

The Timing of Floral Color Change in *Lupinus argenteus*
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Abstract:

This paper describes the timing of floral color change with *Lupinus argenteus* and the behavior of pollinator visitation to manipulated treatments. In theory plants may benefit from color change in two ways. One way is to direct pollinators to rewarding flowers. This may increase the foraging efficiency of the pollinators with the result that before leaving the plant, more flowers are visited. A second benefit plants may get is the prevention of pollen waste on unreceptive stigmas by directing pollinators to receptive flowers (Gori 1989).

Floral color changes with un-manipulated (un-pollinated) controls were compared in terms of the rate of color change to hand-pollinated treatments. Three stages of color were used to assess each stage of change. Field comparisons on 30 inflorescences (15 treatments and 15 controls) revealed that treatments moved to stage two twice as fast than controls but stayed longer in stage two than controls.

Pollinator visitation behavior was observed by painting the banner spot purple (the last color stage) on young flowers that contained pollen. I also painted white spots as a control. My hypothesis assumed that pollinators would avoid the painted purple spots since the purple stage is a cue to pollinators that less pollen exists (Gori 1989). Herbivory was responsible for the loss of pollinator visitation data. I returned to the field sites to find insects inside the netted plant bags along with damage to the painted petals. 95% of pollinator visits were to natural white spotted flowers.

Introduction:

Most plants display floral characteristics to attract pollinators such as bright colored petals, sweet smelling nectar, and protein packed pollen. Color provides advertisement to pollinators over relatively long distances, whereas the ultimate attractant is pollen, nectar, or some other kind of floral reward. Some plants retain flowers long after they have been pollinated to add to the floral display and enhance attractiveness to pollinators (Ida 2003). The retention of fertilized flowers can enhance the pollination success of a whole plant, although the flowers provide little reward and little residual reproductive ability (Ida et al. 2003). In a past study spent flowers were removed from *Lupinus argenteus* and it was observed that pollinator visitation was reduced when spent flowers were removed (Gori 1989).

A color-change in flower parts sometimes accompanies the depletion of pollen and/or fertilization. The pollination efficiency mechanism states that "...floral changes might discourage pollinators from visiting flowers that have already been pollinated..." (Gori 1989). A crucial criterion for flower color of any species is that it "... falls within the spectral sensitivity of

desirable visitors” (Waser 1983). Visits by bumblebees are significantly higher if plant color pattern is accurately associated with the lack of reward than if it is not (Gori 1989).

Flowers in each community have evolved to be visually distinct therefore maximizing the coverage of the pollinator visual spectrum (Waser 1983). Within a single species flower color may fluctuate geographically in an outwardly arbitrary way as a function of the local color environment (Waser 1983). The size of the colored target may influence the approach rate of the pollinator and that rate may be important to plant fitness (Waser 1983). According to Gori, increased pollinator approaches do not explain a color change in flower pattern alone, rather the color change functions in beneficial ways to alter the behavior of pollinators once they actually arrive at the plant (Gori 1983).

The spatial patterns of rewards on an inflorescence often direct pollinator movements in ways that are advantageous to plant fitness. Plant reproductive success may be increased by having distinguishable patterns that guide pollinators which increase their approach rate (Waser 1983). Flower visitation rates may be increased by possessing guides than do flowers without guides. For most flowering plants the flowers on an inflorescence open from bottom to top and as a result the pollinators first deposit the pollen they bring with them on stigmas and pick up pollen to carry to other plants. This morphology increases plant fitness because pollinators tend to work their way upward which reduces skipping and revisiting flowers (Waser 1983). In reference to the predications of the optimal foraging theory, the foraging economics of plants direct pollinators to move down a floral reward gradient and to visit more flowers on an inflorescence before departing (Waser 1983). Greater visitation rates by pollinators usually results in improved male fitness since more pollen is removed and improved female fitness as an outcome of the bigger number and quality of seeds produced (Price et al. 1977).

Aster vimineus a color change occurs where the florets transform from yellow to red. Yellow florets contain more pollen as well as a greater percentage of viable pollen than red florets. All pollinator types consistently visit yellow flowers more than red. *A. vimineus* also retains red florets which contribute to the floral display thus increasing pollinator visitation rates (Niesenbaum et al. 1999).

Lupinus argenteus is one such flowering plant that changes petal color within its lifetime. *Lupinus argenteus* or slivery pine lupine is one of the herbaceous perennials that live in meadows surrounding Gothic, CO. *L. argenteus* is self-incompatible, does not produce nectar, and is pollinated exclusively by several species of *Bombus* (Gori 1989). It has an interesting characteristic in which a spot on one of its petals, the banner petal, changes color from white to purple during the flower’s lifetime. The cause of this color change is not fully understood. The

purple spot may be a cue to pollinators that less pollen exists and it can move on to a non-fertilized flower. From an evolutionary perspective this makes sense. It increases the foraging efficiency of the pollinator and may deter incoming pollen from being wasted on unreceptive stigmas and may also prevent the gathering of unviable pollen (Gori 1989). Pollen viability decreases after anthesis and this is a potential reason why the color change occurs (Gori 1989). Alternatively, pollen deposition on stigmas may initiate color change (Nuttman et al. 2003).

