Bingham

A Comparison of Pollinator Limitation, Self-compatibility, and Inbreeding Depression in Populations of *Campanula rotundifolia* L. (Campanulaceae) at Elevational Extremes

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DRAFT

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Abstract

Campanula rotundifolia is a widespread herbaceous perennial that exists along an elevational gradient in Colorado. Alpine populations of this and other insect pollinated species may suffer from pollinator limitation due to the harsh environment of high elevations. Limited gene flow due to low levels of pollinator activity may increase rates of selfing and biparental inbreeding in alpine populations. This could result in highly inbred populations that have been purged of deleterious recessive alleles and, therefore, may exhibit less inbreeding depression than primarily outcrossed populations of the same species from lower elevations. We determined the levels of inbreeding depression and self-compatibility in high and low-elevation populations of *C. rotundifolia.* Hand self- and outcross-pollinations were performed on plants at high and low elevations. Seed set was determined to assess levels of self-compatibility. Seeds were weighed, germinated, and grown in a common garden to determine levels of inbreeding depression. Plants in all populations were found to be self-compatible, and capable of autogamous seed set. Although visitation rates in the high-elevation population were significantly lower that in low elevation populations, there was no evidence for pollinator limitation in alpine populations. Low-elevation seedlings exhibited significant inbreeding depression for all parameters measured, while the high-elevation seedlings did not.

No management recommendations are indicated.

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Introduction

Plants exhibit a variety of mating systems ranging from those that are obligate outcrossers to those that are predominately selfing and with some displaying mixed mating systems. Levels of outcrossing and the degree of self-compatibility exhibited by species vary among populations of the same species as well as among different species (Barrett and Eckert, 1990). Darwin was among the first to suggest that floral mechanisms promoting cross-pollination might have the selective advantage of avoiding inbreeding depression, and much of the early work on the evolution of plant mating systems focused on the avoidance of inbreeding (Darwin, 1876). Theoretical developments in the field included Fisher's (1941) demonstration that an individual capable of self-pollination experiences a selective advantage over strict outcrossers, in that they contribute one genome to the next generation through each successful pollen grain and two through each successful seed, while an outcrossing individual contributes one through pollen, but only one through each seed. Since then, much of the research on the evolution of plant mating systems has dealt with questions regarding the maintenance of outcrossing in the face of Fisher's "automatic selection" for self-pollination.

One explanation for the maintenance of outcrossing that has received a great deal of attention is based on the observation that offspring produced from matings between close relatives exhibit inbreeding depression. If inbreeding depression results from the expression of deleterious recessive alleles in homozygotes, predominant selfing or predominant outcrossing may exist as alternative stable states of the mating system (Lande and Schemske, 1985). According to this theory, selection acts to maintain outcrossing in historically large populations that experience high levels of inbreeding depression, while populations with a history of population bottlenecks or pollinator failure are selected for self-pollination. Self-pollination in the latter populations evolves due to a reduction in inbreeding depression as deleterious recessive alleles are purged from the population.

Reproductive assurance may also contribute to the evolution of self-pollination (Lloyd, 1979). Self-compatibility may evolve in self-incompatible species due to the selective pressure of pollen-limited seed set (Stebbins, 1957; Wyatt, 1983; Richards, 1986). Pollinator limitation is likely in habitats where animal pollinators are rare (Spears, 1987), during periods of inclement weather (Schemske et al., 1978), or when plant species compete for pollinator service (Levin, 1972). There are many examples of species in which both self-compatible and self-incompatible populations exist, and changes in pollinator availability have been implicated in the shift toward self-pollination in some of these cases (Solbrig and Rollins, 1977; Inoue and Amano, 1986; Inoue, 1988).

Pollinator activity and diversity decline with increasing elevation, and unreliable pollination is widely accepted as one reason for the high frequency of asexual reproduction and self-pollination in plant populations at high latitudes and altitudes (Billings, 1974). Species occurring along elevational gradients consist of populations experiencing different pollinator environments and may, therefore, vary for levels of self-compatibility and inbreeding depression. **This study is the first to explicitly test these predictions by investigating levels of self-compatibility and inbreeding depression in populations of the same species occurring at elevational extremes.**

Using the widespread species *Campanula rotundifolia*, this research addresses the following questions:

1.) Do high elevation populations exhibit greater self-compatibility than low elevation populations?

2.) Do seedlings produced from self-pollinations in alpine populations exhibit less inbreeding depression than those from low elevation populations?

Methods

Study organism

Campanula rotundifolia is a widespread herbaceous perennial with a circumpolar distribution. In North America it occurs from the eastern arctic of Canada south into the mountains of Central Mexico. Along the Front Range of Colorado *C. rotundifolia* has a broad elevational distribution extending from 1500m in the foothills to over 3000m in the alpine tundra.

C. rotundifolia is insect-pollinated, relying almost exclusively on bees for pollination (Shetler, 1979; Bingham pers. obs.). Species composition of *C. rotundifolia* pollinators in Colorado changes with elevation (Bingham, unpub. data). In addition, pollinator visitation rates were found to be significantly lower in alpine as compared to foothill populations in both 1993 and 1994 (Bingham, unpub. data). This species, therefore, provides an excellent opportunity to investigate the possible impacts that changes in the pollination environment may have on the reproductive biology of this species.

Hand Pollinations and Inbreeding Depression

Forty-five plants in each of three low elevation populations (City of Boulder Open Space), and one high elevation population (Niwot Ridge) were bagged to exclude pollinators. Fifteen plants were left untreated to test for autogamous seed set. Fifteen of the remaining thirty plants were used for outcross-pollinations and fifteen for self-pollinations. Three flowers on each of the experimental plants were hand pollinated. An additional fifteen marked, open-pollinated plants were used as controls. Fruits from hand pollinated flowers were allowed to mature and then collected prior to dehiscence. Seed set per fruit and average seed weight were determined for all treatments. Seeds were then germinated and maintained in the University of Colorado greenhouse until harvest. Standard randomization procedures were followed to minimize position effects on developing seedlings. At harvest, leaf number, leaf length and width, petiole length, and above ground dry weight were determined for all surviving seedlings. Levels of inbreeding depression were assessed by comparing seed set, seed weight, percent germination, leaf number, leaf length and width, petiole length and above ground dry weight of seedlings from all treatments.

