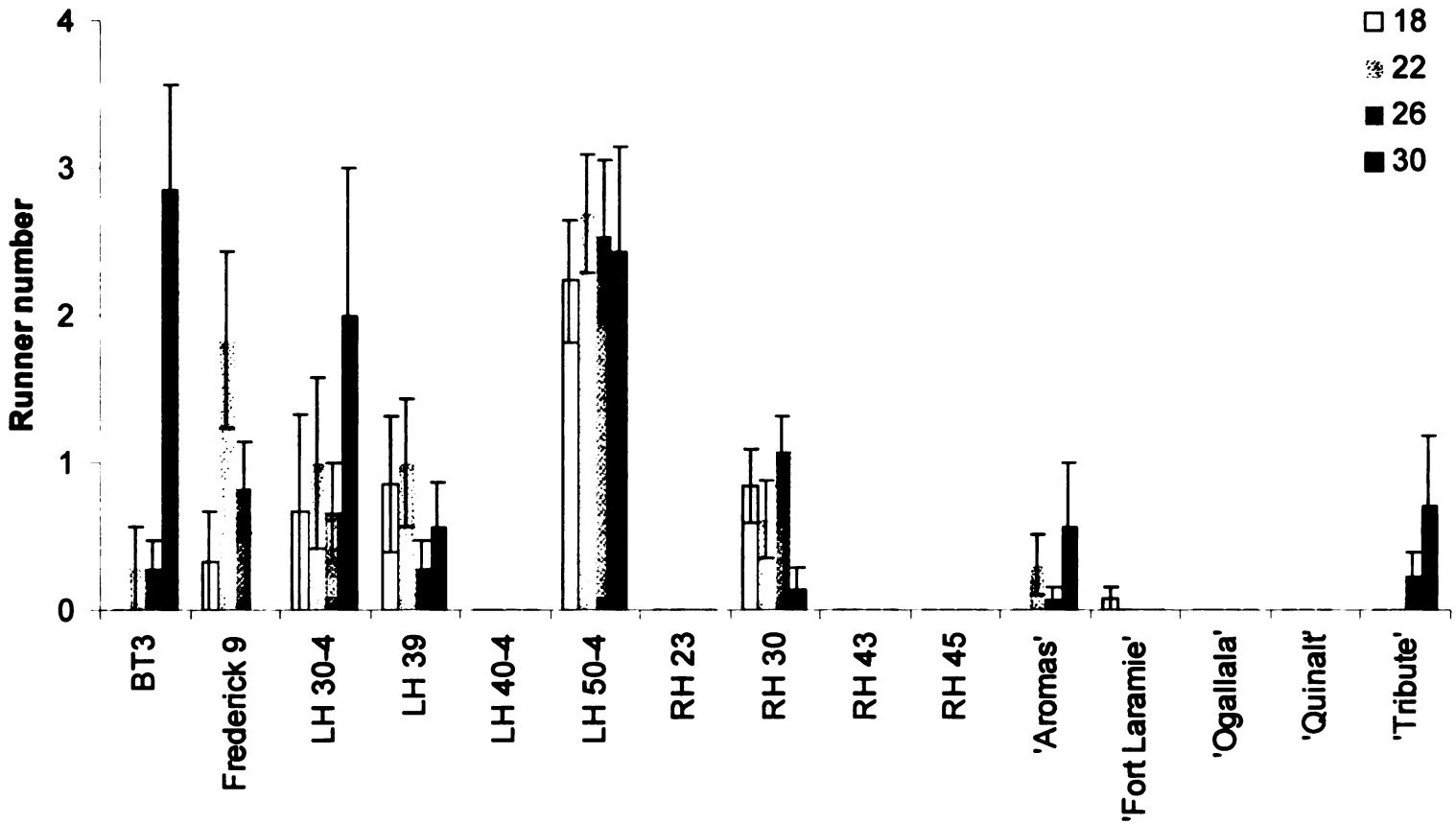


Figure 7. Mean runner number of strawberry genotypes grown in growth chambers at 12 h days and 18, 22, 26, and 30 °C. The bars represent standard errors.



CHAPTER 2

GENETICS OF DAY-NEUTRALITY IN *FRAGARIA ×ANANASSA* AND *FRAGARIA VIRGINIANA*

Introduction

Breeders outside of the Mediterranean climates are very interested in identifying new, much stronger sources of day-neutrality that are also highly resistant to summer heat. To this end, native strawberry clones have been collected from Alaska, Alberta, Minnesota New York, the northern Rocky Mountains, Ontario, Pennsylvania, western North Carolina (Luby et al., 1992; Hancock et al., 1993; Hokanson et al., 1993; Sakin et al., 1997). Over 2,500 native strawberries have now been evaluated for their flowering types and horticultural attributes, and out of this group, several elite day-neutral clones have been selected (Hancock et al., 2001 a and b). These clones originated from a wide geographical range, including climates with high summer temperature (Hancock et al., 2001 a and b; www.berrygenetics.org).

Several different models have been proposed to explain the genetic control of photoperiod control in strawberries. These include: 1) regulation by a single-gene, 2) two complementary genes without modifiers, and 3) two complementary genes with modifiers (Clark, 1927; Powers 1954; Ahmadi et al., 1990). In those models, the genes regulating day-neutrality have been proposed to be dominant, recessive, or both with no maternal effects (Macoun, 1924; Clark 1937). Unfortunately, these studies were conducted using several different genetic sources of day-neutrality.

Most researchers consider a strawberry plant to be DN if it can form flower buds under both long and short day conditions. The most precise method of evaluating day-neutrality is to monitor the plants during the whole growing season in the field; however, several less time consuming methods have been used in greenhouses (Ahmadi et al., 1990). For example, a seedling is considered to be DN if it flowers 3 months after germination or if there are flowers on new runner plants. It is possible that some of the discrepancies in the literature on the genetics of day-neutrality have been caused by the use of different methods of determining day-neutrality. It is also not known how well field and greenhouse are correlated. Since the use of greenhouse and seedling data is the fastest way to identify DNs for cultivar development, knowledge about how well the various methods correlate is critical.

The genetics of day-neutrality has been studied extensively without any consistent results. It was not until the 1970s that the term DN was employed instead of everbearer, but any genotype will be considered as DN; if they were shown to flowered under both short and long days. In the first study done Richardson (1917), European *F.* ×*ananassa* everbearing mutants ('St. Antonie Padoue' and 'Laxton's Perpetual') was used to investigate the genetics of DN. When Richardson made DN x DN and DN x SD crosses he did not get the expected 3:1 and 1:1 ratios, respectively, if day-neutral was a dominant trait. His conclusion was that this trait is controlled by a partially dominant gene with complex interactions.

Using North American *F.* ×*ananassa* mutants that were probably derived from 'Pan American', Macoun (1924) and Darrow (1937) found DN x DN crosses fit a 9:7 ratio and SD x DN crosses fit a 3:1 ratio. This is consistent with a dominant, complimentary

gene model. Clark (1937) also found similar mean values in DN x DN and DN x SD, but he uncovered sufficient differences among cultivars in the proportion of DN progeny to lead him to implicate significant factor interactions. Clark (1937) used North American *F.* ×*ananassa* mutants like Macoun (1924) and Darrow (1937), but his source of DN was probably different as he obtained his day-neutrals from short day 'Mastodon' x short day 'Howard 17' crosses.

Powers (1954) was the first investigator to analyze segregation patterns in DN material derived from *F. virginiana* ssp. *glauca*, as well as North American *F.* ×*ananassa* mutants. He concluded that this trait is control by several (at least six) dominant and recessive genes; and suggested that the dominant genes are not equal in power in conditioning the expression of day-neutrality. He indicated that there were three independent loci and ranked their dominant allele based upon their power in conditioning day-neutral progeny (A' >A > B and C).

Using a mixture of North American *F.* ×*ananassa* clones where the DN gene came from both North American *F.* ×*ananassa* mutants and *F. virginiana* ssp. *glauca*, Orecky and Slate (1967) also provided progeny data that implicated a complimentary genes model. However, they detected significant parent specific deviations from the model and suggested octosomal segregation to explain this variability.

Bringhurst and Voth (1978) examined segregation of DN in populations derived from *F. virginiana* ssp. *glauca*. They found that this trait appeared in about 40% of the offspring in DN x SD crosses and concluded that DN is controlled by a single dominant gene. Ahmadi et al. (1990) later confirmed this in crosses of *F.* ×*ananassa* carrying the Wasatch source with short day types of *F.* ×*ananassa* and *F. chiloensis*. They also found

three dominant genes that control the expression of the day-neutrality trait in native California *F. vesca*. This is in contrast with work by Brown and Wareing (1965) where they found day-neutrality in 'Baron Solemacher' and 'Bush white' of European *F. vesca* to be controlled by single, recessive gene.