This distinct phenotypical characteristic is the object of my investigation. The banner spot change however, may not be due to fertilization. A variety of factors affect the coloring of the spot. In this experiment I explore some factors that influence the timing of color change and pollinator behavior. I examined the banner spot using two treatments such as hand-pollination and painting the color of the banner spot. Specifically, I addressed two questions: First, does the banner spot change color in response to fertilization or is the color change a simple effect of flower senescence? Second, do pollinators respond to floral color change and preferentially visit young flowers before the banner petal changes to purple? Is the colored spot a floral cue to pollinators? I hypothesize that the banner spot will change color regardless of being fertilized and that pollinators discriminate between white and purple colored flowers, choosing white.

Materials and Methods:

Description of Study Site:

This research was conducted in June and July of 2005 in the vicinity of RMBL. The plants used for experimentation were located at three different areas at elevation of 2900 M. The first meadow is located in Gothic meadow on a west facing slope and flat. The second meadow is located on a west facing slope near the kettle ponds, and the third meadow is located on a west facing slope adjacent to Alison Brody's cabin in South Gothic.

Two inflorescences on each plant matched as closely as possible were randomly selected as either a treatment or a control. Standard colored field flags (1 meter) were used to mark the location of each plant. Each inflorescence was covered by transparent nets to allow for sunlight and keep insects out were secured by tying string at the bottom of each bag. This was performed for the hand-pollinating and the painting portions of the experiment.

Hand-pollination:

I examined whether the banner petal changes color as a response to fertilization by hand pollinating flowers and comparing the rate of color change to un-pollinated flowers. To do so I

randomly selected young flowers (that had not been pollinated) to be hand-pollinated on one inflorescence, bagged them, and compared this to flowers that have not been pollinated, also bagged, on a different inflorescence on the same plant. I examined all flowers every 12-14 hours and scored them according to the timing and intensity of the color change. Three stages of color were the determinants of the color change although intermediate colors exist between the three stages. The first stage is white, the second stage is pink, and the third stage is purple. I made these comparisons on 30 inflorescences (15 pairs). I recorded how long it took the banner spot to change from white to purple, the full course of the color change. I choose to hand-pollinate flowers based on how easy it was to push the keel down and in doing so, if the stigma was visible without any pollen traces. This was performed at 8:00a.m. and 7:30pm mountain standard time until 15 inflorescences (67 flowers) were hand-pollinated.

The materials used to hand- pollinate are as follows; an optical binocular magnifier. This was used to magnify the stigma surface and to also see the pollen grains as I brushed them on the stigma. Standard sharp edged tweezers were used to pluck anthers. A white paint marker was used to mark each flower that was hand pollinated.

Painting the banner spot:

To examine whether bees preferentially visit flowers with white banner petals, I manipulated the color change by painting the banner spot purple. Again, I used 15 pairs of inflorescences (two per plant), matched as closely as possible in terms of relative phenology and number of flowers. I randomly assigned them to one of two treatments: on one inflorescence on each plant I painted the banner spot purple on all flowers that had a white banner petal. I also painted an inflorescence white to test if the bees avoided the paint in general. I made these comparisons with naturally colored spots and I observed these plants in the field and compared the total number of visits to color-manipulated inflorescences.

The materials used to paint the banner spot are as follows; a non-toxic acrylic paint was applied to the selected inflorescences. I used blue, red, and white colors and quarter inch paint brush to apply the paint. The paint will not affect pollinator behavior in terms of avoidance (M.V. Price, personal communication).

Statistical analysis:

To examine if color change was triggered by fertilization I performed a one way analysis of variance (ANOVA) with the timing of color change (the three stages) as the response variable and hand-pollination as the treatment. I analyzed pollinator behavior again using an ANOVA.

Results:

45 hand-pollinated flowers (treatments) changed from stage one to stage two twice as fast than the non-pollinated flowers (controls). Treatments however stayed an average of 11 more hours in stage two than did controls. Out of 60 pollinator visits, 52 visits were to natural white spots, 1 visit was to one painted purple spot, and 7 visits were to a natural purple spots.

Discussion:

The results of color change clearly show that pollination has an effect on the rate of color change in *L. argenteus*. In Ida and Kudos' experiment with *Aster vimineus* they calculated the mean duration time of how long each color phase was upon fertilization. Yellow was the first stage and lasted on average for 3.1 days followed by the red phase which lasted 2.1 days. The white stage for *L. argenteus* lasted for 2.03 days while the pink stage lasted for 1.06 days. Non-pollinated *L. argenteus* might move quicker through the color stages possibly because it requires less energy to maintain that color. Pollen viability might be a potential reason why the color change occurs. In Gori and Ida's paper they discuss inviable pollen as being a possible cause for the color change. How is pollen tested for viability? Individuals displaying natural white spots were visited more than any other color combination. This is consistent with the conception that color change discourages pollinators from visiting unreceptive non-rewarding flowers (Gori 1989).

Purple phase flowers did not act as a cue to attract pollinators and seem to indicate a low level of reward thus functioning as a cue to discourage pollinator visitation (Pollinator tenure mechanism). These data are consistent with pollinator foraging efficiency as well as pollination efficiency of the flower. The pollinator can choose which flower to visit based on petal color and this is associated with increased pollen removal. Flower fitness can be increased based on this since bumblebees are the pollinators. Flowers have an improved chance of becoming fertilized if they correctly display their maturation stage to pollinators (Pollination-efficiency mechanism).

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