Results

Pollinator limitation

There were no significant differences in seed set between outcrossed and open-pollinated treatments within any of the populations. However, open-pollinated flowers from SR and BET set significantly fewer seeds than those from TF or NR (Table 1, Figure 1a and 1b). Mean outcross-seed sets were 75.7 (51.4) for BET, 119.9 (39.6) for NR, 143.4 (119.4) for SR, and 128.8 (66.2) for TF (standard deviations in parentheses). Average seed set for open-pollinated flowers was 22.5 (43.0) for BET, 112.3 (94.0) for NR, 44.7 (63.1) for SR, and 121.1 (113.6) for TF.

Self-compatibility

On average, 63% of the low elevation plants tested for autogamy set seed, as compared to only 25% of the alpine plants tested. The high elevation plants that did set seed autogamously, set significantly fewer seeds per fruit than plants at the Betasso site ($p<0.05$). There were no differences in levels of autogamous seed set between any of the other populations. All populations were equally successful at self-pollination, with no significant differences found in the number of self- versus outcross seeds set. Plants from TF and NR produced significantly heavier seeds than plants from the other sites ($p<0.05$), but mean seed weight did not vary significantly among treatments. The interaction term from the ANOVAs were not significant suggesting no difference in the response of high and low elevation plants to selfing as measured by seed set and seed weight.

Seeds from all treatments at all sites successfully germinated (Table 2). The mean percent germination was 22.2% (21.7) for autogamous seeds, 32.6% (22.0) for control seeds, 32.1% (23.6) for selfed seeds, and 42.6% (21.5) for outcrossed seeds.

Inbreeding Depression

Mann-Whitney U-tests detected significant differences between outcrossed and selfed progeny from TF, SR, and BET for nearly all of the variables used to test inbreeding depression. Within each of these populations, selfed offspring had significantly lower dry weights (TF and BET: $p<0.0001$, SR: $p<0.05$) (Figure 3, Table 3), petiole lengths (TF and BET: $p<0.0001$, SR: $p<0.05$) (Figure 5, Table 5), and number of leaves (TF and BET: $p<0.0001$, SR: $p<0.05$) (Figure 6, Table 6) than outcrossed offspring. In addition, selfed seedlings from TF and BET had significantly lower leaf widths than outcrossed seedlings ($p<0.0001$) (Figure 7, Table 7), and selfed seeds from TF had significantly lower percent germination than outcrossed seeds ($p < 0.0001$) (Figure 2, Table 2). There were no significant differences between selfed and outcrossed offspring from NR for any of these variables (Figures 2-7, Tables 2-7).

Discussion

Pollinator Limitation

While it is often assumed that alpine plant populations experience pollinator limitation, we did not find any evidence for such limitation in alpine populations of *C. rotundifolia* in 1994. Open pollinated plants from NR set comparable numbers of seeds to plants which received hand outcross-pollinations. This is in contrast to what has been reported for several other tundra species. Supplemental pollen significantly increased seed set in the alpine species *Polemonium viscossum.* (Galen, 1985), and pollinator limitation has also been implicated in the limited seed set reported for high elevation populations of *Ranunculus flammula*, and *Mimulus primuloides* (Johnson and Cook, 1968; Douglas, 1981).

Resource limitation may also contribute to limited seed set. While we found no differences among treatments for the numbers of seeds set, there were consistent differences among sites. Both open pollinated and outcrossed plants from SR and BET set significantly fewer seeds than plants from TF and NR. These differences suggest that resources may have been limiting at these sites. SR and BET are relatively closed *Pinus ponderosa* habitat in contrast to the open meadow and tundra habitat of TF and NR respectively. In addition, populations of *C. rotundifolia* at SR and BET are much less dense than populations at TF or NR. These observations suggest that differences in resource availability among habitats may have contributed to the differences in seed set found among sites. Further research is needed to clarify the relative importance of resource and pollinator limitation to seed set in *C. rotundifolia.*

Self-compatibility

Most available information on the mating system of *C. rotundifolia* report it to be self-incompatible (Shetler, 1982). However, our results indicate that populations of *C. rotundifolia* in Colorado are capable of self-pollination. Plants from all populations successfully set seed upon self-pollination, and the numbers of selfed seed did not differ significantly from those set by either hand-outcrossed or open pollinated flowers. *C. rotundifolia* is protandrous, meaning that pollen presentation occurs prior to stigma receptivity. While such temporal separation of the sexual phases probably enforces outcrossing, our results indicate that there is no genetic self-incompatibility mechanism present in the populations studied. While the lack of genetic self-incompatibility could increase the probability of a shift toward greater selfing in populations experiencing pollinator limitation, we found no evidence for increased selfing in any of the populations studied. In addition, there was no evidence that plants in alpine populations were more likely to set seed autogamously than foothill plants. In contrast to expectations, fewer alpine plants set seed autogamously than foothill plants. These results, along with those reported above, do not support the hypothesis that alpine populations of *C. rotundifolia* in Colorado have experienced selection for increased self-compatibility or autogamy.

Inbreeding Depression

Perhaps the most interesting results from this study were those for inbreeding depression. Selfed seedlings from low elevation populations exhibited significant reductions in almost all variables measured when compared to seedlings resulting from outcross pollinations (Figures 2-7, Tables 2-7). In contrast, selfed seedlings from alpine populations did not differ significantly from outcrossed seedlings. These results suggest that alpine populations of *C. rotundifolia* may have a history of inbreeding which purged these populations of deleterious recessive alleles. However, results from genetic studies do not indicate differences in past levels of inbreeding in these populations (Bingham, unpub. data). This contradiction points to other possible explanations for the lack of observed inbreeding depression in alpine seedlings . Differences in the expression of inbreeding depression have been found in seedlings grown under greenhouse versus field conditions (Dudash, 1990). It is possible that, in our study, alpine seedlings "perceived" the greenhouse as a relatively benign environment, while foothill seedlings did not. To address this hypothesis further field studies are required.

