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Identifying New Sources of Genes that Determine Cyclic Flowering in Rocky Mountain Populations of *Fragaria virginiana* ssp. *glauca* Staudt

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ABSTRACT. The genes that determine cyclic flowering in all commercially grown cultivars of strawberry (*Fragaria ×ananassa* Duch.) were derived from a single source of *F. virginiana* ssp. *glauca* from the Wasatch Mountains in Utah. To broaden the germplasm base of cyclic flowering cultivars, we evaluated the reproductive characteristics of 5 to 10 colonies of *F. virginiana* ssp. *glauca* from each of 32 Rocky Mountain sites ranging in elevation from 700 to 2900 m. Populations at high and low elevations had high percentages of putative day neutrals with cyclic flowering (43% to 100%) and hermaphrodites (20% to 80%), although most hermaphrodites were only partially fertile. There was also little association between elevation and crown numbers or flower number per cycle, but the total number of flowers per plant was negatively correlated with elevation. Fruit size was not significantly correlated with fruit number. When the data were subjected to a principal component analysis, two distinct groups were identified: one from the Black Hills of South Dakota and the other from low-elevation sites in Idaho and northwestern Montana. These patterns mirrored previously described patterns based on leaf traits.

The introduction of commercially acceptable cyclic flowering cultivars over the last 15 years has had a major impact on strawberry production trends. Whereas strawberry production was once highly seasonal, it is now possible to produce multiple crops across most temperate climates. More than 60% of the hectarage in California is now devoted to cyclic day-neutral flowering types, and regionally adapted day neutrals are available in most other North American locations. Developing new multiple-cropping cultivars has become an important objective of breeding programs throughout the world (Hancock and Simpson, 1995; Hancock et al., 1996).

Genes for cyclic flowering in octoploid strawberry cultivars were derived from three independent sources (Hancock et al., 1990, 1995). First, European cultivars were derived from the 'Gloede' seedling introduced in France in 1866. The second source was a chance seedling or clonal mutation of 'Bismarck' found in New York by S. Cooper in 1898. Most recently, day-neutral cultivars have been derived from native clones of *F. virginiana* ssp. *glauca* (Bringhurst and Voth, 1984; Powers, 1945). In fact, all modern day-neutral cultivars trace to a single selection of *F. virginiana* ssp. *glauca* from the Wasatch Mountains of Utah collected by Bringhurst (Hancock et al., 1995).

While the Wasatch Mountain clone has been used to develop a broad range of cultivars, other *F. virginiana* clones may possess additional useful genes (Hancock et al., 1990; Luby et al., 1991, 1992). Darrow (1966) indicated that *F. virginiana* was a potential source of drought tolerance, resistance to mildew [*Sphaerotheca macularis* (Wallr. ex Fr.) Jacz.], red stele root rot (*Phytophthora fragariae* Hickman), and root knot nematode (*Meloidogyne* ssp.). Powers (1945) observed that Rocky Mountain clones of *F. virginiana* had considerable winter hardiness, tolerance of blossoms to frost, early maturity, and good quality. It has also been suggested that some clones of *F. virginiana* have high photosynthetic heat tolerance (Caldwell et al., 1990; Hancock et al., 1995).

The purposes of this study were to 1) screen Rocky Mountain clones of *F. virginiana* for cyclic flowering and several other critical yield components and 2) determine if there are key geographical or elevational characteristics that are strongly associated with cyclic flowering and yield. The overall intent of the work was to identify useful breeding parents and design a strategy for future germplasm collection.

Materials and Methods

Six to twenty-seven of the original *F. virginiana* clones collected by Luby and Hancock (Luby et al., 1992) from 32 sites in the Rocky Mountains and Great Plains states (Luby et al., 1992; Table 1) were planted in 10 × 10 × 10-cm plastic pots containing sandy loam and placed in a unheated greenhouse without lights at Michigan State Univ., E. Lansing. All plants were adults with five to seven leaves and well-developed root systems. They were field collected in July and August, when night temperatures were approaching freezing, and were stored in ice until planting, 2 to 4 weeks later. Detailed information about characteristics of collection sites is given in Luby et al. (1992). Plants were irrigated with tap water and fertilized as needed. Placement was initially random and they were shifted every 4 months. Greenhouse temperatures fluctuated from 25 to 35 °C during summer and 5 to 15 °C during winter. Light levels varied from 750 to 1300 $\mu mol \cdot s^{-1} \cdot m^{-2}$ during summer and 85 to 225 $\mu mol \cdot s^{-1} \cdot m^{-2}$ during winter.

