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3 The effect of elevation on the phenology and pollination ecology of *Frasera speciosa*
4 (Gentianaceae).

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Abstract

This study was conducted in order to assess the effect of elevation on the phenology and pollinator interactions with *Frasera speciosa* a long-lived monocarp in the Gentianaceae family. *F. speciosa* flowers in a masting event that occurs infrequently. Due to the rarity of a mast, few studies have been conducted regarding the pollination ecology of this species. It was hypothesized that the unique pattern of flowering of *Frasera speciosa* would have an effect on pollinator interaction with this species. Further, it was hypothesized that this pattern of flowering might have developed to reduce the frequency of geitonogamous pollination. Observations were conducted to test the hypotheses at locations that span the elevation range of *F. speciosa* in Colorado. During the course of this study, a potential pollinator asynchrony was discovered. The presence of this asynchrony poses many questions regarding the effect of an increasingly variable climate on the long-term reproductive success of *F. speciosa*.

Keywords – *Frasera speciosa*, monocarp, phenology, pollination biology, pollinator asynchrony, elevation gradient, alpine, sub-alpine.

Introduction

Frasera speciosa is a long-lived perennial monocarp that ranges throughout the Rocky Mountains of the Western United States (Kershaw, MacKinnon, and Pojar 1998). Within its range, *F. speciosa* can be found along a wide gradient of elevation from Montane forests through the alpine. Due to its variation in elevation combined with a unique life history, *F. speciosa* provides a interesting system to study. The question central to this study was the effect of elevation on the phenology and pollinator intersections with this species. I hypothesize that the pattern of flowering of *Frasera speciosa* effects pollinator movement and is an adaptation to reduce geitonogamy. Further, I hypothesize that due to reduced height at higher elevation will have an effect on pollinator interaction with *Frasera speciosa* and there will be a higher incidence of geitonogamous interactions.

To date, there has been no comparison of populations of *F. speciosa* flowering simultaneously at multiple elevations. This is not surprising due to the life history of *F. speciosa*. As a monocarp, *F. speciosa* flowers only once during its life cycle. Using this reproductive strategy enables *F. speciosa* conserve as much energy for reproduction as possible and when triggered by environmental cues release that stored energy into one reproductive effort (Metcalf, Rose, and Rees 2003). *Frasera speciosa* is unusual among monocarps in the length of time it grows before flowering (30 – 50 years (Taylor and Inouye 1985)), and that it flowers in a mast; a widespread flowering of individuals of an appropriate size class, size in this case is number of leaves, which corrispondes with age. This year was such a mast and

1 compared to recent masts this year's event was the largest in over a decade (Inouye,
2 unpublished). Anecdotal evidence shows there is a slight correlation, for the
3 population in the greater Crested Butte area, between a wetter than average
4 summer four years before a mast (Inouye, unpublished). As yet the frequency of
5 masting remains unpredictable, which lends more importance to this study.

6 Due to the rarity of masting events, the study of *F. speciosa* has been confined to
7 population demography or the study of the plant rosette form (Che-Castaldo and
8 Inouye in press, and Geller and Smith 1982). These studies have provided insight
9 into the life history of *F. speciosa* leading up to reproduction. Until it flowers *F.*
10 *speciosa* remains a rosette slowly adding leaves at a predictable rate (Inouye
11 1985). These leaves are pre-formed several years in advance (Inouye 1985). As
12 with its leaves, *F. speciosa* will begin pre-form its inflorescence several years before
13 it flowers (Taylor and Inouye 1986). When flowering occurs, an inflorescence,
14 which can be up to 2.5m tall at lower elevations, emerges from each plant. This
15 study focuses on the pattern of flowering on these large inflorescences, and in turn
16 how that pattern of flowering effects pollinator behavior.

17 One study conducted at the Rocky Mountain Biological Laboratory, focused on the
18 ecology and pollination biology of *F. speciosa* (Beattie et al. 1973). This study, along
19 with a similar one conducted on *Frasera caroliniensis*, a related species that occurs
20 in the eastern United States, (Threadgill, Baskin, and Baskin 1981) provided the
21 foundational methodology utilized during this study. These studies helped to

1 outline the parameters of the natural history of *F. speciosa* by defining the pattern of
2 flowering and effective pollinator size.