This study was repeated during the summer of 1995 with some modifications to the experimental design. Currently, data on seed set and seed weight are being collected. Seeds from hand pollinations will be germinated and grown in the greenhouse during the spring of 1996 in order to repeat the studies on inbreeding depression.

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	Autogamous	Control	Self	Outcross
Betasso	28.435 (23.754)	22.538 (42.990)	39.538 (28.754)	75.667 (51.393)
Niwot Ridge	3.267 (6.486)	112.275 (94.024)	56.316 (57.646)	119.889 (39.602)
Shannahan Ridge	15.860 (28.262)	44.660 (63.105)	73.786 (75.569)	143.400 (119.366)
Tenderfoot	18.621 (30.120)	121.130 (113.630)	85.176 (67.259)	128.769 (66.238)

Table 1: Mean Seed Set for All Treatments at All Sites
Standard Deviations in Parentheses

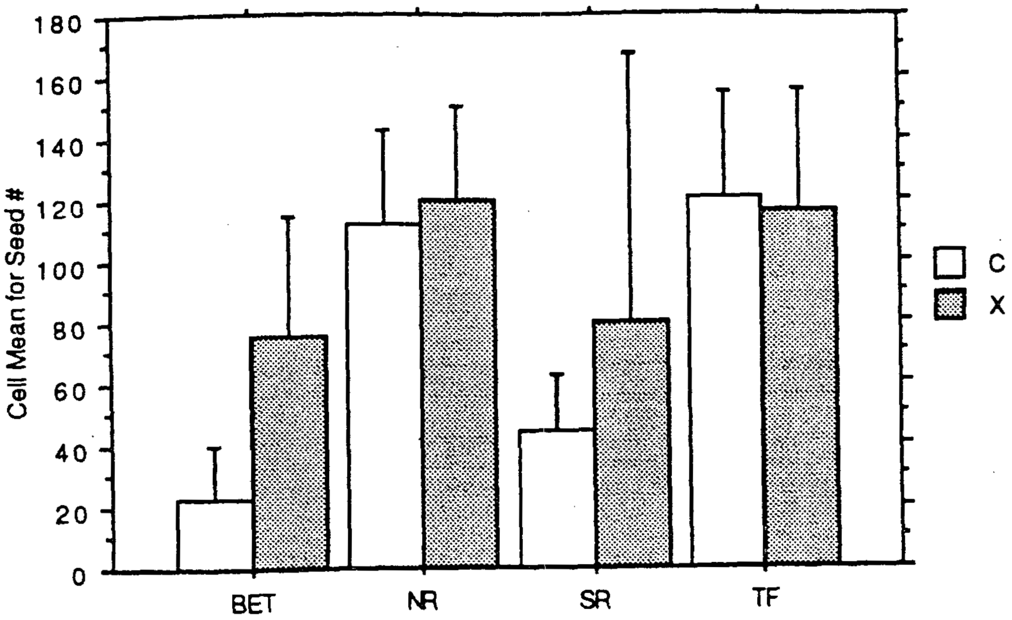


Figure 1a: Control and Outcross Seed Set for All Sites
Error Bars: 95% Confidence Interval