Most recently, Hancock et al. (2002) investigated the inheritance of DN by crossing SD and DN representatives of *F. virginiana* to SD and DN *F. ×ananassa* cultivars. The resulting progeny were evaluated in three different locations (Michigan, Minnesota, Ontario). When DN *F. virginiana* genotypes were hybridized with SD *F. ×ananassa*, they produced ratios which all deviated significantly from a 1:1 ratio indicating photoperiod sensitivity is not controlled by a single dominant gene. The percentage of DN progeny produced by each of the day-neutral *F. virginiana* parents varied widely in a quantitative fashion and, in fact, a few DN progeny (up to 11.5%) were covered in crosses of SD *F. virginiana* x SD *F. ×ananassa* and SD *F. ×ananassa* x SD *F. virginiana*. In addition, they detected significant differences in expression of DN among crosses across locations, with Ontario producing the highest percentage DN progeny.

If all the studies of the genetics of DN are considered together, we must conclude that the genetics of day-neutrality in strawberries is still unknown. No consistent patterns of inheritance have emerged. One of the most important reasons for the discrepancy in the literature is the employment of different sources of DN. *Fragaria virginiana* ssp. *glauca* was used as DN source in different backgrounds (Powers 1954; Orecky and Slate 1967; Bringhurst and Voth 1978), and the *F. ×ananassa* sources of DN also varied greatly. Richardson (1917) used European source came from 'F. de Gaillon', while Macoun (1924) and Darrow (1966) used North American source, 'Pan American', which

was a chance seedling of 'Bismark'. Clark (1937) used a DN source derived from a cross of SD 'Mastodon' x SD 'Howard 17'. In fact, there have been more than six independent and original sources of DN identified in *F. ×ananassa* and transferred to SD types (Darrow, 1966).

It is also possible that differences in evaluation methods and timing have contributed to the variation in segregation ratios. Numerous methods of screening DN have been employed including 1) flowering on mother and runner plants during one summer and fall, 2) flowering patterns across two seasons, 3) how fast seedlings flower, and 4) crossing individuals to SD *F. chiloensis* and analyzing the percentage of day-neutral progeny produced (Nicoll and Galletta, 1987; Ahmadi *et al.* (1990). It is not known how tightly these evaluation methods are associated. Timing of evaluation is critical as well. Ahmadi *et al.* (1990) noted that SD genotypes with little chilling requirement might initiate flower buds in August and flower in November. If the progeny were scored during that time, the genotype could have been misjudged as DN. Also, SD genotypes flower semi-continuously in the second year in mild climates. The various investigators who have studied the genetics of day-neutrality rarely used the same dates. For example, Richardson (1917) scored his genotypes from May to October, while Powers (1954) evaluated them from July to September.

The use of the term "everbearing" has added complexity to the literature regarding the genetics of day-neutrality. Everbearers have been variously described as a plant fruiting more than one time in a year and synonymies as "perpetual", "four-season", "rebloomer", "remontant", "double-cropping", and finally "day-neutral" (Galletta and Bringhurst, 1990). Nicoll and Galletta (1987) stated that the term "day-neutral" and

"everbearer" can be used interchangeably, if DN is used as to a physiological term to denote a relative insensitivity to day length in flower bud initiation and everbearer is used as an agricultural term to indicate a pragmatic expectation of summer strawberry production. However, the distinction should be made clear between DN and everbearer in genetic studies since both the DN and LD physiological classes have been referred to as everbearer in the literature.

In this chapter, experiments were designed to elucidate the genetics of day-neutrality in native clones of *Fragaria*, to more efficiently utilize them in breeding new day-neutral *Fragaria ×ananassa* cultivars. The specific objectives were to determine: 1) the relationship between several different evaluation methods for DN, 2) if greenhouse screens can be used to predict field flowering performance, 3) if day-neutrality is a quantitative or qualitative trait, 4) if there is more than one source of genes for day-neutrality in native North American populations of *F. virginiana*, and 5) if genetic background has an influence on the expression of day-neutrality.

EXPERIMENT 1

Material and Methods

This experiment was designed to evaluate different methods of identifying DN progeny. Segregating populations were constructed in a partial-diallel fashion (Table 8). Information on *F. virginiana* clones can be found at Sakin *et al.* (1997), Hancock *et al.*

(2001 a and b) and www.berrygenetics.com. DHL 1336 ('Tribute' x Montreal River 10) is a selection from the MSU Strawberry Genetics Program.

All parental genotypes were potted onto 18 x 16 x 13 cm pots in the summer of 1999) and placed in a greenhouse at MSU with natural day lights and a mean temperature of ~ 21 °C. Crosses were made by transferring pollen with a camel hairbrush after removal of stamens using sharp tweezers to prevent self-pollination. Fresh pollen was generally collected from open flowers; however in some occasions, pollen was stored in petri dishes at -16 °C for future use. The fruits were harvested when fully ripe and seeds were extracted by smashing the fruits on paper towels. To promote germination, seeds were then placed on soil in pots and held in a growth chamber at 4 °C with continuous inflorescent light and moisture. In February 2000, when the seeds started to germinate, they were placed in a growth room with ~ 18 °C with continuous light.

Each seedling was evaluated using five methods to score day-neutrality: 1) They were planted in March 2000 into 14 x 12 x 12 cm pots and placed in a greenhouse at MSU under long day conditions (13 h days created with ~ 800 μmol s^{-1} m^{-2} of supplementary light) and 18 - 22 °C. Any genotype that flowered within 100 days from germination was considered DN (DN1-100 days to flowering). 2) The seedlings were transplanted in the field at the Southwestern Michigan Research and Extension Center (SWMREC) on 07/25/2000 at 60 x 120 cm spacing and any that flowered in that same

Table 8. The genotypes crossed in a partial-diallel fashion to study the interaction of different evaluation method of day-neutrality.

Fragaria ×ananassa		*Fragaria virginiana*	
Day-neutral or everbearer	Short day	Day-neutral	Short day
'Aromas'	DHL 1336	Frederick 9	Eagle 14
'Fort Laramie'	'Camarosa'	LH 39-15	High Falls 22
'Ogallala'	'Honeoye'	RH 30	Montreal River 10
'Tribute'	'Glooscap'		RH 18

summer before 9/9/2000 were considered DN (DN2-1st year field flowering). 3) Rooted one-year-old runner plants were collected from each of these mother plants and placed in an unheated greenhouse in September 2000, and allowed to flower in the spring of 2001 without supplementary light. Those plants that flowered again before 9/01/01 were considered as DN (DN3-2nd year greenhouse flowering). 4) All of the original field-grown plants were monitored another year for flowering in the spring and summer of 2001. Those that flowered in the spring and again before 9/9/2000 were considered as DN (DN4-2nd year field flowering). 5) During this same period in the field, genotypes that produced flowers on their newly formed runners were considered DN (DN5-runner flowering). In all experiments, each family was maintained in a single plot without replication.

Greenhouse surveys can only be used to identify elite DN progeny if the photoperiod response of progeny is similar in the greenhouse and field, or if there are fewer DN progeny in the greenhouse, those that do produce multiple greenhouse crops are at least the strongest DN genotypes in the field. To investigate this possibility, each field grown genotype was given a flowering strength rating (FSR) of 0 - 10 in the summer of 2001, after its photoperiod sensitivity had been rated in the 2000 greenhouse screens. These plants with no flowers were rated 0, and those with the most were given a 10.

A number of horticulturally important traits were also evaluated for each of the hybrids in the summer of 2001. Crown and runner numbers were counted on 5/6/01, and inflorescence and flowers per inflorescence were recorded on 5/9/01. Four fruits from each plant were harvested on 6/13/01 and weighed to calculate average fruit weight.

The percentage of DN progeny was calculated for each family and the grand mean for each family using each evaluation method was determined. Correlations among these scores were calculated using the SAS program (SAS Institute, Cary, N.C.). The mean and SEs of the horticulturally important traits were also determined for each family.

Result and Discussion

Day-neutrality evaluation methods: Different mean percentages of DNs were observed across the evaluation methods. DN5 (runner flowering) had the lowest overall mean (18%), while DN1 (100 day flowers) had the highest (55%) (Table 9). The greenhouse evaluations produced higher means than the field evaluations (55 and 49% vs. 41 and 40%). Large amounts of variation were observed across families in the mean % DN progeny for each method. In fact, in three of the evaluation methods, the range in family values was 0 - 100% (DN1, 100 days flowers, DN3 - 2nd year field flowering, DN4 - 2nd year greenhouse flowering). DN x DN crosses generated higher numbers of DN progeny than DN x SD crosses for all methods. For example, when DN 'Aromas' was selfed it produced 60, 67, 67, 100, and 33% for DN1, DN2, DN3, DN4, and DN5, respectively, while SD 'Glooscap' x SD RH 18 produced 47, 38, 27, 6, and 0 % (Table 9).

