Pollinator visitation rate and effectiveness vary with flowering phenology

Experiment: Pollinator effectiveness

Creator: M. Kate Gallagher [*1, 2, 3], Diane R. Campbell [1, 2]

[1] Department of Ecology and Evolutionary Biology, University of California, Irvine, CA, USA
[2] Rocky Mountain Biological Laboratory, Crested Butte, CO, USA
[3] The Botanical Garden, School of Plant Sciences and Food Security, Tel Aviv University, Tel Aviv, Israel

*AUTHOR FOR CORRESPONDENCE:
M. Kate Gallagher
Tel: +847 5714839
Email: megankgallagher@gmail.com

Year of study:

2016

Location of study:

Plants used in this experiment were wild *M. ciliata* populations along Rustler's Gulch (38° 59.6'N, 107° 0.5'W; 3,009 m. a.s.l.) and Schofield Pass (39°00'54.98" N, 107° 2'49.40" W; 3,263 m.a.s.l.).

Keywords:

Bombus, *Boraginaceae*, *Mertensia*, mutualism, pollinator effectiveness, pollen deposition, pollinator visitation

Purpose:

To test whether floral visitors differ in their effectiveness as pollinators to the subalpine perennial, *Mertensia ciliata* (Boraginaceae), we measured pollen receipt and seed set resulting from single visits to virgin flowers by bumblebees, solitary bees, and flies.

Abstract:

1. Rising temperatures and changing precipitation patterns associated with climate change are altering the seasonal timing (i.e. phenology) of flower production and of pollinator activity. Phenological shifts may alter pollination success via changes in the total pollinator visitation rate and the types of pollinator visitors, potentially impacting pollen receipt and seed set.
2. We investigated mechanisms by which changes in flowering phenology influences pollination and reproductive success of the subalpine perennial, *Mertensia ciliata* (Boraginaceae). We manipulated flowering onset of potted plants and assessed the frequency and composition of pollinator visitors, as well as pollen receipt and seed set. We also tested whether floral visitors differed in their effectiveness as pollinators by measuring pollen receipt and seed set resulting from single visits to virgin flowers by bumblebees, solitary bees, and flies.
3. Despite a five-fold decrease in pollinator visitation over four weeks, we detected no significant difference in conspecific pollen receipt or seed set among phenology treatments. Measurements of single-visit pollinator effectiveness revealed that, on a per-visit basis, each bumblebee transferred more conspecific pollen than did a solitary bee or a fly. Thus, while the total pollinator visitation rate declined over the season, because the proportion of visits by more effective worker bumblebees increased, differences in flowering phenology had no net effect on seed set
4. Estimating rates of successful pollination from the summed product of each pollinator taxon's effectiveness and per-week visit rate did not accurately predict the observed levels of pollen

receipt, but did predict similar patterns of reproductive success in terms of seed set.

5. This work illustrates the need to consider pollinator effectiveness, both in terms of conspecific and heterospecific pollen deposition as well as seeds produced, along with changes in pollinator visitation and species composition to understand the mechanisms by which shifts in phenology impact levels of pollination in the face of climate change.

Note: For details on phenology manipulation experiment, see:
gallagher&campbell_nonSpatialData_phenologyExperiment.docx
gallagher&campbell_phenologyExperimentData.xlsx

Methodology:

To estimate the single-visit pollinator effectiveness of different insect visitors, in 2016 we measured pollen receipt and seed set resulting from single visits to virgin *M. ciliata* flowers in wild populations at Rustler's Gulch (June 27-July 12) and Schofield Pass (July 12-29).

Experimental Design

Unopened flowering cymes on individual ramets were bagged with fine mesh jewelry bags to provide a supply of virgin flowers. For each single-visit ramet, two additional ramets in the same clone were bagged to serve as controls. The first control group remained bagged throughout the experiment to serve as a control for self-pollination. Non-production of seeds by plants in the bagged-control group would indicate that despite being self-compatible, *M. ciliata* flowers are not self-pollinating and therefore require insect pollination. Cymes in the second control group (hereafter open control) were made available to pollinators during observation periods but not observed to be visited, thus serving as a control for missed visits by observers. Each group of three ramets, including the single-visit ramet and two control ramets, belonged to the same *M. ciliata* clone, and no clone was used for more than one group of three.