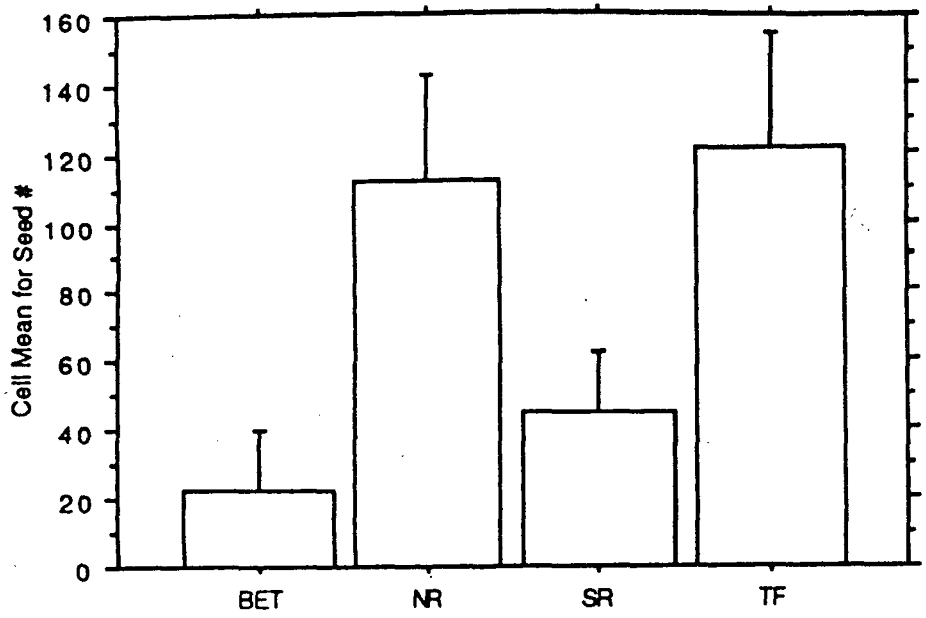


Figure 1b: Control Seed Set for All Sites
Error Bars: 95% Confidence Interval

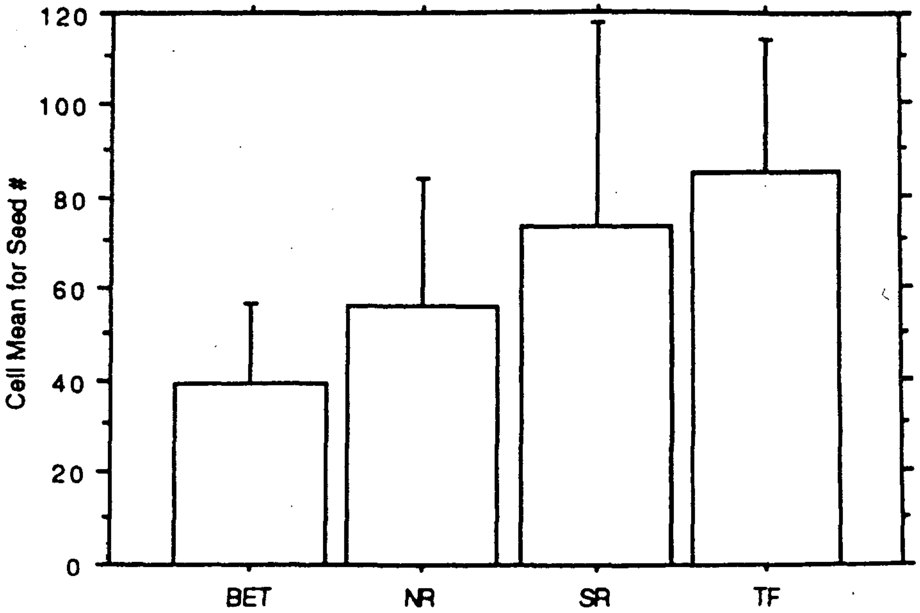


Figure 1c: Self Seed Set for All Sites
Error Bars: 95% Confidence Interval

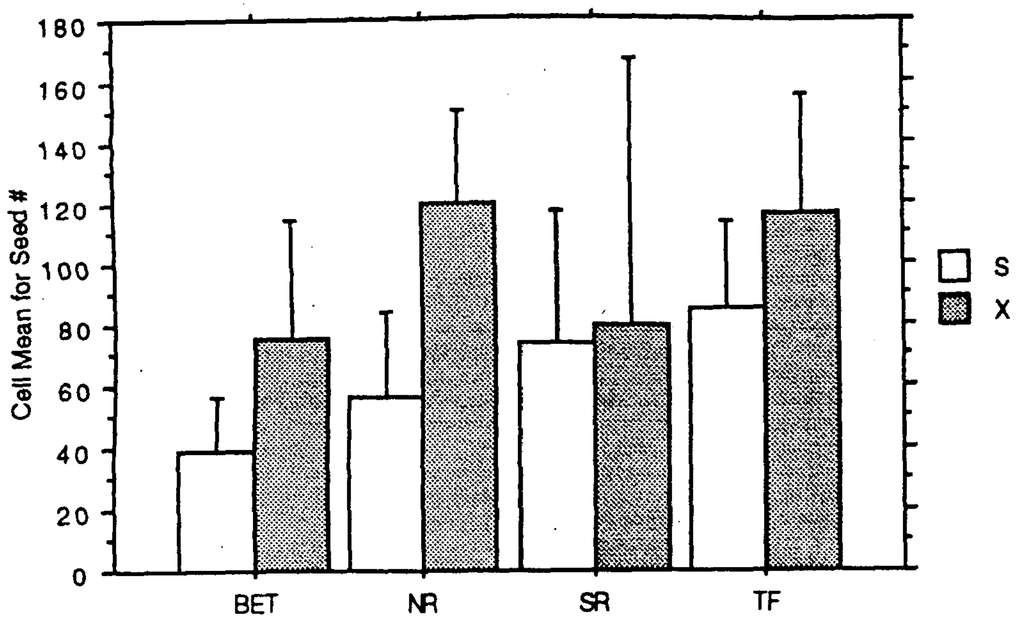


Figure 1d: Self and Outcross Seed Set for All Sites
Error Bars: 95% Confidence Interval

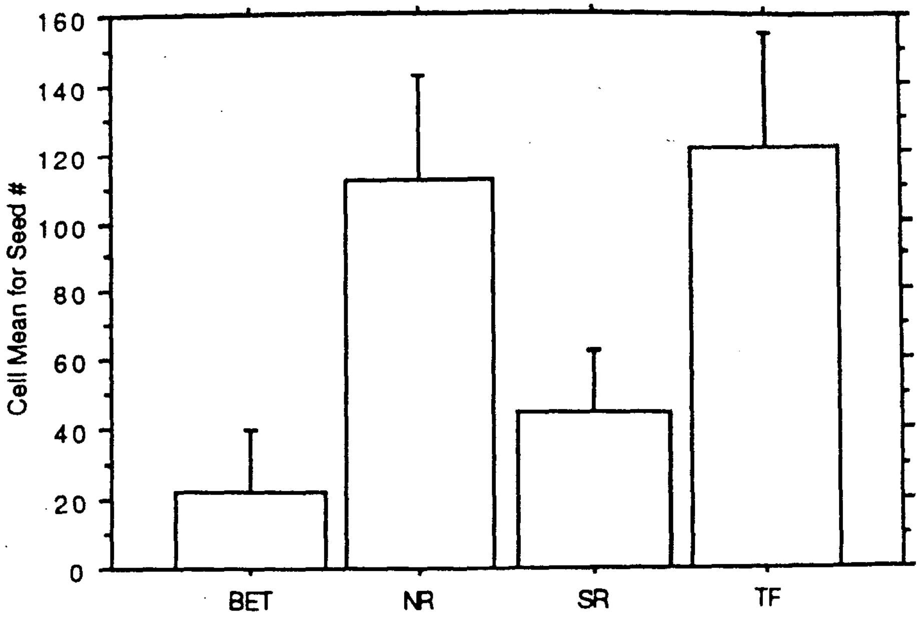


Figure 1e: Autogamous Seed Set for All Sites
Error Bars: 95% Confidence Interval

	Autogamous	Control	Self	Outcross
Betasso	36.309 (20.227)	25.938 (22.792)	24.504 (17.591)	30.154 (25.830)
Niwot Ridge	42.390 (10.762)	45.512 (18.804)	59.548 (18.552)	57.639 (22.831)
Shannahan Ridge	16.781 (20.432)	17.871 (17.702)	34.073 (21.102)	32.166 (5.932)
Tenderfoot	5.124 (6.963)	30.890 (20.476)	17.468 (13.546)	44.688 (14.435)