The plants were maintained in the greenhouse for 2.5 years. From January to December in the second year, data were gathered for several plant characteristics (Table 2). The number of flowering cycles was recorded for each genotype by counting the number of discrete bloom and fruiting periods. For most plants, these cycles were clearly defined. During each flowering cycle, the date of the first flower of each individual plant was recorded, along with total number of flowers generated in each cycle and the width of the first flower that opened. The gender of each plant was also

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Table 1. Location and number of individuals measured for each collection site.

Population	Individuals (no.)	Locale	State	Elevation (m)	Latitude (°N)	Longitude (°W)
LH 3	21	Black Hills	SD	1760	43 40	103 30
LH 4	13	Black Hills	SD	1380	43 30	103 20
LH 5	17	Black Hills	SD	1550	44 10	103 40
LH 6	21	Black Hills	SD	1840	44 20	103 50
LH 7	15	Black Hills	SD	1980	44 30	103 50
LH 8	7	Bighorn	WY	2210	44 40	108 30
LH 9	13	Bighorn	WY	2650	44 30	108 30
LH10	8	Bighorn	WY	1860	44 30	108 40
LH13	24	Shoshone	WY	1980	44 30	109 40
LH14	17	Shoshone	WY	2160	44 40	109 40
LH15	14	Shoshone	WY	2470	44 50	109 40
LH17	13	Shoshone	WY	2900	45 00	109 30
LH22	27	Beaverhead	MT	1670	45 20	111 30
LH24	22	Beaverhead	MT	2160	45 30	113 20
LH26	19	Bitteroot	MT	1980	45 30	114 20
LH28	8	Bitteroot	MT	1460	45 50	114 20
LH29	10	Clearwater	MT	1610	46 40	114 30
LH30	16	Lolo	MT	1060	46 50	114 20
LH32	20	Lolo	MT	820	47 20	115 10
LH34	13	Couer d'Alene	ID	790	47 30	116 20
LH35	7	Couer d'Alene	ID	730	47 50	116 40
LH36	15	Kaniksu	ID	700	48 40	116 50
LH37	4	Couer d'Alene	ID	1580	47 30	115 40
LH38	10	Kootenai	MT	730	47 50	115 40
LH39	18	Kootenai	MT	1065	48 00	115 20
LH40	17	Flathead	MT	1340	48 30	114 10
LH42	6	Flathead	MT	1800	48 40	114 30
LH43	19	Flathead	MT	1465	48 50	114 40
LH44	20	Kootenai	MT	975	48 40	114 50
LH45	19	Glacier	MT	2075	48 40	113 40
LH46	14	Flathead	MT	915	48 00	113 50
LH47	20	Lolo	MT	1220	47 10	113 30
LH48	16	Helena	MT	1870	47 00	112 20
LH49	19	Helena	MT	1430	46 20	111 10
LH50	13	Lewis & Clark	MT	2255	46 40	110 40
LH51	25	Gallatin	MT	1965	46 00	110 20
LH52	10	Gallatin	MT	1615	45 20	110 10

Table 2. Traits measured on Rocky Mountain populations of *F. virginiana* ssp. *glauca*.

Designation	Description
Cyclic flowering (%)	Percentage of cyclic flowering plants in each population
Flowering cycles	Mean number of flowering cycles per plant[z]
Flower number	Mean number of flowers per flowering cycle per plant
Crown number	Mean number of crowns per plant[y]
Hermaprodite fertility	Mean proportion of achenes that developed normally on hermaphodite plants[x]
Hermaprodites (%)	Percentage of hermaprodite individuals in each population
Flower width	Mean width of the first flower of each plant at widest point (mm)
Fruit length	Mean length of the first 5 fruit per plant from calyx to tip (mm)
Fruit width	Mean width of the first 5 fruit per plant at widest horizontal point (mm)
Fruit weight	Mean weight of the first 5 primary fruit (g) per plant

[z]Counted from January to December.
[y]Measured in December.
[x]1 = 0% to 20%, 5 = 80% to 100%.

Table 3. Reproductive characteristics of Rocky Mountain populations of *Fragaria virginiana* ssp. *glauca*.

