

**Pollinator selection by floral traits and color in a hybrid zone of
Ipomopsis aggregata and *I. tenuituba* (Polemoniaceae)**

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Abstract

Albinos plants are usually discriminated by their pollinators. Exist the possibility that other pollinator drive a reproductive isolation on this plants. To observe if an albino of a plant commonly pollinated by hummingbirds can be selected by other pollinator we used the F1s of albinos of *Ipomopsis aggregata* and hybrids of them and *I. tenuituba*. We performed two experimental arrays to observed the selection of the hummingbirds and hawkmoths. The first array was a pale array where we selected albino and hybrid plants with pale flowers. The second array was the opposite, the plants selected were albino and hybrid plants with dark flowers. Also, both arrays had wild types of *I. aggregata* and *I. tenuituba*. In both arrays the hummingbird preferred the dark flowers but in the second array they visited the hybrids almost the same as the *I. aggregata* wild types. The hawkmoths were presented only for the first array where they did not show any preference by the color of the flowers. The hawkmoth can establish a population of albino *I. aggregata* or at less maintain their genes in the populations of *I. aggregata*.

Introduction

Speciation in most of flowering plants has been driven by co-evolution with animals . Pollinator syndromes are a response to selection from pollinators that interact with the plants, depending on the morphology of the pollinator. For example, hummingbird pollinator syndrome has been observed to be red tubular flowers there are also differences in nectar volume & concentration, and in tube width, and hawkmoth pollinator syndrome has been observed to be white tubular flowers. When a plant develops a mutation of flower color or flower morphology,

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its pollinators usually discriminate against the mutation, so those genes will disappear of the population when the mutant plant die. An example of this discrimination is a study of *Delphinium nelsonii* that found that hummingbirds and bumblebees discriminate against albino flowers (Waser and Price 1981). However, in a situation where a mutant phenotype is similar to another pollinator syndrome this mutation can persist.

Ipomopsis hybrid zones have been studied to observe selection on floral traits including color. The common pollinators on the *Ipomopsis* hybrid zones are hummingbirds and hawkmoths (Campbell et al. 1997, Elam and Linhart 1988). The hummingbirds prefer the red flowers of *Ipomopsis* to the white flowers when the reward (nectar) is the same, but if the white flowers have more reward than the red flowers the hummingbirds prefer the white flowers (Meléndez-Ackerman et al. 1997). *Ipomopsis aggregata* is visited more often by the hummingbirds than the *I. tenuituba* and their hybrids under natural conditions, but if all the flowers are painted red the hummingbirds contraction discriminate between *I. aggregata*, *I. tenuituba* or hybrids flowers (Meléndez-Ackerman and Campbell 1998). While hummingbirds prefer red wide corollas, hawkmoths prefer narrow corollas of *Ipomopsis* without discrimination by length or optical density of the corolla (Campbell et al. 1997). The albinos of *I. aggregata* can be discriminated by hummingbirds, but it is possible that hawkmoths can visit this plant and maintain an albino population, although there have not yet been studies to prove the selection of albinos flowers of *I. aggregata* and its hybrids by hummingbirds and hawkmoths.

We expected that dark (red, orange and dark pink) and wide flowers will be visited mainly by hummingbirds, and that pale (white, light yellow and light pink) and thin flowers will be visited by hawkmoths. To test this hypothesis our questions are: 1) is the flower color related

to the pollinator and the quantity of visits? and 2) is the flower's morphology related to the pollinator and the quantity of visits?

Methods

Study System.

Ipomopsis aggregata and *I. tenuituba* are perennial plants that grow in sunny places. They are found in the western United States. *Ipomopsis aggregata* grows in lower elevations in respect to *I. tenuituba* and hybrids that grow between them. *Ipomopsis aggregata* flowers have red, trumpet-shaped, wide corollas and produce more nectar with low sugar concentration than *I. tenuituba*. In contrast, the flowers of *I. tenuituba* that are pale violet to white, longer, narrower and produce less nectar with higher sugar concentration (Meléndez-Ackerman 1997, Campbell et al. 1997). The pollinators of *I. aggregata* are hummingbirds and the pollinators are hawkmoths. Albinos of *I. aggregata* occur naturally, but at low frequency.