Table 2: Mean Percent Germination for All Treatments at All Sites
Standard Deviations in Parentheses

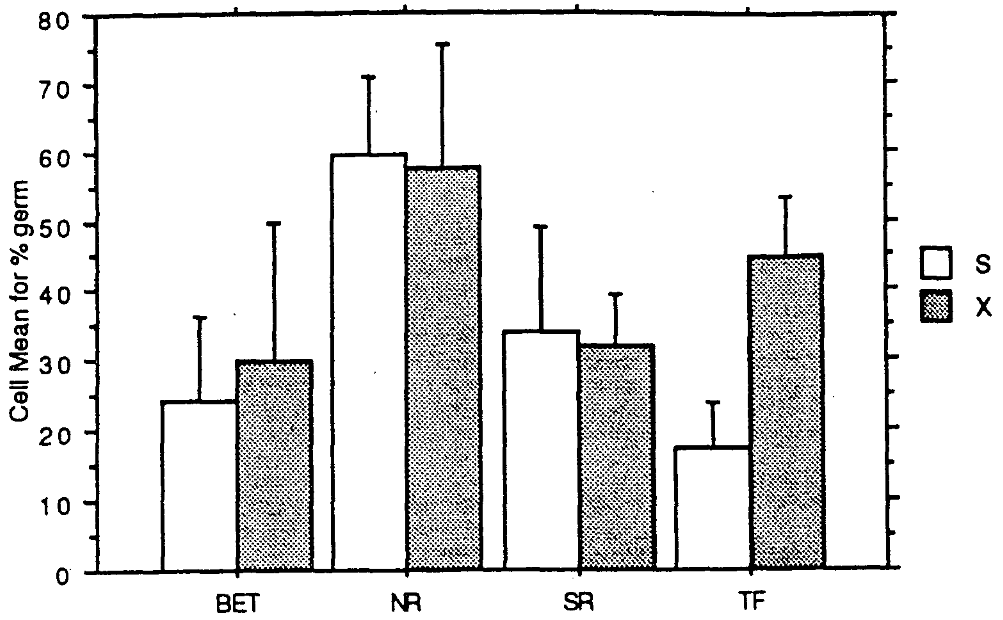


Figure 2: Percent Germination for Self and Outcross Seeds from All Sites
Error Bars: 95% Confidence Interval

	Autogamous	Control	Self	Outcross
Betasso	6.348E-3 (5.902E-3)	.011 (9.970E-3)	4.815E-3(5.976E-3)	.012 (.011)
Niwot Ridge	.018 (9.908E-3)	.014 (.011)	.012 (9.533E-3)	.013 (9.451E-3)
Shannahan Ridge	5.157E-3 (6.005E-3)	6.056E-3 (7.806E-3)	5.295E-3 (5.241E-3)	8.852E-3 (6.706E-3)
Tenderfoot	3.861E-3 (6.381E-3)	.011 (9.842E-3)	4.697E-3 (8.509E-3)	.013 (.011)

Table 3: Mean Dry Weight in Grams for All Treatments at All Sites
Standard Deviations in Parentheses

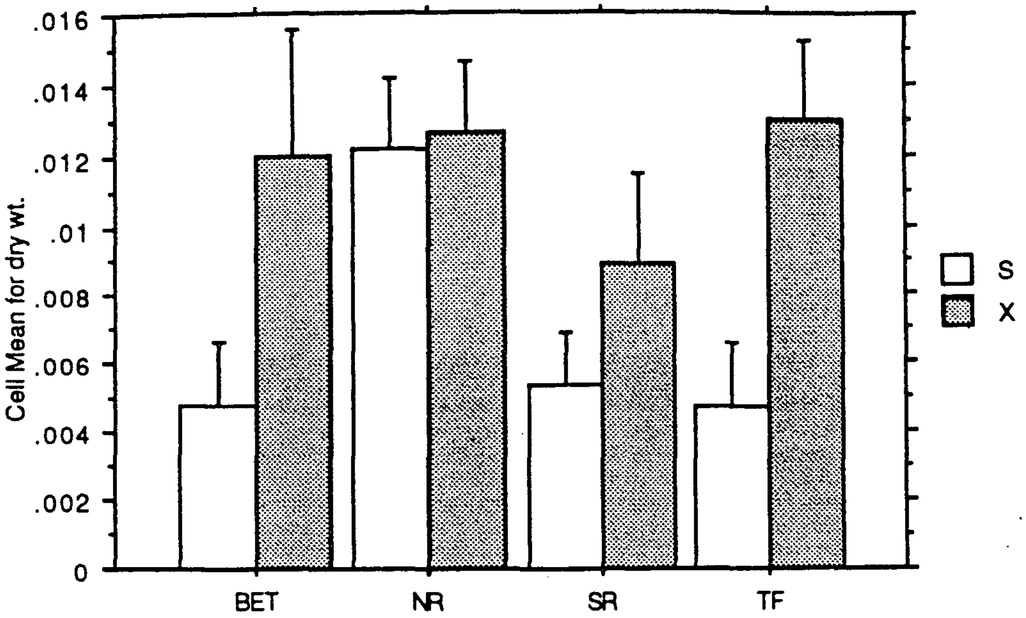


Figure 3: Dry Weight for Self and Outcross Seedlings from All Sites
Error Bars: 95% Confidence Interval