Some of the crosses with high numbers of DN progeny in the field and greenhouse screens did not have any flowers on their runners; for example, 'Aromas' x 'Fort Laramie', and 'Aromas' x DHL 1336 (Table 9). The highest family values for flowers on their newly-formed runners (DN5-runner flowering) were 64, 39, and 38% for 'Tribute' x Frederick 9 (64%), 'Tribute' x RH 18 (39%), and 'Tribute' x Eagle-14 (38%) (Table 9).

'Tribute', which usually forms flowers in its runners, is the common parent in these crosses (Draper et al., 1981; Maas and Cathey, 1987).

The average flowering strength rating (FSR) across all families was 1.2 (Table 9). Eagle-14 x Eagle-14 had the lowest average FSR (mean = 0.0, N = 28), while 'Aromas' x 'Ogallala' (mean = 3.8, N = 9), 'Tribute' x RH 18 (mean = 3.4, N = 38), and 'Tribute' x Eagle-14 (mean = 3.4, N = 39) had the highest FSR rating.

Hundred-day flowering (DN1) was significantly correlated with DN2 (1st year field flowering) (R = 46%, P = 0.003), but not with any of the other methods (Table 10). However, all the other evaluation methods were significantly correlated (Table 10 and Figure 9 and 10). Likewise, the FSR were significantly correlated with all the DN evaluation methods except DN1 (flowering within 100 days) (Table 10). The highest correlation was observed between DN4, 2nd year field flowering, and FSR. This is not surprising, as these data were collected at the same time in the field and a high value for strength of flowering also indicates the plants are strong day-neutrals (Anonymous, 1988). Also, the year-to-year correlation in the field (DN4 vs. DN2) was high (71%, *P* = 0.000), and the greenhouses vs. field evaluations in 2001 were highly significant (73%, *P* = 0.000 (Table 10 and Figure 9).

Horticultural traits: The families had quite variable averages for all of the horticulturally important traits (Table 11). Mean crown number ranged from 2.1 ('Aromas' x 'Ogallala' and 'Aromas' x 'Tribute') to 6.4 ('Fort Laramie' x 'Frederick 9') (Table 11). 'Aromas' x 'LH 39' did not have any runners, while Eagle-14 x Eagle-14 had very high runner numbers (11.5) (Table 11). 'Aromas' x 'Tribute' produced the lowest inflorescence and crown numbers (mean = 4.4), while DHL 1336 x 'Glooscap' had the

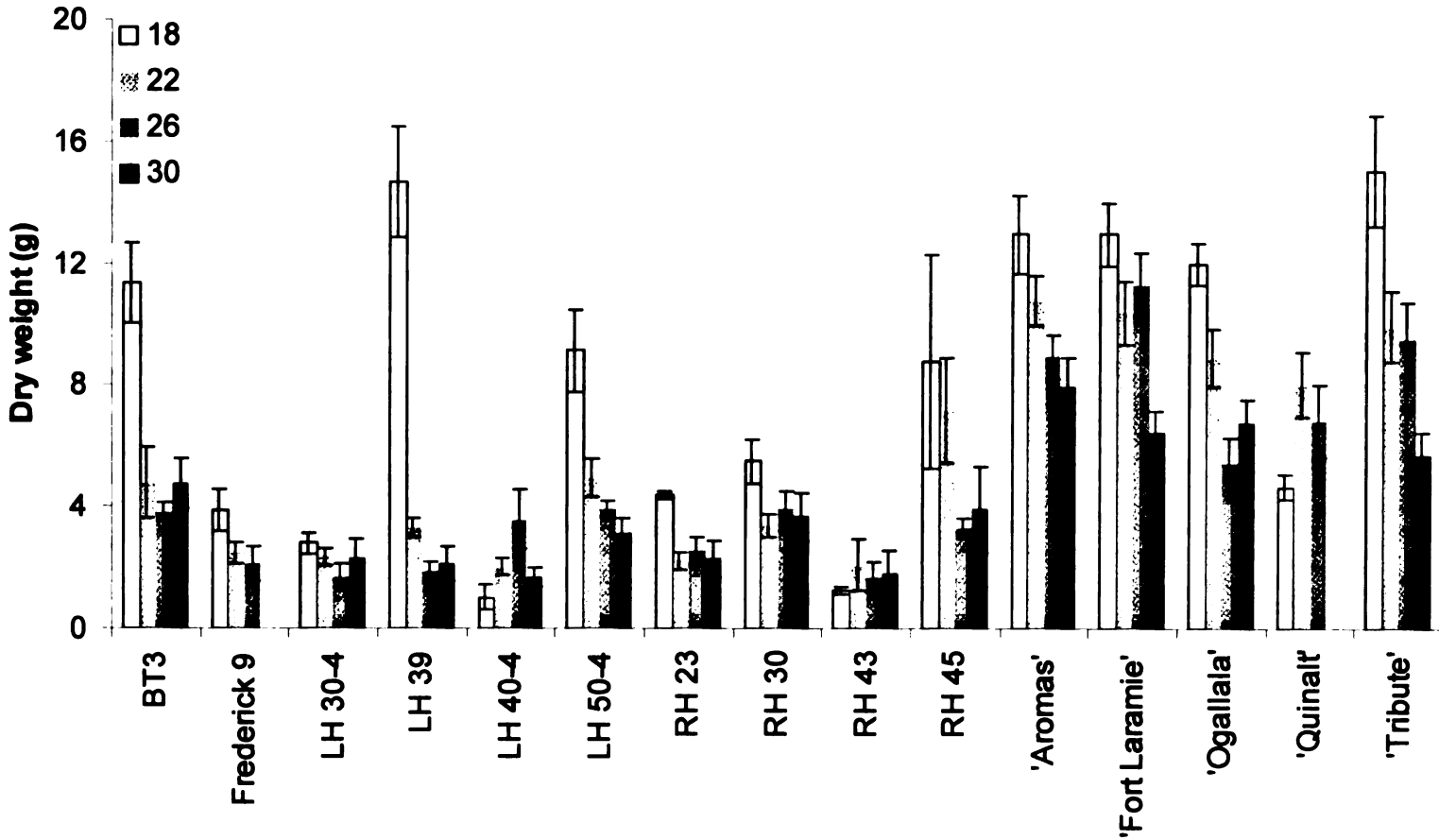
highest numbers (mean = 12.5) (Table 11). The range in flowers per inflorescence was from 3.1 ('Aromas' x 'Ogallala') to 6.7 ('Ogallala' x 'Camarosa') (Table 11). The average fruit weight in Eagle-14 x Eagle-14 was extremely low (mean = 1.4), while 'Camarosa' x 'Glooscap' had the largest fruits (mean = 13.8). Among these traits, only runner number was negatively associated with % DN in the families (Table 12).

Conclusions: These results indicate that scoring DN progeny within 100 d from germination can not be used to predict field performance. Apparently, the speed with which a seedling begins flowering is not tightly associated with photoperiod sensitivity. However, greenhouses can be used to predict field performance, if the flowering behavior of individuals is followed through a whole season. The DN percentage observed in our second year greenhouse screens were highly correlated with the subsequent field evaluations, and the families with the highest flowering strength in the field also had the highest percentage of DNs in both the greenhouse and field screens.

The final decision on whether greenhouses will be utilized in a breeding program will still have to be based on the objective of the breeding program. While the correlation between the field and greenhouse determinations of % DN in families was high, some families which had no DN individuals in the greenhouse, did show some in the field. For example, the second year greenhouse evaluations of DHL 1336 x 'Honeoye' crosses generated no DNs, while 17% were DN were recovered in the field. If one is interested in finding DNs in the broadest range of families, the populations need to be evaluated in the field, probably for multiple years. Additionally, the relationship between DN and number of fruiting cycles was not evaluated. If numbers of cycles is important, populations will need to be screened directly for that characteristic.

Small fruit size is a common problem in DN breeding and it has been suggested that fruit size and day-neutrality are negatively correlated (Dale et al., 2002). We did not find this to be the case in this study (R^2= 0.18, df = 39, P = 0.275) (Table 12). In fact, the only significant correlation observed between day-neutrality and horticulturally important traits was a negative correlation with runner number (- 49%, P = 0.001) that has been previously demonstrated (Hancock et al., 2002). This suggests that with the exception of runner numbers, there are a few negative compensation that will impede DN breeding using the populations in this study. Even though runner number was negatively correlated with %DN, some families produced runners than the current DN cultivars indicating that improved runnering types can be recovered.

Figure 8. Mean dry weight of strawberry genotypes grown in growth chambers at 12 h days and 18, 22, 26, and 30 °C. The bars represent standard errors.