3 **Methods**

4 Study Organism

5 Since phenology and the pattern of flowering are central to this study, it is necessary
6 to define several aspects of the reproductive systems of *Frasera speciosa*. Each
7 inflorescence is composed of multiple whorls of buds. The height of the
8 inflorescence decreased with the elevation of the study site; for the lower
9 population average height was close to 2 meters and for the higher populations
10 average height was close to 0.5 meters. The number of whorls of plants in this study
11 ranged from 20-24 for lower elevations sites and between 13-17 for the alpine
12 population. Elevation was shown to have significance on both height and whorl
13 number (ANOVA for height: $F_{2,53}=659.53$; $p<0.0001$, ANOVA for whorl: $F_{2,53}=230.01$;
14 $p<0.0001$). Each whorl is comprised of between five and nine groupings of buds
15 associated with an individual leaf. The grouping of buds can then be divided into
16 upper, middle, and lower.

17 Unlike other plants with raceme-like inflorescences, *F. speciosa* does not follow a
18 regular pattern of flowering, i.e. in succession from the bottom of the inflorescence
19 to the top. Rather, *F. speciosa* begins its flowering at one of the middle whorls. I
20 noted anecdotally that flowering typically begins on southerly aspects of a whorl.
21 Flowering then progresses simultaneously up and down the inflorescence from the
22 middle. Over the course of the flowering cycle multiple waves of flowering occur.

1 Each wave would correspond to one of the bud levels. The common pattern would
2 be upper buds, followed by lower buds, then middle buds, alternating between
3 lower and middle until flowering was completed.

4 Not only is there a specific pattern to flowering at the inflorescence level individual
5 flowers progress through a regular series of stages. Each stage corresponds to a
6 phase in the presentation of sexual parts (Beattie, Breedlove, and Ehrlich 1973).

7 Upon opening a flower is in the first male phase (M1). In this phase, the flower is
8 fully open but the anthers have not dehisced, exposing pollen. In the second male
9 phase (M2), the anthers have dehisced and pollen is exposed and can be removed by

10 visitors. After about two days in the male phases, the flower begins to close, passing
11 through an inter-phase (I). At this point, the stigma begins to open, becoming
12 receptive to pollen. For this study, inter-phase plants were considered to be

13 receptive to pollen due to the extremely short duration before reaching the female
14 phase. Once in the female phase (F), the flower is closed except for a small opening
15 at the apex of the corolla. In this phase the anthers are excluded, outside of the

16 semi-closed corolla, and the stigma is now fully receptive to pollen. After

17 progressing beyond the male phases, the flower remains open in the inter and

18 female phases for slightly more than a day before finally closing. The sex phases are

19 illustrated in Plate 1.



Plate 1 – The sex phases of *Frasera speciosa*, l – r Male 1, Male 2, Inter-phase, and Female (Culpepper 2010)

Study Locations

Three study populations were chosen at the low, middle, and upper ends of the elevation range of *Frasera speciosa* in the Gunnison basin. The lower population (WGS84 UTM 13S 327540E 4312820N), referred to in this study as Gothic, was at an elevation of 2900m and located in a large montane meadow approximately 1.5km south of the Rocky Mountain Biological Laboratory in Gothic, CO. The mid-elevation population (WGS84 UTM 13S 325720E 4320060N) on the east slope of Mt. Belleview was located in a rocky sub-alpine meadow surrounded by conifer forest at an elevation of 3500m approximately 8km north of Gothic. Both the Gothic and Belleview sites are located in the East River Valley NE of Crested Butte, CO. The high site (WGS84 UTM 13S 371650E 4283720N) was located in an alpine meadow at

1 approximately 3750m near Cumberland Pass, Gunnison County, CO. This alpine
2 population has been the subject of a 30 year demographic study of *F. speciosa* (Che-
3 Castalodo & Inouye in press).

4 Phenology Observations

5 Phenology observations were conducted at regular intervals for both the Gothic and
6 Belleview sites. At the Gothic site 30 plants were originally chosen for phenology.
7 This sample was later reduced to 20 due to the loss of a few plants to severe
8 herbivory and frost damage. It was also determined that additional plants would
9 not enhance the data set. At the Belleview site 20 plants were selected originally
10 and one plant was lost due to herbivory during the study.