Experimental Arrays.

The study was made from July 6, 2004 to August 10, 2004 in Gothic, Colorado. We prepared two arrays in a meadow about 1 km south of the Rocky Mountain Biological Laboratory (RMBL). The meadow had a variety of natural flora including *I. aggregata*, Asteraceae, grasses, and others. The arrays were set in a place where no *I. aggregata* were too close (this is an awkward sentence – how close is too close? It would be better to simply describe the site and state approximately how far away the nearest *aggregata* were).

Each array consisted of 24 potted plants: six albino *I. aggregata* x albino *I. aggregata* F₁s (AA), six albino *I. aggregata* x *I. tenuituba* F₁s (AT), six *I. aggregata* wild type (W) and six *I. tenuituba* wild type (T) (all these plants were emasculated to prevent gene flow to the natural

population in the study site). The plants were organized randomly leaving a meter between each plant in an area of six meters per four meters (6m x 4m).

The plants used that involve albinos crosses are the result of experimental crosses made in 2002 that were collected from the Upper Gunnison Basin (East River, Slate River, and Irwin Lake). The crosses of the albinos (AA) collected from different location produce a wide range of color mutants, from pale yellow to dark orange / red flowers and the cross made of albinos from the same place produce mainly pale yellow flowers. The crosses made using albinos *I. aggregata* x *I. tenuituba* wild types (AT) produce colors between pale yellow and pink. The crosses made between albinos and wild types *I. aggregata* (WA) produced colors nearly identical to wild type *I. aggregata*. Also, we collected *I. aggregata* (W) and *I. tenuituba* (T) wild types from the field.

The albino and hybrids plants were sorted to obtain one array with pale yellow flowers and another with dark orange and red flowers. The first array consisted of pale albino and pale hybrids plus *I. aggregata* and *I. tenuituba* wild types, and the second array consisted of dark albinos and dark hybrids plus *I. aggregata* and *I. tenuituba* wild types

Pollinator Observations.

The field data were collected for two hours in the mornings (0700-0900h) and two hours in the evenings (1930-2130h) until we get at less 20 foraging bouts or more by hummingbirds to each array and until hawkmoths disappeared. On each observation day collected data of number of flowers and the location of the plant on the array. Also, for each visitor we recorded the time, type of visitor, sequence of plants visited on the array and the number of flowers visited on each plant.

Floral Traits.

We took five morphological measurements (corolla length and width, shorter and longer stamen length, and style length) of 3 – 4 flowers for each plant. Nectar production rate over 48 hours and sugar concentration were measured using 3 flowers per plant. We measured sugar concentration using a refractometer. Height and number of flowers were measured on each plant used in the arrays every day an array observation was conducted. Analyses were performed on means of these measurements for each plant.

To measure the corolla color we extracted the pigment of three flowers per plant for 24 hours in 10 ml ice-cold methanol:HCl (97:3). Then we measured the absorbance of 3 ml of the extract at wavelengths corresponding to the peak absorbance for *I. aggregata*, *I. tenuituba* and their hybrids (508nm, 528nm, 520nm respectively) using a Milton Roy Model 401 Spectrophotometer. Corollas were air dried and weighed. In addition to corolla absorbance, reflectance spectra were obtained for 1 flower each from all plants used in the arrays using an Ocean Optics Model 2000 Spectrometer.

Data Analysis.

We performed a canonical discriminant analysis to distinguish the 5 types of plant in the study (AA, AT, WA, W, T) according to morphometric, absorbance, and nectar traits. A canonical discriminant analysis calculates a hierarchical set of orthogonal linear equations each of which maximizes the variance between group centroids in multivariate space.

Pollinator visitation data were tabulated for each type of array (light and dark), and expressed as both total visits to each type of plant and number of pairwise transitions between types of plants, separately for each kind of pollinator (hummingbirds and hawkmoths). Also, number of flowers visited by each kind of pollinator were totaled for each plant in every array.