Montreal River 10 x RH 18	13	53	13	8	10	20	13	8	13	8	13	0.2
'Aromas' x RH 18	12	50	12	58	8	50	12	58	12	50	12	1.5
'Fort Laramie' x 'Camarosa'	12	75	12	33	12	50	12	33	12	8	12	0.5
'Fort Laramie' x Eagle 14	11	85	11	55	9	56	11	64	11	27	11	1.6
'Ogallala' x 'Camarosa'	14	47	14	64	9	89	14	50	14	21	14	1.9
'Tribute' x 'Honeoye'	40	10	40	10	38	37	40	18	40	10	40	0.2
'Tribute' x Eagle 14	8	57	8	50	8	100	8	75	8	38	8	3.4
'Tribute' x Montreal River 10	11	45	11	36	9	44	11	45	11	36	11	1.8
'Tribute' x RH 18	18	35	18	39	17	65	18	61	18	39	18	3.4
Frederick 9 x 'DHL 1336'	15	33	15	53	13	62	15	53	15	20	15	2.3
'Honeoye' x Frederick 9	22	75	22	32	18	44	22	18	22	14	22	0.8
RH 30 x Montreal River 10	10	100	10	30	9	44	10	50	10	30	10	1.0
Total/mean	641	55	641	41	533	49	641	40	639	18	641	1.2

[1]Flowering within 100 days from germination in a greenhouse in 2000.
[2]Flowering before 9/9 in field in 2000.
[3]Flowering under both short and long days in a greenhouse in 2001.
[4]Flowering under both short and long days in field in 2001.
[5]Flowering on their newly-formed runner in field in 2001.
[6]Flowering strength ratio of 0-10 (10 having the most flowers during the second cycle of flowering) in field in 2001.

Table 10. Correlation coefficients, significance, and number of individuals in strawberry families grown in a greenhouse at Michigan State University, East Lansing, Mich., and in the field at the Southwest Michigan Research and Extension Center Benton Harbor, Mich. in 2000 and 2001.

	DN2[2]	DN3[3]	DN4[4]	DN5[5]	FSR[6]
DN1[1]	0.46	0.26	0.27	0.16	0.06
	0.003[7]	0.125	0.095	0.320	0.726
	(39)	(36)	(39)	(39)	(39)
DN2		0.71	0.71	0.33	0.54
		0.000	**0.000**	**0.042**	**0.000**
		(36)	(39)	(39)	(39)
DN3			0.73	0.45	0.74
			0.000	**0.005**	**0.000**
			(36)	(36)	(36)
DN4				0.70	0.85
				0.000	**0.000**
				(39)	(39)
DN5					0.58
					0.000
					(39)

[1]Flowering within 100 days from germination in a greenhouse in 2000.
[2]Flowering before 9/9 in field in 2000.
[3]Flowering under both short and long days in a greenhouse in 2001.
[4]Flowering under both short and long days in field in 2001.
[5]Flowering on their newly-formed runner in field in 2001.
[6]Flowering strength ratio of 0-10 (10 having the most flowers during the second cycle of flowering) in field in 2001.
[7]Significant P values, at 0.05, are bolded.

Figure 9. Association of several day-neutrality scoring methods in strawberry families grown in a greenhouse at Michigan State University, East Lansing, Mich., and in the field at the Southwest Michigan Research and Extension Center Benton Harbor, Mich. (DN1-Flowering within 100 days from germination in a greenhouse in 2000; DN2-Flowering before 9/9 in field in 2000; DN3-Flowering under both short and long days in a greenhouse in 2001; DN4-Flowering under both short and long days in field in 2001).

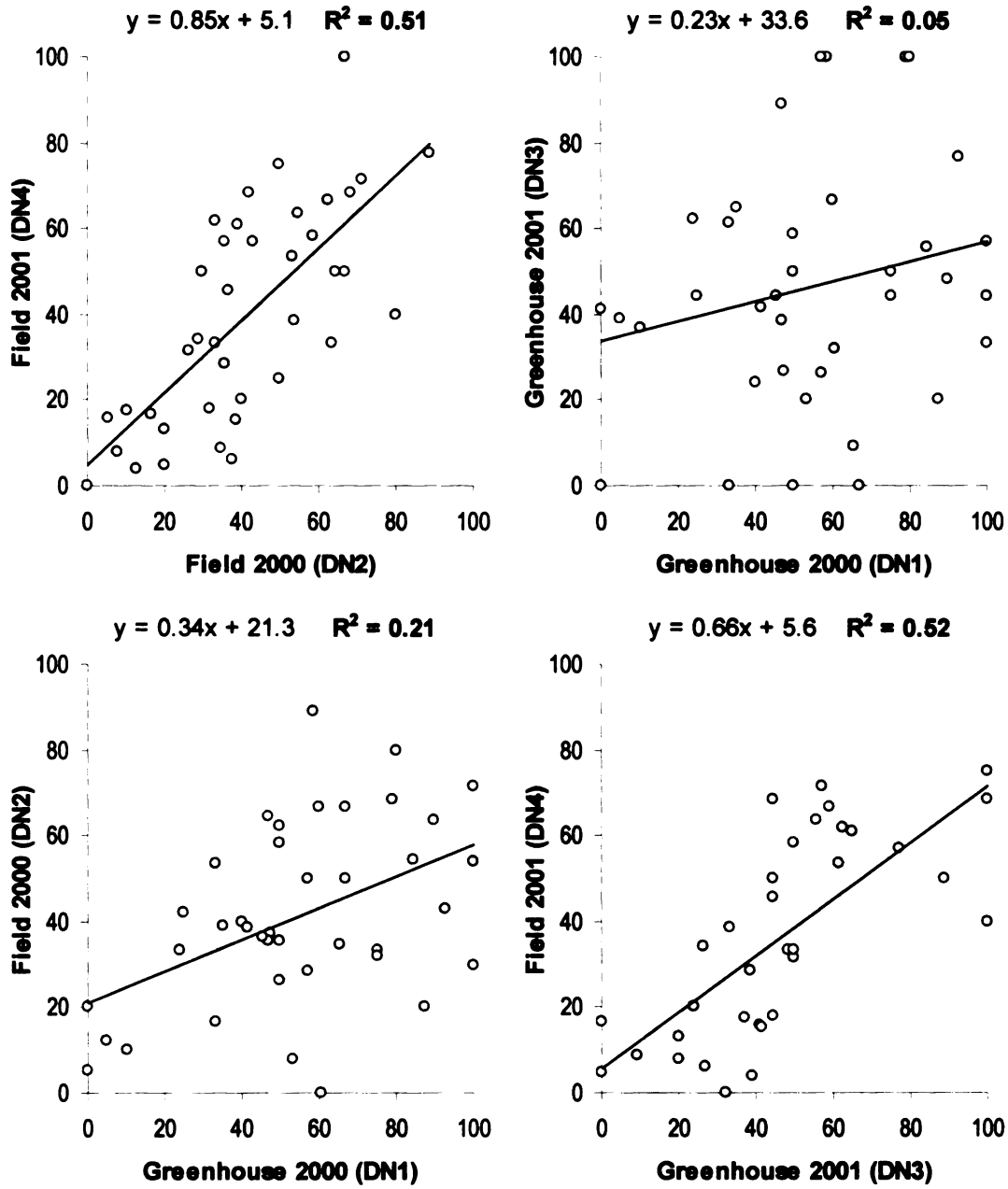


Figure 10. Association of several day-neutrality scoring methods in strawberry families grown in a greenhouse at Michigan State University, East Lansing, Mich., and in the field at the Southwest Michigan Research and Extension Center Benton Harbor, Mich. (DN4-Flowering under both short and long days in field in 2001; DN5-Flowering on their newly-formed runner in field in 2001; Flowering strength ratio of 0-10 (10 having the most flowers during the second cycle of flowering) in field in 2001).