11 In Gothic, phenology observations began on 16JUN10 and continued until 28JUL10.
12 Throughout this period, an observation was made every other day. At the Belleview
13 site, phenology observations were conducted between 23JUN10 and 29JUL10 and
14 were made three times per week during this time. Observations were missed
15 between 17JUL10 and 25JUL10 due to personal injury. Because the Cumberland
16 Pass site was not readily accessible, it was decided to experiment with the usage of
17 time-lapse cameras. Two Plantcams by the company Wingscapes (Alabaster, AL)
18 were placed at the site. These cameras were programmed to take a picture every
19 10 minutes. The cameras were first placed on the site on 03JUL10 and allowed to
20 take pictures until 30JUL10.

21 The methodology used for the phenology observations was designed to capture a
22 broader range of floristic change than a simple count of open flowers. Each study

1 plant was divided into its number of whorls and the counts were recorded for each
2 whorl. Further, the grouping of buds that emerge from the base of each leaf in a
3 whorl was then divided into upper, middle, and lower buds. Flowers were counted
4 according to its sex phase, as described by Beattie, Breedlove, and Ehrlich. In
5 addition to Male 1 (M1), Male 2 (M2), Inter-phase (I), and Female (F), and additional
6 phase of Closed (C) was added. This method of counting, while time consuming,
7 provided increased detail in the pattern of flowering on an individual. This pattern
8 was then used in subsequent pollinator observations and later fruit collection.

9 Pollinator Observations

10 Pollinator observations were conducted concurrently with the phenology
11 observation at the Gothic and Bellevue sites. It was necessary to conduct these
12 together so an accurate account of flowers in each phase was known per each whorl a
13 potential pollinator visited. These observations were conducted three times per
14 week at each site during the flowering period. As with the phenology observations,
15 pollinator observations were missed between 17JUL10 and 25JUL10 due to injury.
16 The observations were conducted twice on each day; once in the morning and once
17 in the afternoon. The same plants used for phenology tracking were also used for
18 these observations. The sample plants were then subdivided into two smaller
19 groups of 10. For each day of observation the groups were alternated between
20 morning and afternoon observations. To further account for pollinator variability
21 due to the time of day, the start time of each observation period was moved forward
22 during the week. For instance, on day one of a week's observations the morning

1 would begin at 0800 and the afternoon at 1300. Then for the final observation of
2 the week, the morning session would have moved to 1000 and afternoon to 1500.
3 For each observation period, each plant in the sub-group was observed for 10
4 minutes. During that time, each visit of a potential pollinator was recorded. The
5 pollinator was classified as *Bombus* queen, *Bombus* worker, other Hymenoptera,
6 Lepidoptera, and other. For each of the pollinator categories, only visitors of
7 effective pollination size, Beattie, et al 1973, were recorded. A visit was considered
8 a landing on an open flower. If a pollinator flew away from the plant, it was
9 considered gone; however, if a pollinator flew between whorls, that was counted as
10 intra-plant movement. Of primary interest were the movements of a pollinator on a
11 whorl. For each visitor, the numbers of flowers visited per sex phase were recorded.
12 Also for each visitor the movements between whorl were also recorded.