	Autogamous	Control	Self	Outcross
Betasso	8.388 (5.649)	11.639 (8.774)	5.452 (4.198)	13.050 (10.689)
Niwot Ridge	20.667 (6.351)	13.698 (10.506)	12.314 (9.537)	13.881 (10.533)
Shannahan Ridge	6.452 (7.205)	7.317 (5.941)	5.818 (5.982)	7.536 (4.985)
Tenderfoot	5.857 (5.669)	10.952 (8.652)	6.598 (8.403)	12.351 (9.061)

Table 4: Mean Petiole Lenth in Millimeters for All Treatments at All Sites
Standard Deviations in Parentheses

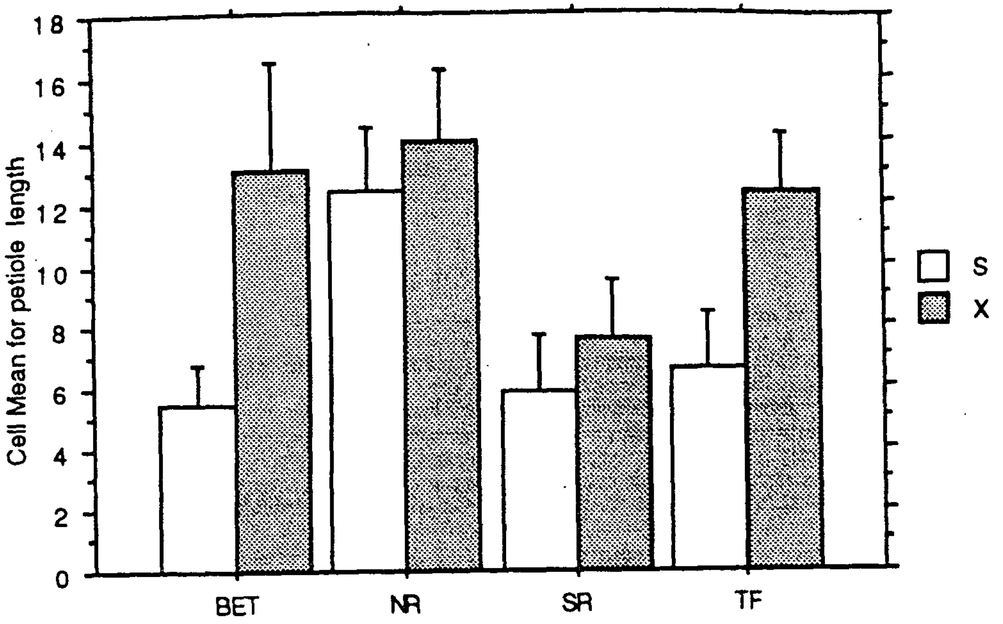


Figure 4: Petiole Length for Self and Outcross Seedlings from All Sites
Error Bars: 95% Confidence Interval

	Autogamous	Control	Self	Outcross
Betasso	7.478 (3.226)	8.944 (3.861)	6.619 (2.905)	9.525 (3.580)
Niwot Ridge	11.667 (.577)	9.129 (3.592)	9.163 (9.362)	8.810 (3.239)
Shannahan Ridge	6.500 (2.597)	7.594 (3.809)	6.773 (2.684)	8.429 (3.338)
Tenderfoot	5.714 (2.563)	8.742 (3.511)	5.902 (2.938)	9.330 (3.720)

Table 5: Mean Leaf Length in Millimeters for All Treatments at All Sites
Standard Deviations in Parentheses

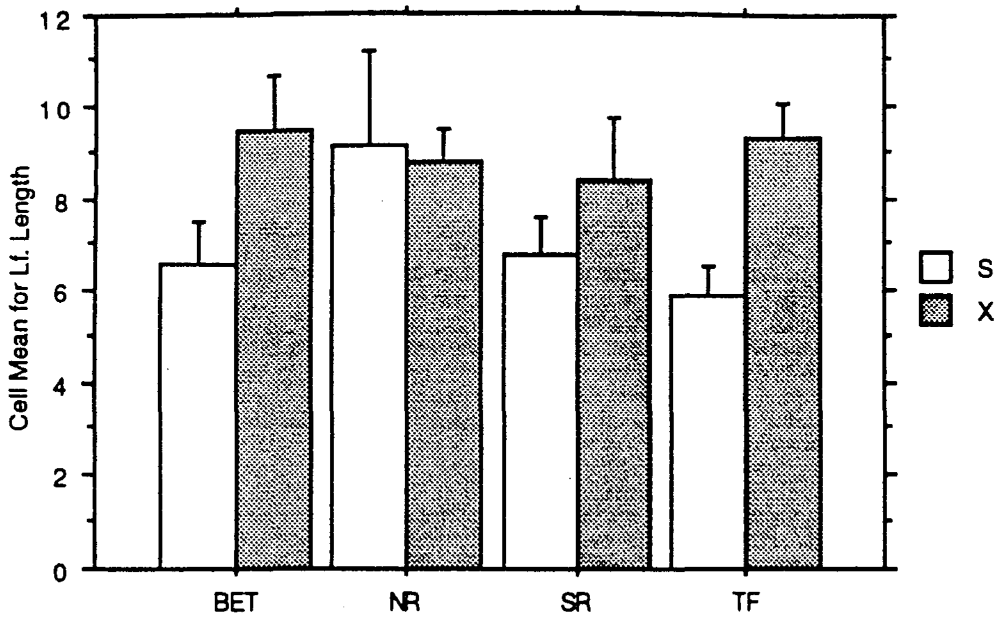


Figure 5: Leaf Length for Self and Outcross Seedlings from All Sites
Error Bars: 95% Confidence Interval

	Autogamous	Control	Self	Outcross
Betasso	2.403 (1.045)	3.028 (1.383)	2.022 (1.238)	2.857 (1.317)
Niwot Ridge	4.433 (1.528)	4.230 (2.073)	4.079 (2.018)	4.440 (2.257)
Shannahan Ridge	2.302 (1.264)	2.231 (1.294)	2.059 (1.555)	2.633 (1.245)
Tenderfoot	1.750 (1.488)	2.736 (1.333)	1.942 (1.099)	3.305 (1.495)