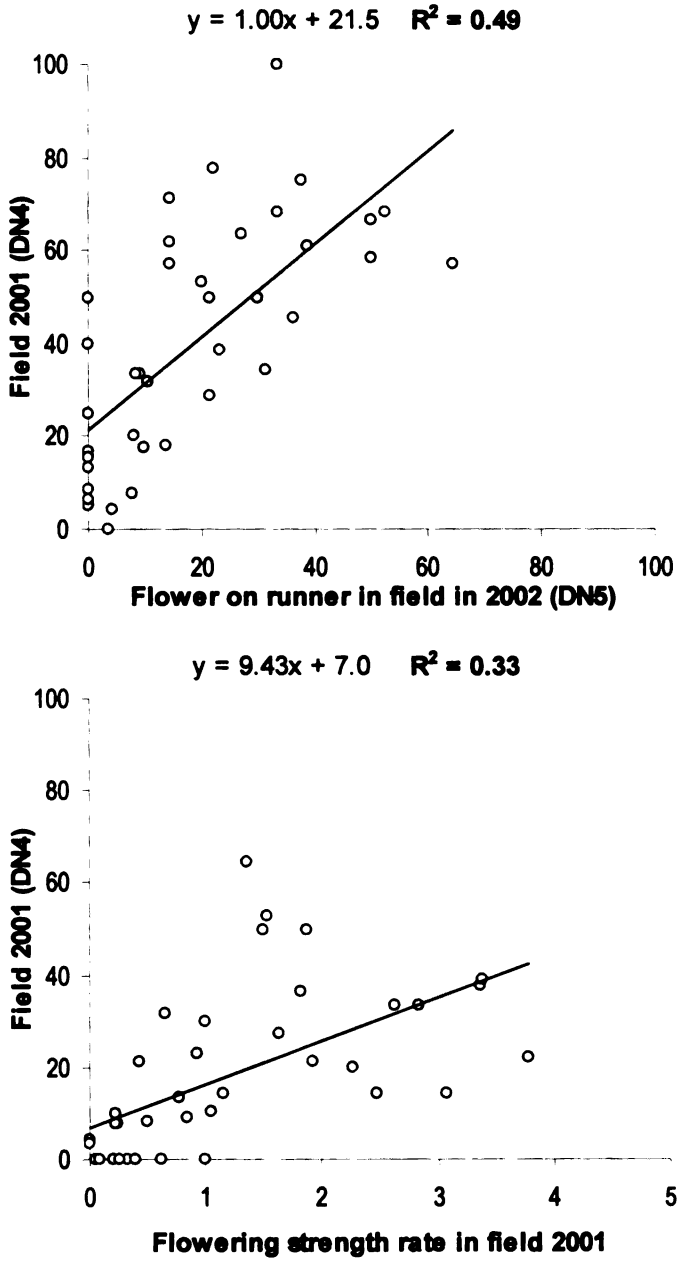


Table 11. Mean and standard deviations for horticulturally important traits in strawberry families grown in a greenhouse at Michigan State University, East Lansing, Mich., and in the field at the Southwest Michigan Research and Extension Center Benton Harbor, Mich. in 2001.

Family	Crown number	Runner number	Inflorescence number	Flower/ inflorescence	Average fruit weight (g)
'Aromas' x 'Aromas'	2.2 ± 0.8	0.8 ± 1.0	8.5 ± 1.6	4.5 ± 1.0	9.6 ± 1.5
'Aromas' x 'Fort Laramie'	3.0 ± 1.1	1.3 ± 1.8	8.3 ± 3.2	4.3 ± 0.8	9.2 ± 3.3
Aromas' x 'Ogallala'	2.1 ± 1.3	0.8 ± 0.7	5.2 ± 2.9	3.1 ± 0.6	8.9 ± 3.3
'Aromas' x 'Tribute'	2.1 ± 1.2	0.6 ± 1.0	4.4 ± 2.6	3.4 ± 1.2	7.9 ± 2.7
'Aromas' x LH 39	2.4 ± 1.1	0.0 ± 0.0	4.5 ± 2.1	3.6 ± 1.6	7.0 ± 3.1
'Fort Laramie' x 'Tribute'	3.0 ± 0.9	0.4 ± 1.0	6.7 ± 2.1	4.6 ± 0.9	12.4 ± 3.9
'Fort Laramie' x Frederick 9	6.4 ± 2.9	6.7 ± 3.8	12.0 ± 3.6	3.9 ± 1.1	3.1 ± 0.4
'Tribute' x Frederick 9	4.7 ± 1.1	4.9 ± 1.8	7.3 ± 1.8	3.7 ± 1.0	3.8 ± 1.7
LH 39 x LH 39	3.8 ± 1.9	3.8 ± 3.6	7.5 ± 5.1	2.8 ± 0.9	3.9 ± 3.0
RH 30 x RH 30	4.3 ± 1.2	4.0 ± 2.9	12.1 ± 5.3	3.6 ± 1.0	1.7 ± 2.6
DHL 1336 x DHL 1336	3.3 ± 1.1	2.1 ± 1.6	5.6 ± 2.4	5.8 ± 2.0	9.5 ± 4.0
'Camarosa' x 'DHL 1336	3.1 ± 1.0	6.7 ± 5.2	8.5 ± 2.5	4.9 ± 1.4	10.2 ± 4.2
DHL 1336 x 'Honeoye'	2.5 ± 0.9	0.7 ± 0.8	5.0 ± 2.1	3.8 ± 0.8	7.1 ± 1.5
DHL 1336 x 'Glooscap'	4.0 ± 0.8	9.7 ± 4.1	12.5 ± 4.2	4.2 ± 1.0	11.2 ± 3.0
DHL 1336 x RH 18	3.4 ± 1.2	5.1 ± 2.3	7.5 ± 4.4	3.3 ± 0.9	2.4 ± 1.7
'Camarosa' x 'Honeoye'	3.5 ± 2.1	3.1 ± 2.5	8.0 ± 3.3	4.6 ± 1.2	11.1 ± 2.7
'Camarosa' x 'Glooscap'	4.2 ± 1.4	2.4 ± 2.9	8.6 ± 3.0	6.1 ± 1.2	13.8 ± 6.9
'Camarosa' x Eagle 14	3.8 ± 1.7	8.2 ± 3.0	8.6 ± 2.8	4.0 ± 0.9	5.4 ± 2.1
'Camarosa' x Montreal River 10	4.1 ± 1.2	3.7 ± 2.6	8.7 ± 3.5	4.3 ± 1.1	7.4 ± 2.6
'Camarosa' x RH 18	3.9 ± 1.2	2.3 ± 1.2	9.3 ± 2.9	4.5 ± 1.1	5.1 ± 2.6
'Honeoye' x Eagle 14	4.1 ± 1.1	5.6 ± 3.2	7.0 ± 2.9	3.5 ± 0.7	2.8 ± 1.2
'Honeoye' x RH 18	4.2 ± 0.8	5.6 ± 1.1	5.4 ± 2.3	3.4 ± 0.5	3.6 ± 1.5
'Glooscap' x RH 18	4.3 ± 1.4	6.9 ± 3.7	11.8 ± 4.1	4.2 ± 0.9	5.2 ± 0.9
Eagle 14 x Eagle 14	4.1 ± 2.6	11.5 ± 6.4	7.4 ± 4.5	3.4 ± 1.3	1.4 ± 1.0

High Falls 22 x High Falls 22	4.5 ± 1.1	9.3 ± 3.7	7.0 ± 3.1	3.2 ± 1.2	2.7 ± 1.3
High Falls 22 x Montreal River 10	3.3 ± 0.9	5.5 ± 2.8	8.6 ± 3.1	4.1 ± 1.0	2.8 ± 1.8
Montreal River 10 x Montreal River 10	3.4 ± 0.9	5.4 ± 2.2	8.8 ± 4.4	4.3 ± 2.1	2.8 ± 1.0
Montreal River 10 x RH 18	3.4 ± 1.4	2.2 ± 2.0	5.9 ± 2.8	4.1 ± 1.0	5.2 ± 2.5
'Aromas' x RH 18	4.0 ± 1.7	2.2 ± 2.6	7.9 ± 4.3	4.1 ± 1.6	4.9 ± 1.2
'Fort Laramie' x 'Camarosa'	5.2 ± 1.3	4.8 ± 2.4	11.9 ± 3.2	3.3 ± 0.5	3.8 ± 2.5
'Fort Laramie' x Eagle 14	5.2 ± 1.7	7.3 ± 3.8	10.8 ± 4.2	3.5 ± 1.4	4.2 ± 1.7
'Ogallala' x 'Camarosa'	4.1 ± 1.7	3.9 ± 4.2	10.8 ± 3.1	6.7 ± 2.8	6.8 ± 2.3
'Tribute' x 'Honeoye'	4.4 ± 1.3	5.7 ± 3.0	8.6 ± 3.0	4.1 ± 0.9	7.5 ± 3.3
'Tribute' x Eagle 14	4.0 ± 1.2	3.4 ± 1.3	7.5 ± 3.0	4.8 ± 1.2	2.9 ± 0.6
'Tribute' x Montreal River 10	3.8 ± 1.0	4.1 ± 3.1	8.1 ± 3.9	4.3 ± 1.2	5.3 ± 2.9
'Tribute' x RH 18	4.0 ± 0.8	2.9 ± 3.4	6.9 ± 2.8	3.9 ± 1.0	3.6 ± 1.7
'Frederick 9 x DHL 1336	3.7 ± 1.3	2.9 ± 2.9	7.9 ± 2.5	4.5 ± 1.1	7.1 ± 3.9
'Honeoye' x Frederick 9	2.9 ± 0.9	6.0 ± 5.2	5.6 ± 2.5	2.8 ± 1.1	3.3 ± 2.0
RH 30 x Montreal River 10	2.2 ± 0.8	4.3 ± 2.7	5.4 ± 2.7	6.4 ± 1.5	2.8 ± 2.3

Table 12. Correlation between percentage of day-neutral progeny and several horticulturally important traits in strawberry families grown in a greenhouse at Michigan State University, East Lansing, Mich., and in the field at the Southwest Michigan Research and Extension Center Benton Harbor, Mich. in 2001 and 2001.