Pollinator visit data

Bags were removed from flowering cymes on single-visit and open control ramets during observation periods. We recorded the number of flowers available on single-visit and open control ramets during each observation period. Once a single visit to a single flower was received on the single-visit ramet, the visited flower was marked on the calyx with permanent marker and both the open control and single-visit ramet were re-bagged to prevent further pollination and loss of seeds. For each visitor, we recorded the pollinator identity and number of flowers visited. Only insects that crawled inside of a corolla were counted as visitors. When a single visit was observed to an open control ramet, we re-designated that ramet as a single-visit ramet and marked the visited flower(s).

We monitored 235 marked flowers from 95 ramets that received single visits, as well as 65 open control and 74 bagged control ramets. The most common pollinators, bumblebees (*Bombus spp.)* and flies (Muscoidea, hereafter flies), accounted for 97.5% of visits, with solitary bees (*Osmia spp*. 2.1%) and a syrphid fly (Syrphidae 0.4%) making up the rest. We excluded the syrphid fly from our analyses.

Floral trait measurements

For single-visit ramets, we measured corolla width and length of marked flowers, or if marked flowers were withered or had fallen off, we calculated mean corolla width and length from up to five randomly selected flowers on the same ramet. In a few cases, more than one flower was visited on the single-visit ramet. When this happened, we marked the calyx of each visited flower, and used mean trait values (e.g., pollen receipt, seed set, etc.) for these ramets in our analyses.

Fitness component measurements

To measure conspecific and heterospecific pollen receipt, we collected stigmas from marked flowers on single-visit ramets, as well as one randomly selected flower from each open control ramet. Stigmas were collected after the corollas fell from the flowers and stigma squashes were made with fuchsin gel (Kearns & Inouye 1993). For an average of 15.5 ± 3.4 flowers per visitor/control type we counted the number of conspecific and heterospecific pollen grains using a compound microscope at 200×.

For each single-visit ramet, we counted the number of seeds produced per marked flower and, when a ramet had more than one marked flower, calculated mean seed set of marked flowers as (number of mature seeds / number of marked flowers). For each open and bagged control ramet, we calculated mean seed set per flower as (number of mature seeds / number of bagged flowers). Mature seeds were collected in coin envelopes and transported to the University of California, Irvine to be weighed. We calculated mean seed mass per flower as (mass of collected seeds / number of collected seeds). Ramets that failed to set seed because of herbivory or accidental damage were excluded from analyses.

Who collected the data?

M. Kate Gallagher, Allison Hacker, Hannah Clements, Wilnelia Recart, Syed Haque

What is in empty cells?

9999999

Variable Descriptions

Workbook: gallagher&campbell_pollinatorEffectivenessData.xlsx

All Data

Worksheet: Exp2.4_2016_AllData

plantID – Plant Identification Number

Plant Identification Number.

This is a six-digit number randomly assigned to each plant (133###). In a few cases, plant IDs include letters (133###a, 133###b, etc.).

Type: Numeric (Factor)

week – Observation week

Observation week

Observations were made over five weeks at Rustler's Gulch (June 27-July 12) and Schofield Pass (July 12-29).

Type: Numeric (Factor)

site – Site

Location of plant.

Plants were located at two sites. (RG = Rustler's Gulch; SP = Schofield Pass).

Type: Text

treat – Plant treatment

Plant treatment

Plants were assigned to one of three treatments (V = visited; O = Open control; B = Bagged control). See methods for description of controls. Pollen deposition was counted for open and visited flowers only.

Type: Text

visID – Pollinator Visitor Identity

Type of pollinator visitor or control

Pollinator visitors were identified to genus, except for flies which were identified to superfamily (Bombus; Muscoidea; Osmia; Open = Open control; Bagged = Bagged control).

Type: Text

ColDate.PolObs – Pollinator observation date

Date the pollinator observations were made.