13 Demography

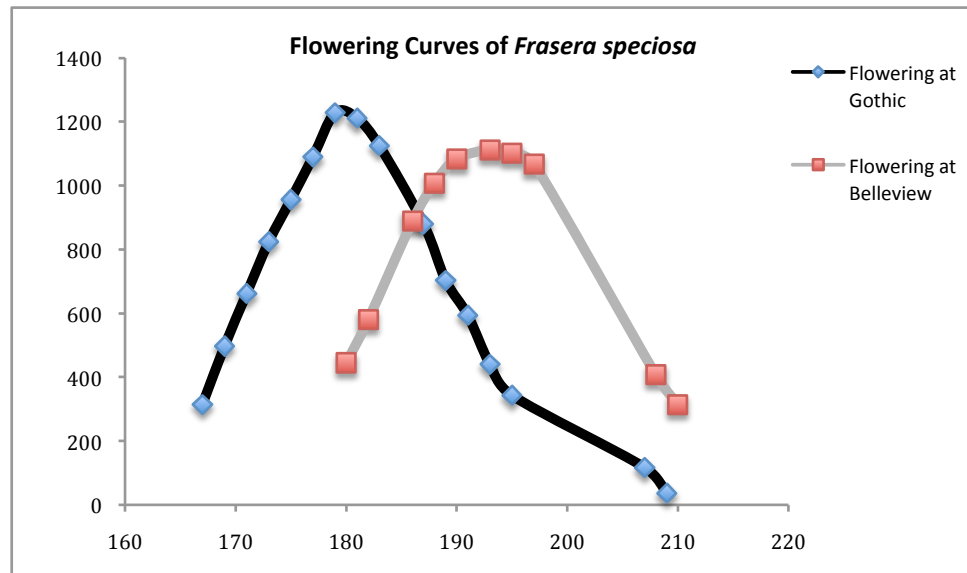
14 Several demographic metrics were recorded for each study site. The primary
15 demographic measurement being the height of each sample plant. Heights were
16 recorded for each phenology observation and growth curves for the Gothic and
17 Belleview sites were compiled. For Cumberland Pass, height was recorded on each
18 visit but only the final height was used in comparison to the other populations. In
19 addition to height, the number of whorls, the average number of leaves per whorl,
20 the distance to the nearest flowering neighbor, and the distance to the nearest non-
21 flowering neighbor were recorded for each study plant. Non-flowering neighbors
22 were classified as those of sufficient age class to potentially flower, i.e. greater than

1 14 leafs per rosette (Taylor and Inouye 1985). Stand densities were also collected at
2 each site. To assess stand density, three 10m by 10m subplots were randomly
3 selected at each site. Within each subplot the number of flowering and the number
4 of non-flowering, yet of age class that could flower, were counted.

5 **Results**

6 Phenology

7 Detailed analysis of the phenology and pollinator data are still underway; however, I
8 present here some preliminary findings regarding... With regard to the phenology
9 of *F. speciosa*, initial analyses indicated that there was no difference in the rate and
10 pattern of flowering at the Gothic and Belleview sites, (Fig. 3). The flowering curves
11 for both sites were similar; each curve climbed quickly to a peak in flower numbers
12 across the study population and then gradually tapered off. The only difference in
13 the curves is the initial flower date and a slight compression of flowering duration at
14 the Belleview site (Fig. 3). Due to the small sample size at Cumberland Pass,
15 flowering curves could not be derived; however, the same trend as in the lower
16 populations was seen upon review of the time-lapse images. The period of
17 flowering was much shorter for the alpine plants due to shorter stature, 13-17
18 whorls, than the lower populations, 20-24 whorls.



1

2 Figure 3 – The flowering curves for the Gothic and Belleview study sites, total open
3 flowers per study population per Julian day.

4 Qualitative analysis showed a similarity in the pattern of flowering within
5 inflorescences at both the Gothic and Belleview sites. This pattern was also
6 observed for two plants under remote observation in the alpine population.
7 Flowering for all observed plants began at a whorl in the middle of the inflorescence
8 and progressed upward and downward. This pattern of flowering occurred in four
9 to five distinct waves.