	Crown no.	Runner no.	Inflorescence no.	Flower/inf.	Fruit weight (g)
% Day-neutral progeny	0.00	-0.49	0.00	0.17	0.18
	0.985	**0.001**[1]	0.991	0.296	0.275
Crown no.		0.54	0.67	-0.11	-0.34
		0.000	**0.000**	0.499	**0.033**
Runner no.			0.43	-0.21	-0.42
			0.006	0.206	**0.007**
Inflorescence no.				0.11	-0.05
				0.496	0.771
Flower/inf.					0.42
					0.007

[1]Significant correlations, at 0.05, are bolded.

EXPERIMENT 2

Material and Methods

To investigate the genetics of day-neutrality, two groups of segregating populations were constructed using a diallel design: 1) DN x SD crosses, and 2) DN x DN crosses (Table 13). An elite DN *F. virginiana* genotype, RH 23 (Hancock et al., 2002) was also crossed to some genotypes. Crosses were made as previously described in Experiment 1. The fruit were harvested and seed were extracted and placed on soil in pots and held in a growth chamber at 4 °C with continuous inflorescent light. When the seeds germinated in summer 2001, the seedlings were placed in a growth room ~18 °C with continuous inflorescent light (~600 μmol s^{-1} m^{-2}). When they had reached the 4 - 6 leaf stage, they were potted into 14 x 12 x 12 cm pots and placed in a greenhouse at ~18 °C and 13 h long day conditions maintained by high intensity lamps (~800 μmol s^{-1} m^{-2}). Plants began to flower on 01/28/02 and by mid-April all flowering had ceased. In May 2002, segregating populations were planted at the MSU Horticulture Farm in a completely randomized design. Each family was divided into four replications and the row spacing was 180 cm x 60 cm. Flowering was then monitored on a weekly basis from July 26 to August 24 in the field. Similar to DN4 (2^{nd} year field flowering) of Experiment 1, genotypes were considered as DN if they flowered under the short days of spring before 5/30/02 (<14 h) and the long days of summer after 7/24/02 (>15 h).

Table 13. The family numbers of genotypes crossed in a partial-diallel fashion to study the genetic of day-neutrality in strawberries.

	Day-neutral						
	F. ×ananassa			*F. virginiana*			
Genotype	'Aromas'	'Fort Laramie'	'Tribute'	Frederick 9	LH 50-4	RH 23	RH 30
Short day							
F. ×ananassa							
'Allstar'		53	86		89	52	
'Chandler'	53	71	72			36	89
'Honeoye'			71	54			72
F. virginiana							
Eagle-14		71	54	54			
FRA 0368	54		29		72	53	
MR 10				72			
Day-neutral							
F. ×ananassa							
'Aromas'		18					70
'Fort Laramie'			30				54
'Tribute'			52		36	72	71
F. virginiana							
Frederick 9					53		72
LH 50-4					36		51
RH 23							
RH 30							

Chi-square tests were constructed for each family using either a 1:1 (DN x SD crosses) or 3:1 ratio (DN x DN crosses), assuming DN was regulated by a single dominant gene (Ahmadi et al., 1990). The tests were also made using pooled data (all DN x all DN, all DN *F. virginiana* x all DN *F. virginiana*, and all DN *F. virginiana* x all DN *F. ×ananassa*).

Fragaria ×ananassa x *F. virginiana* in both DN x SD and DN x DN groups were combined to construct Analysis of Variance tables to test the significance of general and specific combining abilities using the SAS GLM procedure (SAS, 1990). To increase normality, the percentage of day-neutral progeny in each family was $\sqrt{\arcsin}$ transformed, although the means are presented as untransformed data. Both *F. ×ananassa* and *F. virginiana* genotypes were considered as fixed in the analysis.

Results and Discussion

Chi-square analyses: Overall, 58% of the progeny were DN in the combined DN x SD crosses (Table 14). This percentage is significantly higher than the 50% that is expected if DN is regulated by a single dominant gene. The *F. ×ananassa* x *F. ×ananassa* (59%) and *F. ×ananassa* x *F. virginiana* (62%) crosses also averaged significantly more DN progeny than expected under the single–gene model. The average percentage of DN progeny in the *F. virginiana* x *F. virginiana* crosses (48%) did not vary significantly from a 1:1 (Table 14).

Most of the individual families did not fit the single gene model for the inheritance of DN. A continuous variation in % DN progeny in the *F. ×ananassa* x *F. virginiana*

families was observed, ranging from 30% ('Allstar' x LH 50-4) to 87% ('Tribute' x Eagle-14) (Table 14 and Figure 11). All but one of the *F. virginiana* x *F. virginiana* families (FRA 0368 x LH 50-4, 36%) differed significantly from the expected 1:1 ratio (Table 14). The *F. ×ananassa* x *F. ×ananassa* families generally fit a 1:1 model except 'Allstar' x 'Fort Laramie' and 'Tribute' x 'Chandler'.

Overall, 70% of the progeny were DN in the combined DN x DN crosses; which is significantly lower than the expected 75% (3:1 ratio) under the single, dominant gene model (Table 15). While the mean of the *F. ×ananassa* x *F. ×ananassa* crosses did not differ significantly from the expected values (76% vs. 75%), the average percentage of DN in the *F. ×ananassa* x *F. virginiana* crosses was significantly higher (83%) and the *F. virginiana* x *F. virginiana* crosses was significantly lower (48%) than expected (Table 15). In particular, the selfing of 'Tribute' produced extremely high numbers of DN progeny (88%) and the highest number of DN for any family was observed in 'Tribute' x RH 23 (93%). All crosses in the *F. virginiana* x *F. virginiana* group had significantly lower numbers of DN progeny than expected (<63%) (Table 15). Similar to the DN x SD families, the DN x DN families displayed continuous variation in their percentage of DN progeny (Figure 12).

General and Specific Combining Abilities: The analysis of variance indicated that general combining ability (GCA) for DN was significant in both *F. ×ananassa* and *F. virginiana* (Table 16). Likewise, specific combining ability (SCA) was significant (P = 0.000) in both species. The largest proportion of the variance component was in SCA (34%), while $GCA_{F.\ \times ananassa}$ and $GCA_{F.\ virginiana}$ tabulated 22 and 20% of the variation, respectively (Table 16). A continuous pattern of variation in parental means was

observed for the percentage of day-neutral progeny produced, ranging from 55.1% ('Honeoye') to 85.0% ('Tribute') among *F.* ×*ananassa* cultivars and from 49.5% (LH 50-4) to 76.0% (Eagle-14) in *F. virginiana* genotypes (Figure 13).

Conclusions: Such a wide range in the percentage of DN progeny found in the various families suggests that day-neutrality in octoploid strawberries is not regulated by a single, dominant gene. Several different kinds of observations support this conclusion. 1) Less than half the families produced 1:1 or 3:1 ratios of day-neutral plants. 2) DN *F. virginiana* genotypes produced significantly different percentages of DN progeny than DN *F.* ×*ananassa* cultivars. 3) Two different DN parents crossed to the same short day genotype produced different percentages of DN progeny. For example, when 'Tribute' and 'Aromas' were crosses to the same SD genotype, 'Tribute' consistently generated more DN progeny than 'Aromas'. 4) Some of the day-neutrality sources were more powerful than others in producing of day-neutral progeny (e.g., 'Tribute' > 'Aromas' and RH 23 > Frederick 9). Both general and specific combining abilities for DN were significant. 5) None of the DN parents produced 100% DN progeny, which would be expected if there were homozygous dominant DN individual. Repeated selfing of DN parents has increased the percentage of DN progeny, but no true DN breeding genotypes have been produced (Shaw, personal communication). 6) In a previous study, SD x SD crosses generated some (up to 8.2%) DN progeny (Hancock et al., 2002). Such SD genotypes were not expected from SD x SD crosses under the single, dominant gene model.

In the literature reporting the genetics of the everbearing trait in strawberries, only the latest paper from the University of California-Davis suggests that multiple cropping is

regulated by a single dominant allele at a single nuclear gene locus (Ahmadi et al., 1990). The other studies do not always distinguish between DN and LD sources of the everbearing trait, but they uncover a wide array of segregation patterns that did not fit a single, dominant gene model (Hancock, 1999). The segregating populations that Bringhurst's group used to test their genetic hypothesis must have differed from all the others in that it contained a unique source of DN from them or it was fixed for the other quantitative trait loci (QTL) associated with photoperiod sensitivity. All their DN parents must have been heterozygous.