Date format: dd-mmm-yy

Type: Date

flrsAvail – number of flowers available

Number of marked flowers available to the pollinators or within the control bags on the day of observations were made.

Type: Numeric

flrsVisited – number of flowers visited by pollinators

Number of flowers receiving single visits per ramet.

Once a single visit to a single flower was received on the single-visit ramet, the visited flower was marked on the calyx with permanent marker and both the open control and single-visit ramet were re-bagged to prevent further pollination and loss of seeds. For each visitor, we recorded the pollinator identity and number of flowers visited. Only insects that crawled inside of a corolla were counted as visitors. In some cases more than one flower was visited on the single-visit ramet. When this happened, we marked the calyx of each visited flower, and used mean trait values (e.g., pollen receipt, seed set, etc.) for these ramets in our analyses.

Type: Numeric

noFlrs – number of flowers marked/bagged

Number of marked flowers on single-visit ramets or bagged flowers on control ramets.

Type: Numeric

seedCnt – Seed Count

Total number of seeds counted per marked flower for single-visit ramets or total seeds on bagged flowers of control ramets.

Type: Numeric

Measurement unit: Seeds

seedPerFlr – Seeds per flower

Number of seed produced per flower = seedCnt / noFlrs

For each single-visit ramet, we counted the number of seeds produced per marked flower and, when a ramet had more than one marked flower, calculated mean seed set of marked flowers as (number of mature seeds / number of marked flowers). For each open and bagged control ramet, we calculated mean seed set per flower as (number of mature seeds / number of bagged flowers).

Type: Numeric

Measurement unit: Seeds per flower

mcorL – mean corolla length

Mean corolla length of visited flowers and open-control flowers.

For single-visit ramets, we measured corolla width and length of marked flowers, or if marked flowers were withered or had fallen off, we calculated mean corolla width and length from up to five randomly selected flowers on the same ramet.

Type: Numeric

Measurement unit: mm

mcorW – mean corolla width

Mean corolla width of visited flowers and open-control flowers.

For single-visit ramets, we measured corolla width and length of marked flowers, or if marked flowers were withered or had fallen off, we calculated mean corolla width and length from up to five randomly selected flowers on the same ramet.

Type: Numeric

Measurement unit: mm

polDepMC– Pollen Deposition Mertensia ciliata

Count of *M. ciliata* pollen on the slide.

Type: Numeric

Measurement unit: pollen grains

polDepNonMC– Pollen Deposition non-Mertensia ciliata

Count of non-*M. ciliata* pollen on the slide.

Type: Numeric

Measurement unit: pollen grains

sCnt.mass – Seed Count of weighed seeds

Count of seeds collected for weighing.

Type: Numeric

Measurement unit: Seeds

sMass – Seed Mass

Mass of seeds collected.

Mature seeds were collected in coin envelopes and transported to the University of California, Irvine to be weighed.

Type: Numeric

Measurement unit: Milligrams

msMass – Mean Seed Mass

Mean seed mass of collected seed = sMass / sCnt

We calculated mean seed mass per flower as (mass of collected seeds / number of collected seeds). Ramets that failed to set seed because of herbivory or accidental damage were excluded from analyses.

Type: Numeric

Measurement unit: Milligrams

wDate – Weigh Date

Date the seeds were weighed.

Date format: dd-mmm-yy

Type: Date

wInit – Weigh Initials

Initials of the person weighing the seeds.

AS = Aurash Siah

BO = Brittany Ottoson

KG = Kate Gallagher

Type: Text

Seed Set

Worksheet: Exp2.4_2016_seedCnt

plantID – Plant Identification Number

Plant Identification Number.

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Type: Numeric (Factor)

week – Observation week

Observation week

Observations were made over five weeks at Rustler's Gulch (June 27-July 12) and Schofield Pass (July 12-29).

Type: Numeric (Factor)

site – Site

Location of plant.

Plants were located at two sites. (RG = Rustler's Gulch; SP = Schofield Pass).