10 Pollinator Interactions

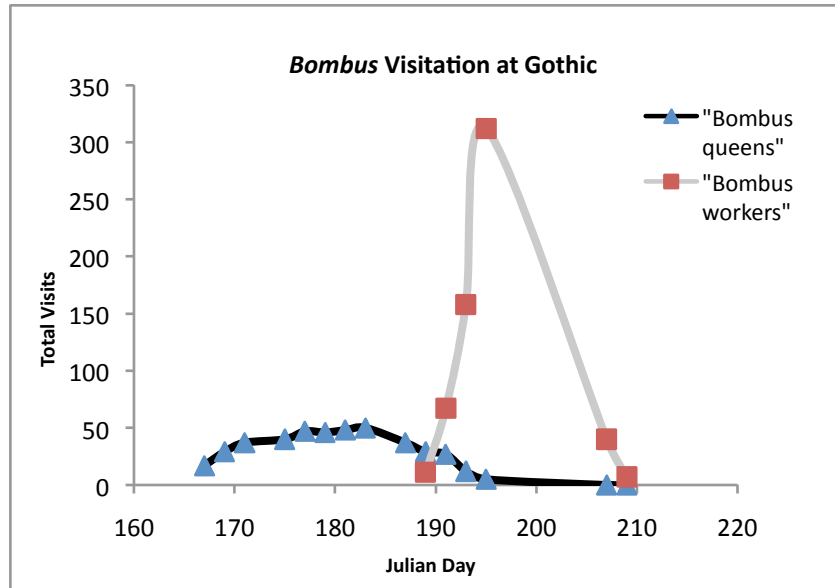
11 Pollinator observations of both the Gothic and Belleview sites showed similarities in
12 the types and sequence of pollinators (Table 1). The dominant pollinator type was
13 *Bombus* presenting in two waves. Early visits were primarily by *Bombus* queens and
14 later visits by *Bombus* workers. The visitation rates of other potential pollinators

1 were minimal in comparison to those of *Bombus* and for preliminary analysis have
2 been omitted.

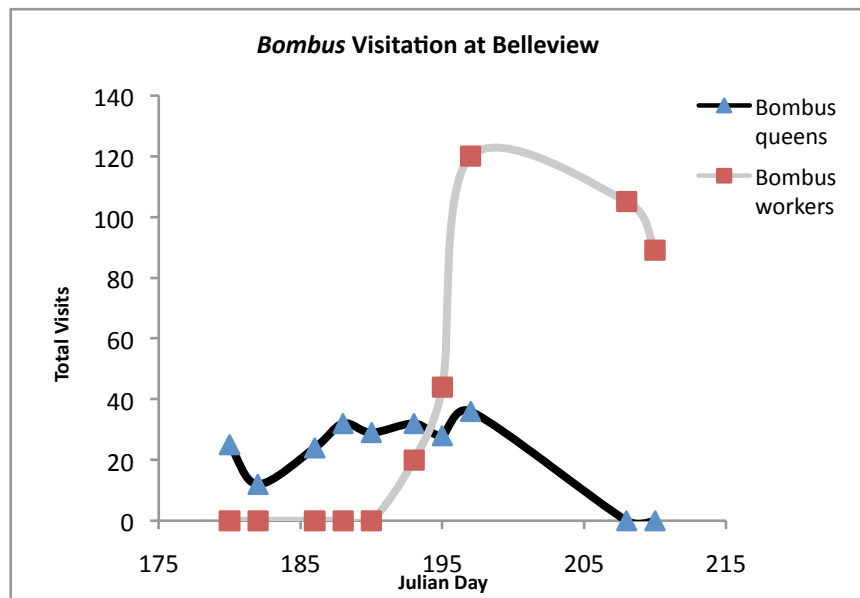
3 Looking specifically at *Bombus* queens and workers, similar trends were seen for
4 both sites. During much of the flowering cycle there were consistent visits by
5 *Bombus* queens, 4-5 visits per 10 minute interval, at both sites. In addition, there
6 was a sharp increase of *Bombus* worker interactions toward the end of the flowering
7 period (Figs 4&5). The rapid decline in visits by *Bombus* workers was not due to the
8 removal of *Bombus* workers from the study system, but rather from the decline in
9 number of available flowers. This spike of visitation at the end of flowering, seen at
10 both the Gothic and Belleview sites, suggests a possible asynchrony between *F.*
11 *speciosa* and its principle pollinators (Figs 6&7). I will use the proportion of
12 successful seed production (proportion of ovules fertilized) by early verses late
13 flowers to examine this apparent pollinator asynchrony. I did not record data on
14 nocturnal pollinators, but did observe moths on the plants in the morning, so it is
15 possible that early-season pollination is accomplished at night. Reduced seed set in
16 early-season flowers will indicate an asynchrony between *F. speciosa* and its
17 pollinators that should warrant further study in future mast years.