Pollinator fidelity (tendency of a pollinator to overvisit one type of plant) was tested using a Chi-squared Goodness of Fit test (Zar 1999). Pollinator constancy (tendency of a pollinator to move from one type of plant to another of the same type) was tested using a G-test of independence on 4x4 contingency tables (Zar 1999). The effect of floral and plant traits on visit number by each type of pollinator was assessed using multiple regression, with morphology, plant height, number of flowers, 48-hr nectar production, sugar concentration, and maximum absorbance/dry weight as independent variables.

Goodness of Fit and G-tests were performed in Microsoft Excel. The canonical discriminant analysis was performed in SPSS.

Results

Floral Traits.

The canonical discriminant analysis show us the floral traits of *I. tenuituba* plants are completely different from the others types of plants. The hybrids (AT) are separate of the others groups but some plants overlap with the albinos *I. aggregata*. Some of the albinos *I. aggregata* (AA) are overlapping with some WA *I. aggregata* and some hybrids (AT). The WA *I. aggregata* are overlapping with the AA and with the *I. aggregata* wild types.(Figure 1)

The reflectance of the plants used in the first array (pale flowers) show that the albinos *I. aggregata*(AA), hybrids (AT) and *I. tenuituba* (T) follow the same pattern. Their reflectance was high in the wavelength of blue (40 – 75%) to red (65 – 90%). The *I. aggregata* wild types reflected less blue, green, and yellow (aprox. 20%, 10%, 10% respectively) but orange and red reflected almost the same than the other types (aprox. 75%) (Figure 2).

In the second array (dark flowers) the albinos *I. aggregata*(AA), hybrids (AT) and *I. aggregata* wild types follow the same pattern. Their reflectance was low in the wavelength of blue, green, and yellow (aprox. 15 – 25%, 10 – 15 , 10 – 15 % respectively), and orange and red reflectance was high(65 – 80%). The *I. tenuituba* (T) reflected more in the wavelength of blue(aprox. 65%) to yellow (aprox. 55%) but red was almost the same than the other types (aprox. 80%) (Figure 3).

Pollinator selection.

In the first array (pale flowers) we observe 20 foraging bouts by hummingbirds in 14 hours of observation. They show fidelity to the *I. aggregata* wild types (red flower plants) that obtain 32 of 37 visits, and discriminate against pale flowers of the albinos *I. aggregata* (AA), hybrids (AT) and *I. tenuituba* (T) that obtain 2, 0 and 3 visits respectively (Table 1a). In this array we observe 7 foraging bouts by hawkmoths. They did not show fidelity to any type of plants. They visited 6 *I. aggregata* wild types, 11 *I. tenuituba* (T), 7 *I. aggregata* (AA) and 3 hybrids (AT) (Table 1b). In the second array (dark flowers) we observe 28 foraging bouts in 6 hours of observation. They show fidelity to the albinos *I. aggregata* (AA) that obtain 24 visits. The *I. aggregata* wild types and hybrids were visited according to the expect (14 visits) were they obtain 17 and 14 visit respectively. The *I. tenuituba* (T) were discriminate obtaining 1 visit (Table 1c). No hawkmoth was observe during the observations of the second array.

The hummingbirds and hawkmoths did not show constancy in any one of the two arrays (Table 2a,b,c)

Discussion

Floral Traits.

The floral traits of the plants product of the cross using albinos *I. aggregata* have a lot of overlapping specially albinos with hybrids and WA *I. aggregata*, and WA *I. aggregata* with *I. aggregata* wild types. The overlapping between albinos and hybrids can be caused of the pale flowers that produced both types of plants. The overlapping between albinos and WA *I. aggregata* was caused by the dark flowers product of both crosses. Also the width and length of the corolla can be having a significant influence to produce this overlap. The high overlap between the *I. aggregata* wild types and the WA *I. aggregata* tell us that they are really similar because the albinisms apparently is recessive. The morphology of the *I. tenuituba* (T) was the cause been separated from the others groups because its color was almost the same as the hybrids, that was its closer group.