Table 6: Mean Number of Leaves for All Treatments at All Sites
Standard Deviations in Parentheses

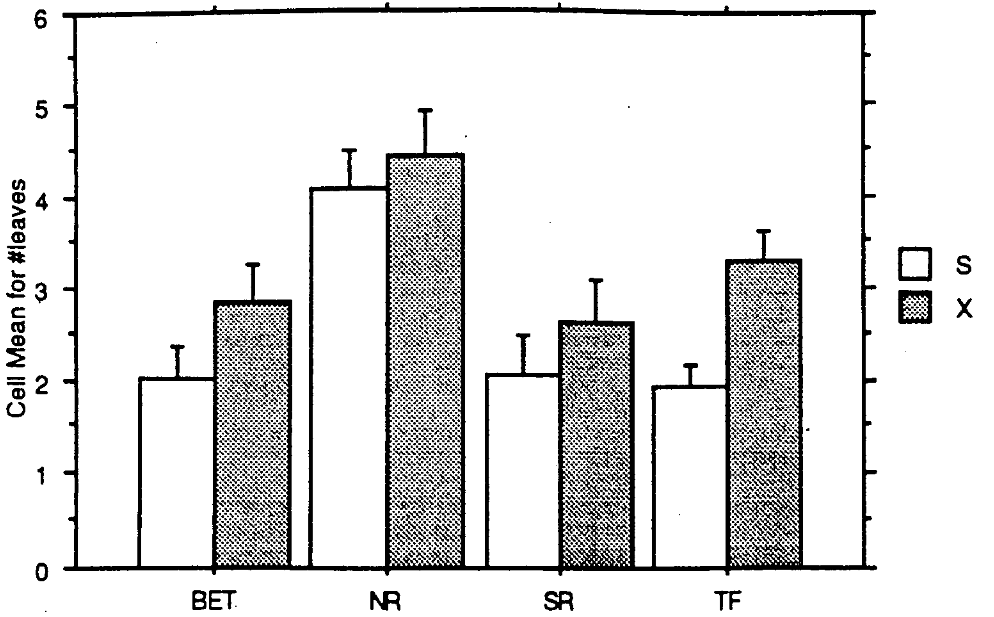


Figure 6: Number of Leaves for Self and Outcross Seedlings from All Sites
Error Bars: 95% Confidence Interval

	Autogamous	Control	Self	Outcross
Betasso	9.791 (4.820)	11.944 (5.966)	8.524 (4.759)	13.325 (6.257)
Niwot Ridge	18.667 (1.155)	12.996 (5.812)	11.349 (5.110)	12.845 (5.374)
Shannahan Ridge	8.476 (4.484)	9.485 (4.987)	8.727 (4.546)	11.000 (5.150)
Tenderfoot	7.286 (5.024)	12.158 (5.939)	7.573 (5.092)	13.394 (5.973)

Table 7: Mean Leaf Width in Millimeters for All Treatments at All Sites
Standard Deviations in Parentheses

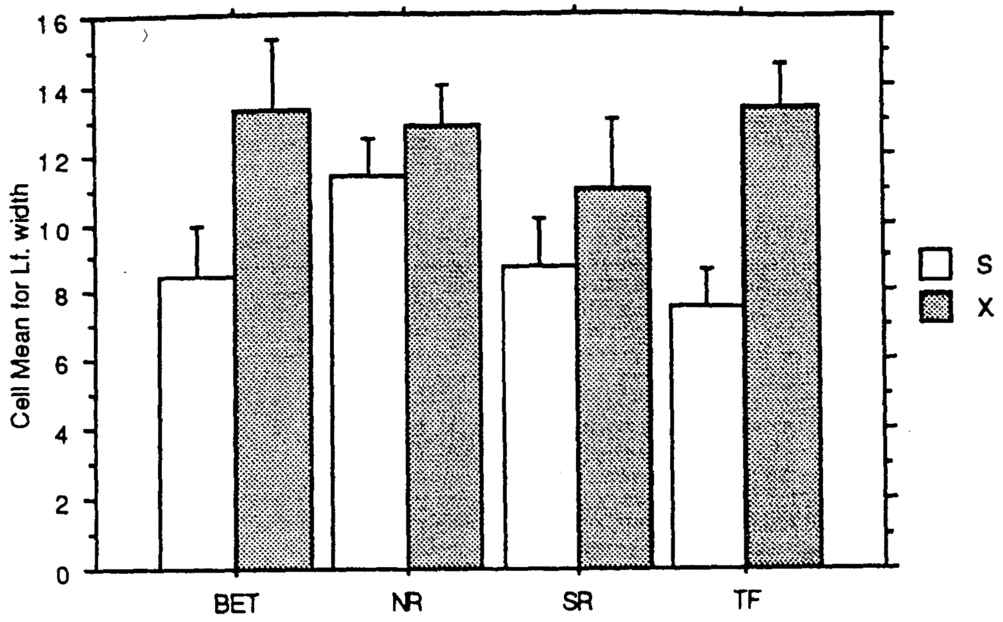


Figure 7: Leaf Width for Self and Outcross Seedlings from All Sites
Error Bars: 95% Confidence Interval

BET	NR	SR	TF
3.981E-5 (1.224E-5)	5.930E-5 (1.517E-5)	3.981E-5 (1.003E-5)	4.819E-5 (6.934E-5)

Table 8: Mean Seed Weight in Grams for All Sites
Standard Deviations in Parentheses