Population	Cyclic flowering (%)	Flowering cycles	Flowers (no.)	Crowns (no.)	Flower width (mm)	Hermaphrodites (%)	Fertility of hermaphrodites	Fruit length (mm)	Fruit width (mm)	Fruit wt (mg)
LH3	100.0	2.4	13.1	2.6	16.4	41	1.1	10.8	9.0	310
LH4	84.6	2.4	19.7	3.6	13.1	43	2.0	10.9	10.6	510
LH5	64.7	2.1	17.0	2.9	16.2	25	2.0	11.5	11.5	510
LH6	71.4	2.2	17.3	2.8	15.8	41	1.6	10.4	10.4	500
LH7	66.7	1.9	11.3	2.5	16.5	36	1.0	11.3	9.4	340
LH8	62.5	2.3	16.0	3.4	15.0	20	2.0	17.0	9.0	290
LH9	62.5	2.5	15.3	3.7	15.7	50	1.8	12.4	10.2	480
LH10	60.0	2.6	17.2	2.9	16.3	66	3.5	10.8	10.0	450
LH13	62.5	2.4	15.3	3.2	15.5	40	2.2	10.3	10.4	450
LH14	76.5	2.4	13.8	3.7	16.3	61	1.6	11.2	10.8	520
LH15	73.3	2.5	16.3	2.4	19.4	45	1.4	12.9	11.3	510
LH17	76.9	2.3	15.1	2.4	18.6	20	1.0	10.8	9.2	410
LH22	63.0	2.4	15.0	3.3	17.4	31	1.6	9.6	9.6	390
LH24	72.7	2.4	18.5	4.8	15.9	50	2.0	11.0	9.1	390
LH26	89.5	2.2	17.3	3.7	16.4	20	2.0	10.9	9.5	470
LH29	60.0	2.3	19.9	4.2	16.4	57	2.3	8.6	9.9	290
LH30	87.5	2.6	20.4	3.5	18.6	66	2.3	10.4	9.1	400
LH32	70.0	2.2	21.1	3.2	18.6	80	1.9	11.1	9.1	430
LH34	84.6	2.9	26.3	4.4	17.5	75	2.1	10.7	9.4	430
LH35	100.0	2.3	21.8	3.5	22.6	70	2.7	10.8	10.1	480
LH36	80.0	2.4	20.3	2.8	16.9	58	2.1	9.8	8.9	360
LH37	100.0	2.1	16.7	3.7	17.4	25	1.0	11.6	9.5	460
LH38	90.0	2.4	25.8	3.9	18.4	70	2.4	10.8	9.0	330
LH39	57.9	2.2	17.4	3.6	19.4	53	3.0	12.4	9.9	520
LH40	82.3	2.5	22.6	4.1	17.9	33	2.0	15.2	10.6	900
LH43	73.7	2.2	19.8	3.7	17.5	47	2.0	12.1	10.6	510
LH44	85.0	2.3	18.8	4.0	15.5	30	2.0	12.2	10.2	550
LH46	100.0	2.2	18.1	4.5	18.6	47	1.7	11.2	8.8	400
LH47	75.0	2.1	19.0	3.9	18.2	31	1.8	11.5	9.3	390
LH48	43.8	1.9	19.9	3.8	15.3	25	1.0	10.7	9.0	390
LH49	73.7	2.3	20.3	3.9	18.2	40	2.3	11.2	9.5	420
LH51	84	2.2	19.3	3.5	16.3	45	2.0	10.2	9.5	500
LSD	---	0.5	3.5	0.9	2.1	---	1.3	3.2	1.3	180