In this study with a broader range of germplasm, the inheritance of DN appeared quantitative with quite variable numbers of DN being produced from the various parental combinations. Similar results were observed in another study, when crosses between DN *F.* ×*ananassa* and DN *F. virginiana* fit a 3:1 ratio in only 3 out of 10 combinations (Hancock et al., 2002). When Barritt *et al.* (1982) tested the genetics of day-neutrality using the eastern genotypes, they also obtained complex ratios, as did Powers (1954) when he examined segregation ratios in a population that contained DN genes from both *F.* ×*ananassa* 'Pan American' and *F. virginiana* ssp. *glauca*. Similar to results of present study, Powers found continuous variation in % DN progeny and suggested two or more dominant genes and at least four recessive genes regulating expression of day-neutrality. Powers also suggested that the effects of dominant alleles are not equal and the effects of the genes are cumulative.

It is possible that some of the variation we recovered among families for percent DN progeny was created by the genomic structure of strawberry. The single, dominant gene model was tested assuming complete diploidization of strawberry genome;

however, the pairing relationship octoploid strawberries have not been completely resolved. Strawberry is considered to be an autopolyploid (Hancock, 1999) so variation in meiotic configurations of chromosomes might be expected, resulting in occasional aberrant segregation ratios. Arulsekar and Bringhurst (1981) used isozyme data to document disomic inheritance in strawberries, but they only evaluated a few loci.

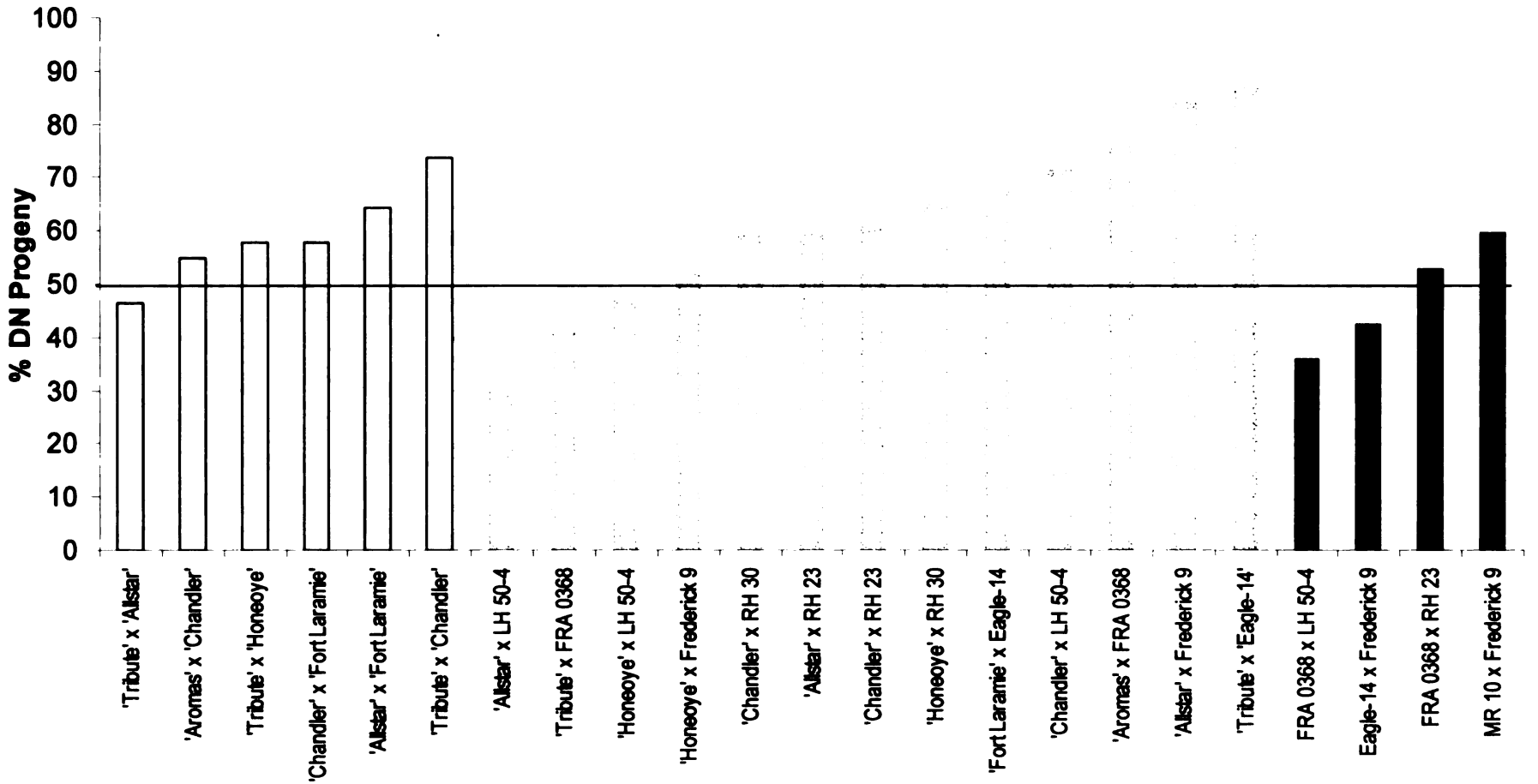
In conclusion, the results indicate that the genetics of day-neutrality is quantitative in strawberry. The numerous investigators who obtained different segregation ratios were probably studying different QTLs controlling day-neutrality and in many instances were using different evaluation methods. It is recommended that quantitative approaches be utilized in breeding day-neutral strawberries. To produce families with the highest proportion of DN progeny, parents should be selected which carry the highest number of QTL for DN.

Table 14. Proportion of short day (SD) and day-neutral (DN) progeny generated in SD x DN crosses. Chi-square tests were made assuming DN was regulated by a single dominant gene (1:1). The families were grown in a completely randomized design at the Michigan State University Horticulture Farm, East Lansing, Mich. in 2002.

Family	SD	DN	Total	%DN	χ^2	*P*
F. ×ananassa* x *F. ×ananassa						
'Tribute' x 'Allstar'	46	40	86	0.47	0.4	0.518
'Aromas' x 'Chandler'	24	29	53	0.55	0.5	0.492
'Tribute' x 'Honeoye'	30	41	71	0.58	1.7	0.192
'Chandler' x 'Fort Laramie'	30	41	71	0.58	1.7	0.192
'Allstar' x 'Fort Laramie'	19	34	53	0.64	4.2	**0.039**[1]
'Tribute' x 'Chandler'	19	53	72	0.74	16.1	**0.000**
Total	168	238	406	0.59	12.1	**0.001**
F. ×ananassa* x *F. virginiana						
'Allstar' x LH 50-4	62	27	89	0.30	13.8	**0.000**
'Tribute' x FRA 0368	17	12	29	0.41	0.9	0.353
'Honeoye' x LH 50-4	38	34	72	0.47	0.2	0.637
'Honeoye' x Frederick 9	26	28	54	0.52	0.1	0.785
'Chandler' x RH 30	36	53	89	0.60	3.2	0.072
'Allstar' x RH 23	21	31	52	0.60	1.9	0.166
'Chandler' x RH 23	14	22	36	0.61	1.8	0.182
'Honeoye' x RH 30	25	47	72	0.65	6.7	**0.010**
'Fort Laramie' x Eagle-14	23	48	71	0.68	8.8	**0.003**
'Chandler' x LH 50-4	20	51	71	0.72	13.5	**0.000**
'Aromas' x FRA 0368	13	41	54	0.76	14.5	**0.000**
'Allstar' x Frederick 9	11	60	71	0.85	33.8	**0.000**
'Tribute' x Eagle-14	7	47	54	0.87	29.6	**0.000**
Total	313	501	814	0.62	43.4	**0.000**
F. virginiana* x *F. virginiana						
FRA 0368 x LH 50-4	46	26	72	0.36	5.6	**0.018**
Eagle-14 x Frederick 9	31	23	54	0.43	1.2	0.276
FRA 0368 x RH 23	25	28	53	0.53	0.2	0.680
Montreal River 10 x Frederick 9	29	43	72	0.60	2.7	0.099
Total	131	120	251	0.48	0.5	0.487
Grand total	612	859	1471	0.58	41.5	**0.000**

[1]P values indicating significant variation from a 1:1 model, at 0.05, are bolded.

Figure 11. Percentage of day-neutral progeny in strawberry families of DN x SD crosses grown in a completely randomized design at the Michigan State University Horticulture Farm, East Lansing, Mich. in 2002. The white column represents *F. ×ananassa* x *F. ×ananassa*, while gray and black columns represent *F. ×ananassa* x *F. virginiana* and *F. virginiana* x *F. virginiana* crosses. The line represents the expected percentage assuming day-neutrality was regulated by a single dominant gene.