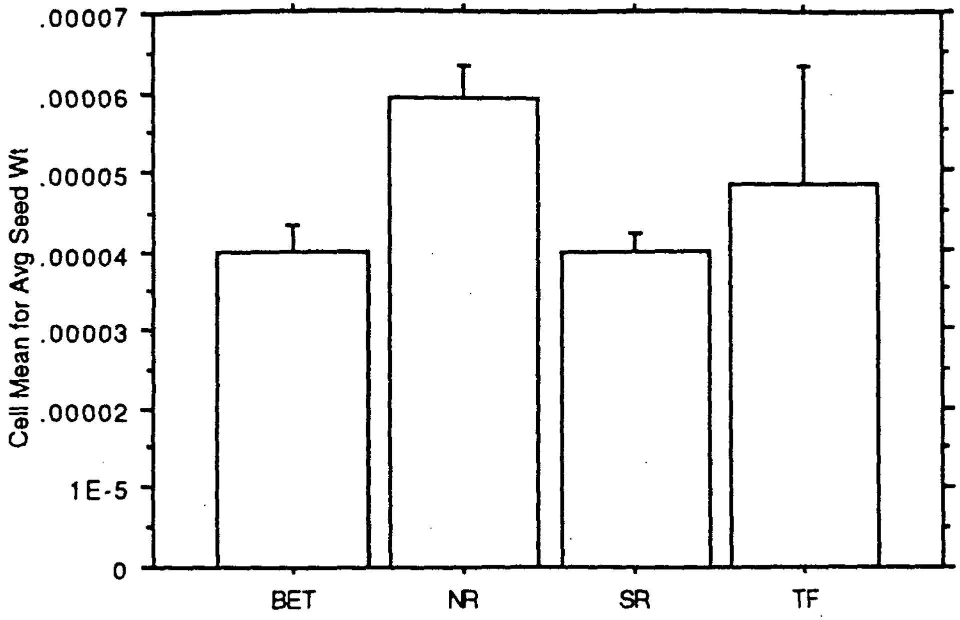
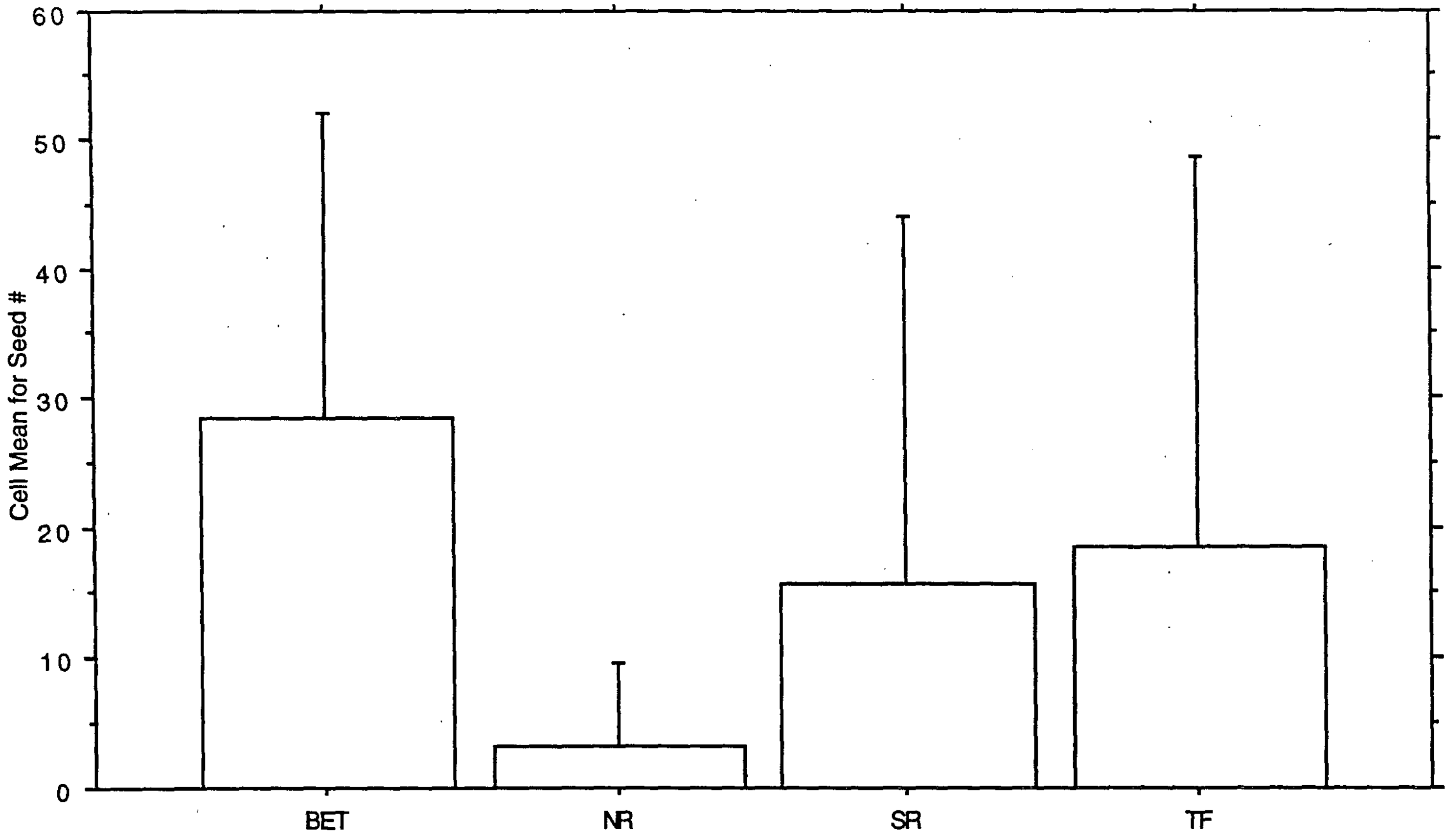


Figure 8: Seed Weight for All Sites
Error Bars: 95% Confidence Interval

Aut Seed Set '94


Cell Bar Chart
Grouping Variable(s): Site
Error Bars: ± 1 Standard Deviation(s)