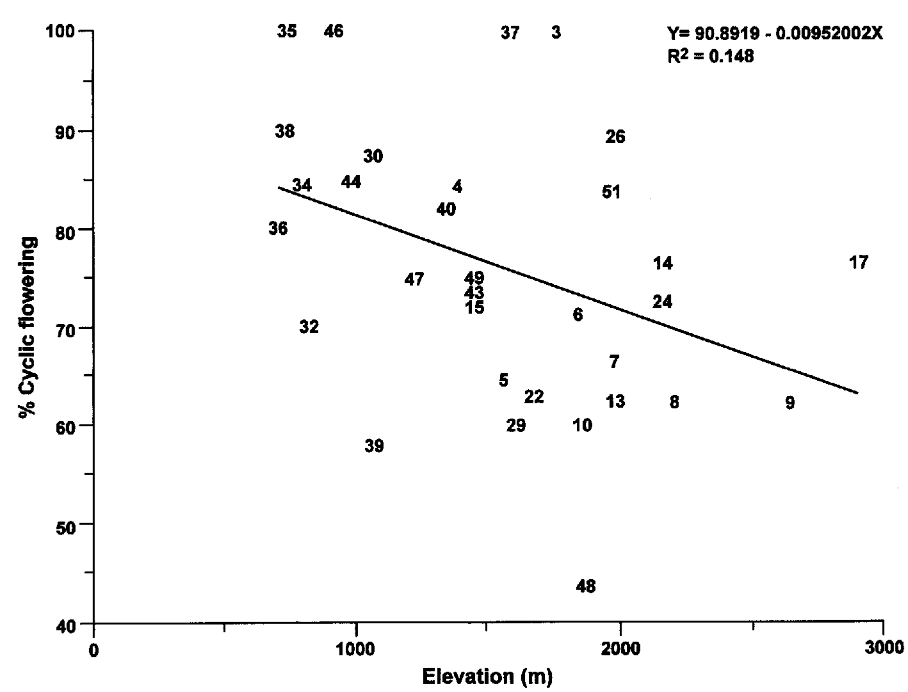

Fig. 1. Relationship between elevation and the percentage of cyclic flowering individuals in Rocky Mountain populations of *Fragaria virginiana*.

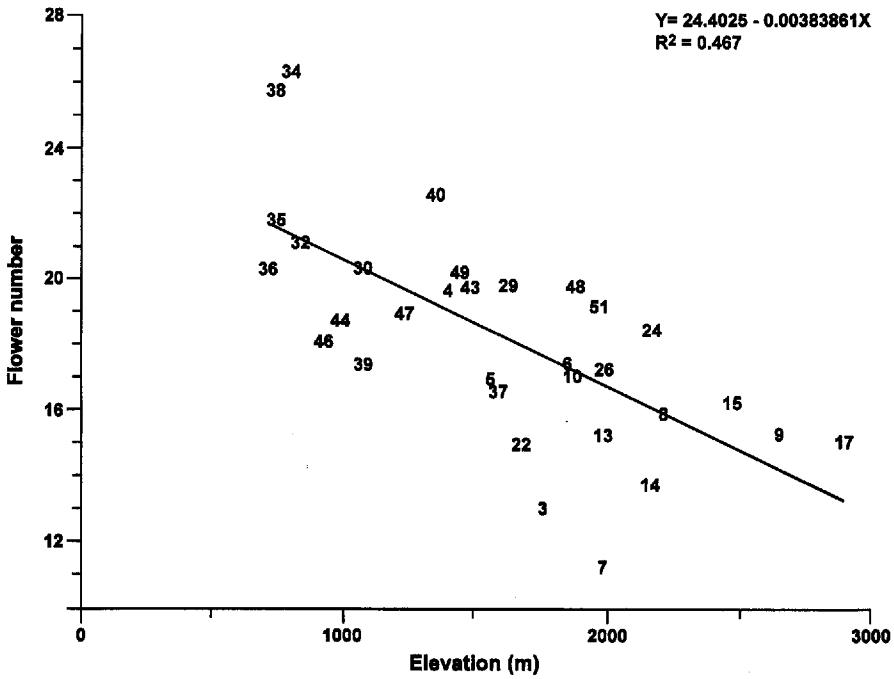

Fig. 2. Relationship between elevation and mean flower number in Rocky Mountain populations of *Fragaria virginiana.*

determined by the presence or absence of anthers and whether fruit were produced after hand pollination.

All open flowers of each genotype were pollinated with a camel hair brush at 3- to 4-d intervals with composites of pollen gathered from individuals from the same site. The fertility of hermaphrodites was rated on a 1 to 5 scale as the average proportion of achenes that developed normally on all the open flowers of each genotype: 1 = 0% to 20% and 5 = 80% to 100%. The first five fully red berries were harvested from each plant at 5- to 7-d intervals and weighed and measured for length from calyx to tip (mm) and width at the widest horizontal point (mm). Crowns were counted on all genotypes in December.

Runners were not counted, as few stolons were produced during the course of this study. Runner formation may have been restricted by the continual cyclic production of flowers in our greenhouse, where temperatures averaged above freezing even during the coolest months of winter. In field plantings, we have observed runners on Rocky Mountain clones, although stolon numbers were limited compared to typical short-day plants.