Type: Text

treat – Plant treatment

Plant treatment

Plants were assigned to one of three treatments (V = visited; O = Open control; B = Bagged control). See methods for description of controls. Pollen deposition was counted for open and visited flowers only.

Type: Text

visID – Pollinator Visitor Identity

Type of pollinator visitor or control

Pollinator visitors were identified to genus, except for flies which were identified to superfamily (Bombus; Muscoidea; Osmia; Open = Open control; Bagged = Bagged control).

Type: Text

flrsAvail – number of flowers available

Number of marked flowers available to the pollinators or within the control bags on the day of observations were made.

Type: Numeric

flrsVisited – number of flowers visited by pollinators

Number of flowers receiving single visits per ramet.

Once a single visit to a single flower was received on the single-visit ramet, the visited flower was marked on the calyx with permanent marker and both the open control and single-visit ramet were re-bagged to prevent further pollination and loss of seeds. For each visitor, we recorded the pollinator identity and number of flowers visited. Only insects that crawled inside of a corolla were counted as visitors. In some cases more than one flower was visited on the single-visit ramet. When this happened, we marked the calyx of each visited flower, and used mean trait values (e.g., pollen receipt, seed set, etc.) for these ramets in our analyses.

Type: Numeric

noFlrs – number of flowers marked/bagged

Number of marked flowers on single-visit ramets or bagged flowers on control ramets.

Type: Numeric

seedCnt – Seed Count

Total number of seeds counted per marked flower for single-visit ramets or total seeds on bagged flowers of control ramets.

Type: Numeric

Measurement unit: Seeds

seedPerFlr – Seeds per flower

Number of seed produced per flower = seedCnt / noFlrs

For each single-visit ramet, we counted the number of seeds produced per marked flower and, when a ramet had more than one marked flower, calculated mean seed set of marked flowers as (number of mature seeds / number of marked flowers). For each open and bagged control ramet, we calculated mean seed set per flower as (number of mature seeds / number of bagged flowers).

Type: Numeric

Measurement unit: Seeds per flower

Init – Initials

Initials of the person taking the measurements.

KG = Kate Gallagher

WR = Wilnelia Recart

CW = Clarissa Whiting

Type: Text

date - Date

Date the data were recorded

Date format: dd-mmm-yy

Type: Date

note – Note

Notes

We noted seeds collected, lost, or other notes about collecting seeds.

Type: Text

Pollen deposition

Worksheet: Exp2.4_2016_pollenDep

plantID – Plant Identification Number

Plant Identification Number.

This is a six-digit number randomly assigned to each plant (133###). In a few cases, plant IDs include letters (133###a, 133###b, etc.).

Type: Numeric (Factor)

week – Observation week

Observation week

Observations were made over five weeks at Rustler's Gulch (June 27-July 12) and Schofield Pass (July 12-29).

Type: Numeric (Factor)

site – Site

Location of plant.

Plants were located at two sites. (RG = Rustler's Gulch; SP = Schofield Pass).

Type: Text

treat – Plant treatment

Plant treatment

Plants were assigned to one of three treatments (V = visited; O = Open control; B = Bagged control). See methods for description of controls. Pollen deposition was counted for open and visited flowers only.

Type: Text

visID – Pollinator Visitor Identity

Type of pollinator visitor or control

Pollinator visitors were identified to genus, except for flies which were identified to superfamily (Bombus; Muscoidea; Osmia; Open = Open control; Bagged = Bagged control).

Type: Text

polDepMC– Pollen Deposition Mertensia ciliata

Count of *M. ciliata* pollen on the slide.

Type: Numeric

Measurement unit: pollen grains

polDepNonMC– Pollen Deposition non-Mertensia ciliata

Count of non-*M. ciliata* pollen on the slide.

Type: Numeric

Measurement unit: pollen grains

init – Counting Initials

Initials of the person counting the pollen.

MKG = M. Kate Gallagher

Type: Text

date – Counting Date

Date the pollen were counted.

Date format: dd-mmm-yy

Type: Date

note – Pollen counting note

Notes.

Notes regarding the counting process (i.e., irregularities, etc.).

Type: Text