18 Based on review of the photographic recorded of Cumberland Pass, similar trends
19 were seen in pollinator interactions. The visitation rate of queens remained steady
20 throughout the flowering period with a rapid increase in worker visits later in
21 flowering. The major difference in the pollinator interactions between the sub-
22 alpine and alpine was the influence of higher weather variability in the alpine

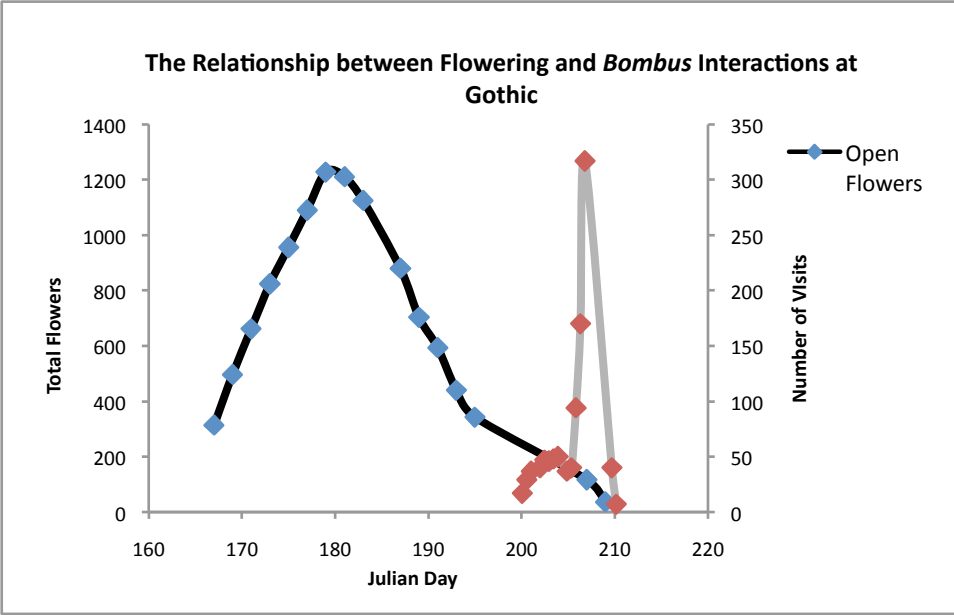
1 (Körner 1999). The analysis of the visitation rates across the three study sites is
2 ongoing.



3
4 Figure 4 – Visitation rates of *Bombus* queens and worker for the Gothic site.



5
6 Figure 5 - Visitation rates of *Bombus* queens and worker for the Bellevue site.



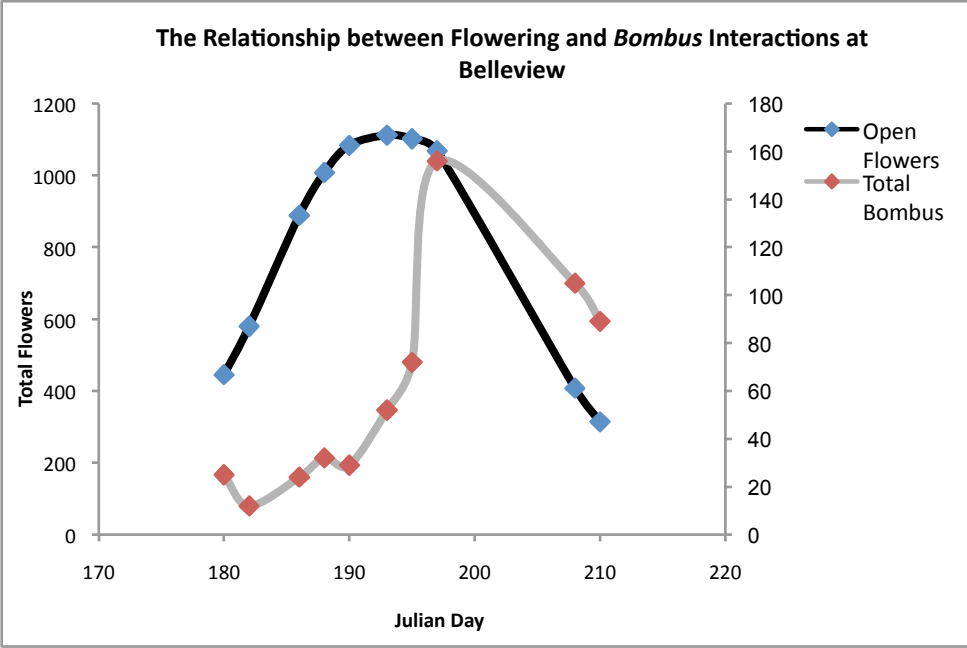
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Figure 6 – The asynchrony between the peak flowering of *F. speciosa* and total

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Bombus at the Gothic site.