Analysis of variance and LSD comparisons were performed using SAS (SAS Institute, Cary, N.C.). Principal component analysis was done on the means of each characteristic within each population using the FACTOR procedure of the same program.

Results and Discussion

All of the measured traits varied significantly across populations (Table 3). The average number of flowering cycles ranged from 1.9 to 2.9, while the percentage of multiple-cropping individuals in each population varied from 43% to 100%. The highest number of flowering cycles was observed in LH 34 (Cour d'Alene), LH30 (Lolo), and LH10 (Bighorn). There was not a significant correlation between elevation and percentage of cyclic flowering plants or number of flowering cycles, although low-elevation sites tended to have more cyclic flowering plants than high-elevation sites (Fig. 1).

The most productive clones appeared to originate from low elevations. There was little association between elevation and crown number or flower number per cycle; however, total number of flowers was negatively correlated with elevation (Fig. 2). LH 34 (Cour d'Alene) and LH 38 (Kootenai) had the most flowers per cycle (Table 3). Hermaphrodite percentage and fertility also tended to be higher at low elevation, although the trend was not significant (data not shown). The highest percentage of hermaphrodites was found in populations LH 32 from Lolo (10%) and LH 34 from Cour d'Alene (75%). The most fertile hermaphrodites were found in LH 10 (Bighorn). No apparent shifts in gender occurred during the course of the study.

Fruit weight was not significantly correlated with flower or crown number (Table 4). This suggests that large-fruited breeding parents that have other desirable characteristics can be selected. Site LH 40 (Flathead) plants had unusually large mean fruit weight (0.9 g) for *F. virginiana* ssp. *glauca* and still had the third highest number of flowers (Fig. 3, Table 3). Flower size was not significantly correlated with fruit weight (Table 4), so it cannot be used to predict fruit weights.

Four principal components (PCs) explained 72% of the total

Table 4. Correlation between various reproductive traits in Rocky Mountain populations of *F. virginiana* ssp. *glauca*. Significant relationship at $P<0.05$ are bold.

	Cyclic flowering (%)	Flowering cycles	Flowers (no.)	Crowns (no.)	Flower width	Hermaphrodites (%)	Fertility of hermaphrodites	Fruit length	Fruit width
Cyclic flowering	---								
Flowering cycles	0.18								
Flower number	0.23	0.34							
Crown number	0.14	0. 10	**0.53**						
Flower width	0.33	0.09	0.29	–0.02					
Hermaphrodites (%)	0.14	**0.49**	**0.49**	0.15	0.37				
Hermaphrodite fertility	–0.09	**0.40**	**0.44**	0.24	0.19	**0.52**			
Fruit length	–0.06	0.01	–0.07	0.04	–0.03	–0.33	–0.02		
Fruit width	–0.20	0.07	–0.16	–0.21	–0.11	–0.08	0.12	0.15	
Fruit weight	0.09	0.12	0.17	0.12	0.08	–0.10	0.13	**0.38**	**0.6**

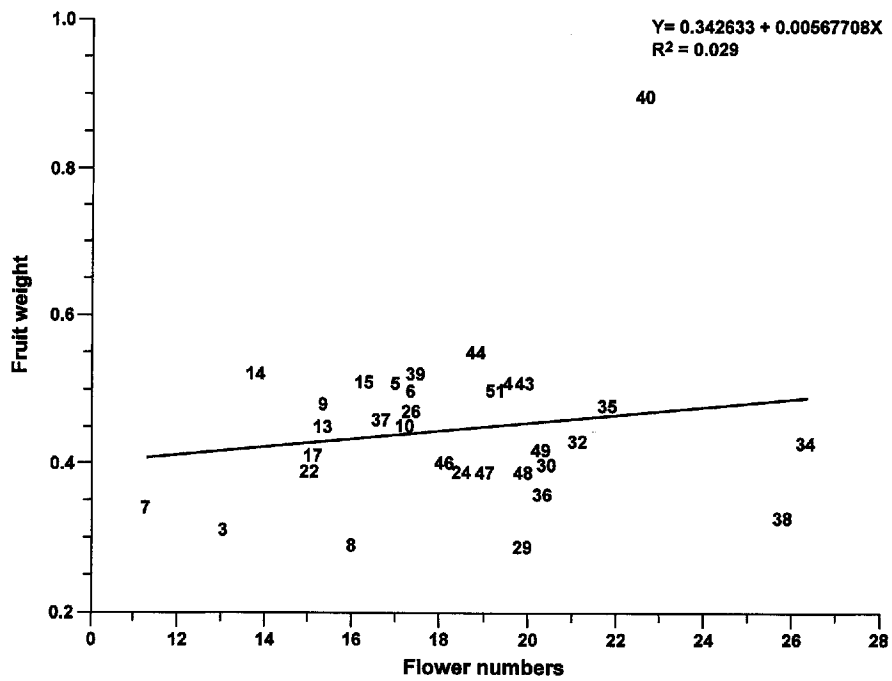

Fig. 3. Relationship between flower numbers and mean fruit weight in Rocky Mountain populations of *Fragaria virginiana*.