The flowers of the three types of plants, albinos, hybrids and *I. tenuituba* (T), with pale flowers in the first array reflected the wavelength of most of the visible light of the spectra that produced a white or really pale color on the flowers. In the second array three of the types of plants albinos, hybrids and *I. aggregata* wild types (W), reflected wavelength that produce a red color which can be more attractive to the hummingbirds than the pale or white. The flower color of the hybrids in this second array were like the *I. tenuituba* but it reflectance were like the *I. aggregata* wild types.

Pollinator selection.

The hummingbirds discriminated to the pale flowers in the first array. In the second array they visit preferred the dark flowers but they visited hybrids, that their color were like the *I. tenuituba*, almost in the same amount that the *I. aggregata* wild types. The reflectance of

flowers can be influencing the selection of the hummingbirds. Also the corolla the hybrids is wider than the corolla of the *I. tenuituba* and produce more nectar so they can be associating the width of the plant and their reward (Meléndez-Ackerman and Campbell 1998). They can be discriminating against the *I. tenuituba* because the their corolla is too thin and too long to them, and their efficiency of nectar foraging in this plants is less than in flowers with wide and short corollas(Grant and Temeles 1992). In contrast, the hawkmoths do not show any preferences by color or morphology in the first array. They visited all the four types of plant in the array because they did not have the restriction that have the beak of the hummingbirds which impede the access to narrow corollas. The hawkmoths can be driven to the reproductive isolation of the albinos of *I. aggregata* and populations of albinos can be establish eventually.

Reference

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Appendix

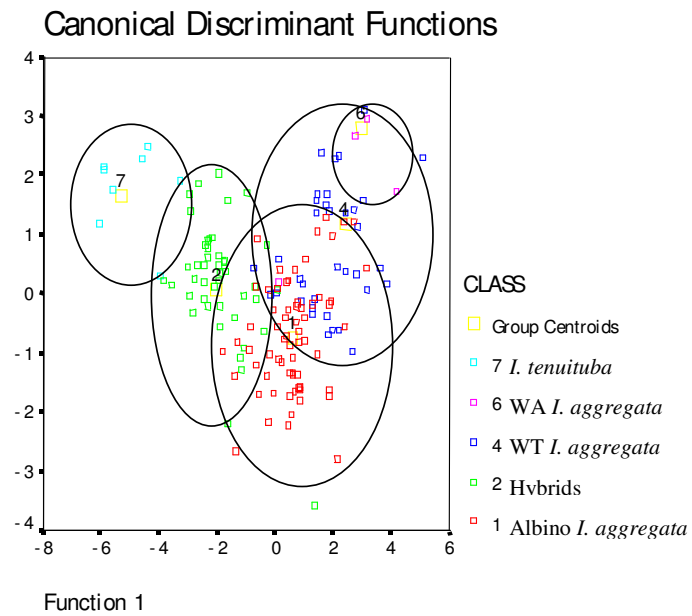


Figure 1: Canonical discriminant analysis for the floral traits of the three different types of plants product of the crosses with albinos of *I. aggregata* (albinos (AA), (WA), hybrids (AT)), and the two wild types, *I. aggregata* (W) and *I. tenuituba* (T).

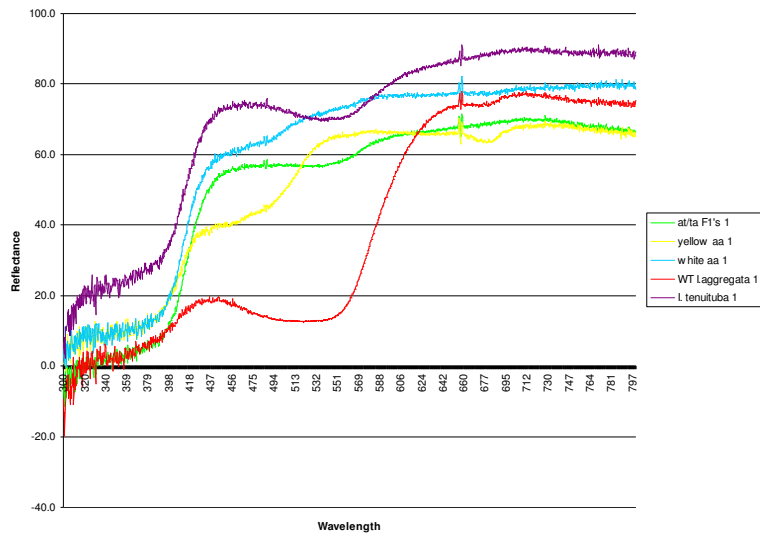


Figure 2: Reflectance of the for types of plants in the first array. The reflectance of the *I. aggregata* wild types is the only different.