4

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Figure 6 – The asynchrony between the peak flowering of *F. speciosa* and total

6

Bombus at the Belleview site.

1

2 It was hypothesized that the pattern in which *Frasera speciosa* flowers would have
3 effect on the intra-plant movements of pollinators. Further, it was hypothesized
4 that this pattern of flowering would perhaps discourage geitonogamous pollen
5 movement. Detailed analysis of pollinator movement data are pending; however,
6 anecdotally, it appeared that the pattern of flowering along the inflorescence did not
7 affect vertical movement of visitors. It is possible that rates of inter-whorl
8 movements might decline with increasing vertical distance between whorls with F-
9 phase flowers. I will explore this possibility using linear regression. I will also
10 quantify the probability of geitonogamous pollen movement using within-whorl and
11 within-plant proportions of flowers in each phase, and compare expected rates of
12 geitonogamy under various null hypotheses of pollinator movement to observed
13 patterns of movement.

14 Anecdotal evidence also indicated that there was a definite flower sex phase
15 preference by both *Bombus* queens and workers. There were no recorded
16 interactions between *Bombus* queens and F-phase flowers. It was not until the
17 appearance of *Bombus* workers at both the Gothic and Bellevue that interactions
18 with F-phase flowers were recorded. It is possible that the small opening in the
19 apex of F-phase corollas excludes *Bombus* queens. There were also far fewer
20 interactions between queens and inter-phase flowers. This trend also indicates the
21 possibility of pollinator asynchrony.

22

Discussion

1 As shown in this study, there was little differentiation between the pattern of
2 flowering and pollinator interaction between the Gothic and Belleview study sites.
3 It would be valuable, for a future study of *F. speciosa*, to sample at multiple sites per
4 elevation. In addition, because a different methodology was used for the
5 Cumberland Pass (alpine) site, it would be beneficial to conduct phenology and
6 pollinator observations that replicate the system used for the lower elevation study
7 organisms.

8 The most interesting finding of this study is the presence of a potential pollinator
9 asynchrony. If it can be shown through further analysis that the offset between the
10 emergence of primary pollinators, *Bombus*, and the flowering of *Frasera speciosa*,
11 what then are the implications for the reproductive success of this *F. speciosa*. Is
12 this asynchrony a normal occurrence for this species? Unfortunately, there is no
13 literature that has recorded the timing of flowering and pollinator visitation for past
14 masting years. Could increasing asynchrony due to warmer spring temperatures
15 affect the long term success of this species, or does it not matter at all due to the
16 monocarpic reproductive strategy of *F. speciosa*.

17 Due to its infrequent masting, *Frasera speciosa* could pose a valuable species to
18 track the increasing variability in climate. Unlike species that reproduce annually,
19 *F. speciosa* has the potential to experience widely different climatic conditions each
20 time it reproduces. Will the masting of this species become more infrequent due to
21 a loss of its current trigger to begin the pre-formation of an inflorescence? Will
22 masting become more erratic across its range? Conversely, will masting become

1 more dispersed temporally, i.e. will there be an increase in the number of plants that
2 flower outside of a masting year?

3 Considering the effect of climate change on *F. speciosa*, it would be important to
4 continue and expand upon this study. Replication of the phenology and pollinator
5 observations is needed for the existing sites but should be expanded to include
6 multiple sites at the various elevations. A future study should also be conducted
7 across a wider geographic range thus encompassing an increased variety of local
8 climatic conditions. It would also be interesting to link climate data from across the
9 range of *F. speciosa* to the findings of this year's and subsequent studies. *F. speciosa*
10 could present itself as a valuable study species with regard continuing climatic
11 variability. It will be interesting to compare this year's mast to the next one in
12 perhaps 5-7 years.

13 **Acknowledgements**

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15 opportunity and the facilities for conduction this undergraduate research project. I
16 also wish to thank George Aldridge for helping me narrow down and develop this
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