variance among the 32 population means (Table 5). PC1 accounted for 28% of the variation and appeared to be a multivariate measurement of hermaphrodite percentage, hermaphrodite fertility, and several flower characteristics (flower number, flower width, and flowering cycle). Fruit characteristics (fruit weight, fruit length, and fruit width) contributed to most of the variation in PC2 (19% of the total variation). The variation in PC3 stemmed primarily from crown number and the percentage of multiple croppers (13% of the total variation), while flower width was the biggest contributor to variation in PC4 (12% of the variation). PC1 showed a significant association with elevation (Fig. 4), while the other PCs did not (data not shown).

There were two groups of collections distinguished by relating PC1 to geographical location (Fig. 5). One group included collections from the southeastern extent of the Rocky Mountain flora and the Black Hills (South Dakota), which had negative values for PC1. The other group represented collections from low-elevation sites in Idaho and northwestern Montana. The Black Hills populations tended to have the fewest flowers, flowering cycles, and fertile hermaphrodites, while the low-elevation Idaho and Montana populations had the most. These patterns of variability in reproductive traits mirror those previously reported for leaf morphological traits in the same populations (Hokanson et al., 1993). Obvious clustering of populations by elevation or latitude plus longitude were not observed with PCs 2, 3, or 4 (data not shown).

Based on these observations, the best place to find new fruitful, cyclic flowering genotypes is probably at low elevations. These sites have about the same proportion of multiple-cropping genotypes as high-elevation sites, but they have plants with more flowers, larger fruit, and a higher proportion of fertile hermaphrodites. The relative rankings of these characteristics among genotypes has held constant in preliminary field trials (data not shown). Overall, low-elevation sites in Idaho and northwestern Montana may be the most productive source of multiple-cropping types, since individuals from these sites tended to have many flowers, multiple flowering cycles, and high hermaphrodite fertility. Finding breeding parents with large fruit and many flowers should be possible, as the largest-fruited population (LH 40) also had many fruit (Fig. 3).

While we have not examined the biotic and abiotic tolerances of the low-elevation populations, they should be a rich source of adaptive genes. The sites in Idaho and northwestern Montana represent a wide range of environments including mature forests, gravel roadsides, and clearcuts (Luby et al., 1992). Slopes varied from flat to >20%. Soils ranged from deep, moist red clays to gravelly, alluvial and deep sandy loams. While the high-elevation sites may be a better source of genes for resistance to temperature extremes (Hancock et al., 1995), the low-elevation sites still contain sufficient variability to dramatically expand the current day-neutral breeding pool of multiple-cropping types.

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Table 5. Coefficients of the eigenvectors, eigenvalues, and percent of total variance for each of the first five principal components (PCs).

	Eigenvector coefficients				
Variable	PC 1	PC 2	PC 3	PC 4	PC 5
XI—Cyclic flowering (%)	0.21	–0.11	0.52	0.44	–0.44
X2—Flowering cycles	0.37	0.14	–0.22	0.07	–0.55
X3—Flower No.	0.48	0.03	0.20	–0.22	0.8
X4—Crown No.	0.29	0.02	0.40	–0.58	–0.03
X5—Flower width	0.29	–0.05	0.18	0.56	0.58
X6—Hermaphrodite (%)	0.48	–0.12	–0.33	0.12	0.02
X7—Hermaphrodite fertility	0.41	0.17	–0.33	–0.18	0.28
X8—Fruit Length	–0.10	0.43	0.35	–0.10	0.24
X9—Fruit width	–0.08	0.58	–0.29	0.17	–0.10
X10—Fruit weight	0.07	0.63	0.20	0.11	–0.05
Eigenvalues	2.77	1.87	1.31	1.19	0.8
Percentage of total variance	28	19	13	12	8