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'Honeoye' x LH 50-4	38	34	72	0.47	0.2	0.637
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'Honeoye' x RH 30	25	47	72	0.65	6.7	**0.010**
'Fort Laramie' x Eagle-14	23	48	71	0.68	8.8	**0.003**
'Chandler' x LH 50-4	20	51	71	0.72	13.5	**0.000**
'Aromas' x FRA 0368	13	41	54	0.76	14.5	**0.000**
'Allstar' x Frederick 9	11	60	71	0.85	33.8	**0.000**
'Tribute' x Eagle-14	7	47	54	0.87	29.6	**0.000**
Total	313	501	814	0.62	43.4	**0.000**
F. virginiana* x *F. virginiana						
FRA 0368 x LH 50-4	46	26	72	0.36	5.6	**0.018**
Eagle-14 x Frederick 9	31	23	54	0.43	1.2	0.276
FRA 0368 x RH 23	25	28	53	0.53	0.2	0.680
Montreal River 10 x Frederick 9	29	43	72	0.60	2.7	0.099
Total	131	120	251	0.48	0.5	0.487
Grand total	612	859	1471	0.58	41.5	**0.000**

[1]P values indicating significant variation from a 1:1 model, at 0.05, are bolded.

Figure 12. Percentage day-neutral progeny in strawberry families of DN x DN crosses grown in a completely randomized design at the Michigan State University Horticulture Farm, East Lansing, Mich. in 2002. The white column represents *F.* ×*ananassa* x *F.* ×*ananassa*, while gray and black columns represent *F.* ×*ananassa* x *F. virginiana* and *F. virginiana* x *F. virginiana* crosses. The line represents the expected percentage assuming day-neutrality was regulated by a single dominant gene.

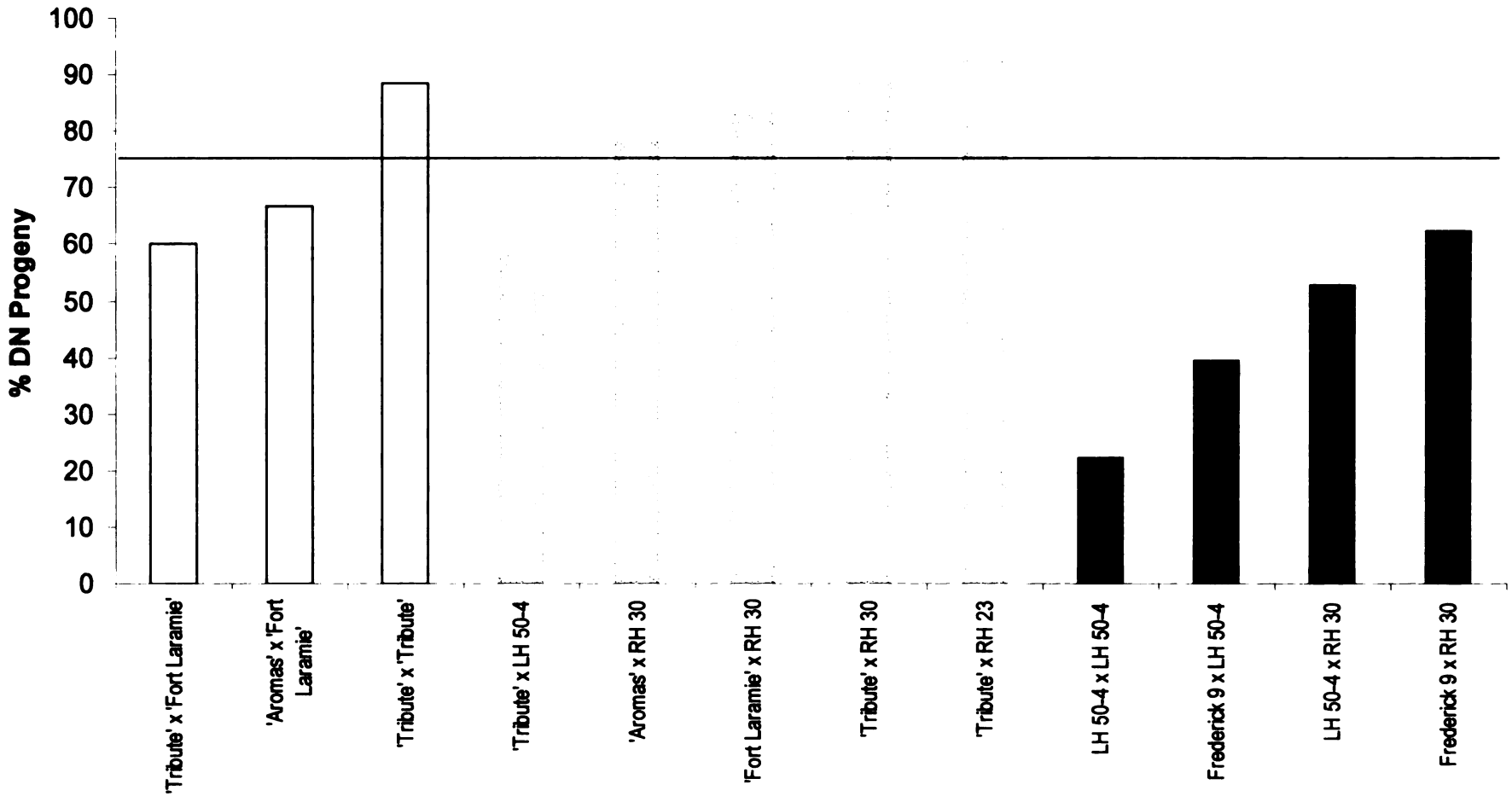
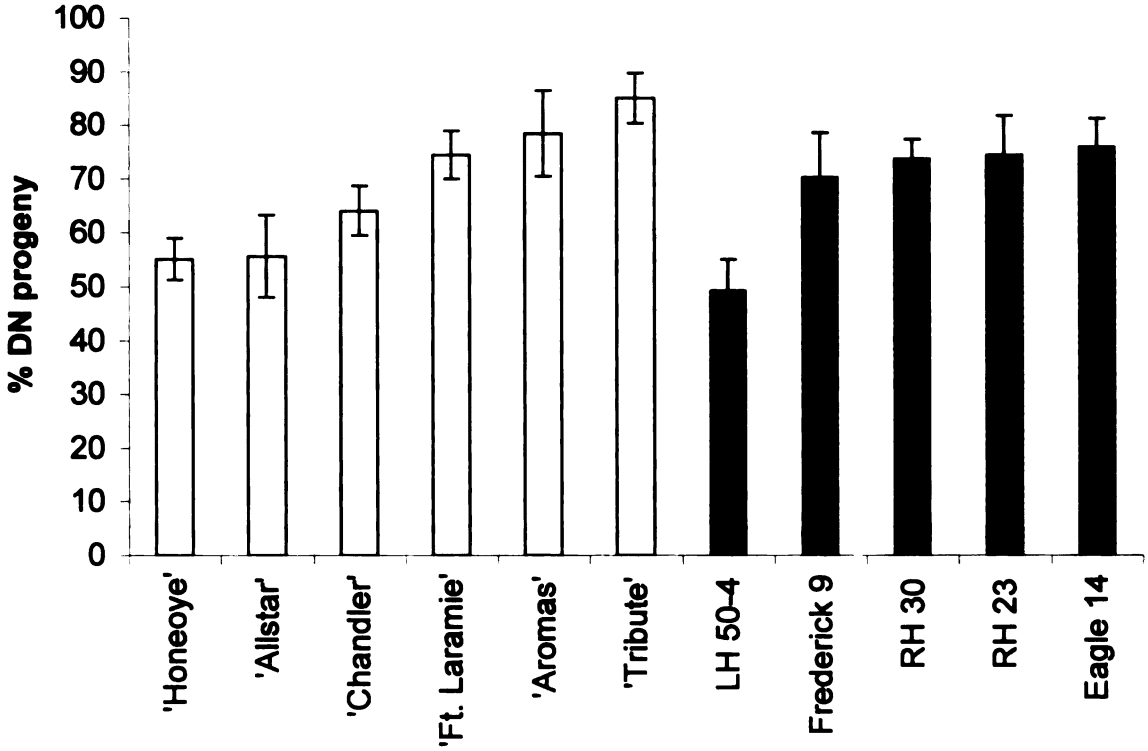


Table 16. General and specific combining ability (GCA and SCA) for percent day-neutral progeny in *F.* ×*ananassa* x *F. virginiana* families grown in a completely randomized design at the Michigan State University Horticulture Farm, East Lansing, Mich. in 2002.

Source	df	Mean squares	F value	*P*	Variance component and percentage
GCA (*F.* ×*ananassa*)	5	928.5	5.55	**0.001**[1]	107.2 (22%)
GCA (*F. virginiana*)	4	993.7	5.94	**0.001**	100.3 (20%)
SCA	6	914.4	5.47	**0.000**	165.1 (34%)
Error	42	167.2			117.7 (24%)

[1]Significant P values, at 0.05, are bolded.

Figure 13. Percentage of day-neutral progeny in strawberry families grown in a completely randomized design at the Michigan State University Horticulture Farm, East Lansing, Mich. in 2002. The white column represents *F.* ×*ananassa* cultivars, while black columns represent *F. virginiana* genotypes. The bars represent standard errors.



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