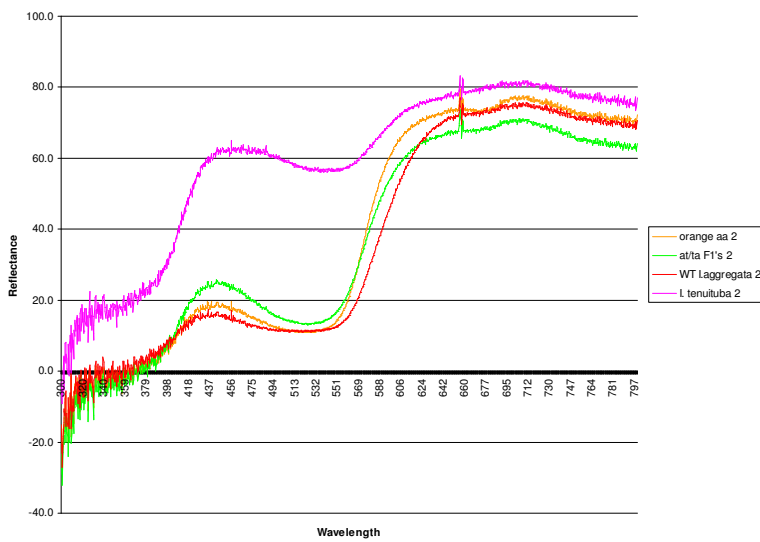


Figure 3: Reflectance of the for types of plants in the second array. The reflectance of the *I. tenuituba* is the only different.

Table 1: Total visits of the pollinators to the plants in the array. (a) Visits of the hummingbirds in the first array ($X^2 = 75.10810811$, $p = 3.44E-16$, $df = 3$). (b) Visits of the hawkmoths in the first array ($X^2 = 4.851852$, $p = 0.182972$, $df = 3$). (c) Visits of the hummingbirds in the second array ($X^2 = 19.85714$, $p = 0.000182$, $df = 3$).

(a)

	w	t	a	h
obs.	32	3	2	0
exp.	9.25	9.25	9.25	9.25

(b)

	w	t	a	h
obs.	6	11	7	3
exp.	6.75	6.75	6.75	6.75

(c)

	w	t	a	h
obs.	17	1	24	14
exp.	14	14	14	14

Table 2: Contingency tables of the movements of the pollinators between the plants in the array to determine their fidelity. (a) Movements of the hummingbirds in the first array ($p = 0.477306$). (b) Movements of the hawkmoths in the first array ($p = 0.7297001$). (c) Movements of the hummingbirds in the second array ($p = 0.471261$).

(a)

	w	t	a	h	Total Rows
o	18.5	2.5	0.5	0.5	22
w	12.5	1.5	1.5	0.5	16
t	2.5	0.5	0.5	0.5	4
a	0.5	0.5	1.5	0.5	3
h	0.5	0.5	0.5	0.5	2
Total Columns	16	3	4	2	25

(b)

	w	t	a	h	Total Rows
o	1.5	2.5	4.5	0.5	9
w	2.5	1.5	0.5	1.5	6
t	2.5	3.5	2.5	1.5	10
a	1.5	3.5	1.5	1.5	8
h	0.5	2.5	0.5	0.5	4
Total Columns	7	11	5	5	28

(c)

	w	t	a	h	Total Rows
o	6.5	0.5	14.5	8.5	30
w	4.5	1.5	4.5	2.5	13
t	0.5	0.5	1.5	0.5	3
a	3.5	0.5	3.5	2.5	10
h	4.5	0.5	2.5	2.5	10
Total Columns	13	3	12	8	36