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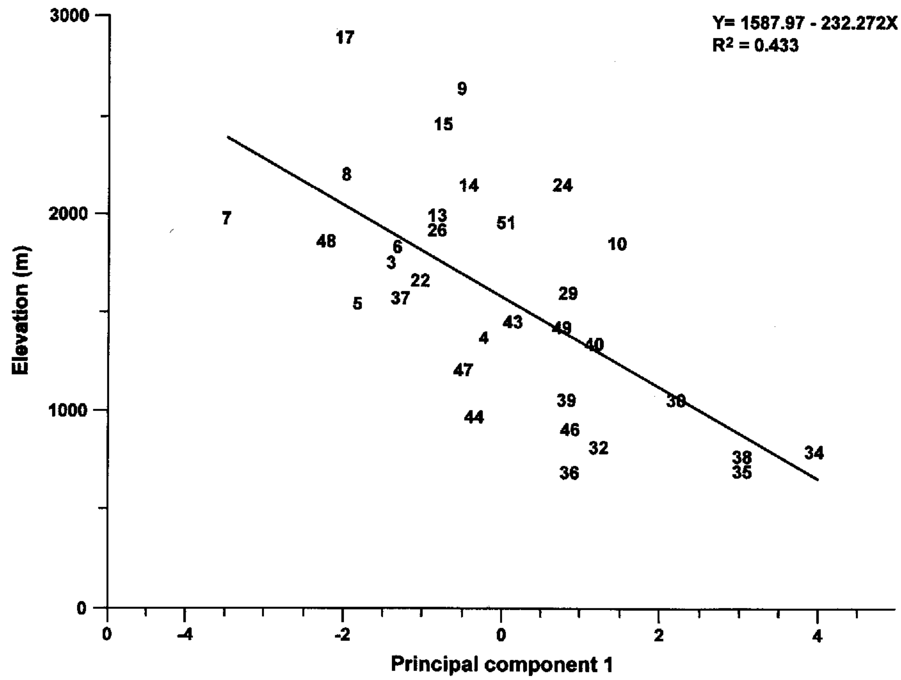

Fig. 4. Relationship between the first principle component (PC1) coordinate and elevation in Rocky Mountain populations of *Fragaria virginiana*.

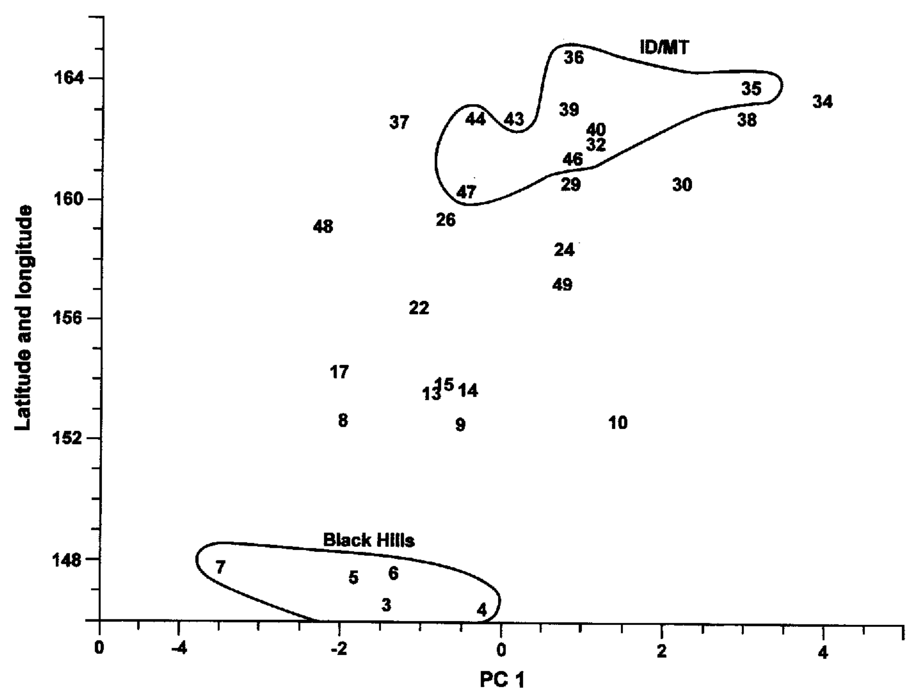

Fig. 5. Relationship of first principle component (PC1) coordinate with the geographical cline of the collection sites from southeast to northwest (represented as the sum of latitude and longitude).