

HEFTY VS WIMPY:
A BIOMECHANICAL HYPOTHESIS FOR PLANT POLLINATOR ASSOCIATIONS

By

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ABSTRACT

Although research has suggested that biomechanics of flowers are linked to energetics in pollinator foraging behavior, no studies have quantified foraging choices based on differences in biomechanics among floral species.. Here we examined pollinator foraging behavior on the co-occurring clover species *Trifolium parryi* and *T. dasyphyllum*, which naturally differ in stem strength and overall sturdiness. We documented foraging behaviors of the long-tongued alpine bumblebee *Bombus balteatus*, which readily forages on either *Trifolium* species but normally prefers the sturdier *T. parryi*. Through artificial manipulation of stem and pedicel sturdiness of the weaker clover, *T. dasyphyllum*, we tested whether flower biomechanics influence foraging transitions and proportion of visits between the two clover species.

Queen *B. balteatus* foraging in mixed species arrays with altered *T. dasyphyllum* flowers demonstrated increased preference for and fidelity to *T. dasyphyllum* compared to queens foraging in control arrays. Overall, strengthening the weaker flowers resulted in queens demonstrating random foraging among *T. dasyphyllum* and *T. parryi* inflorescences. These results suggest that floral preferences of long-tongued pollinators such as *B. balteatus* are driven by biomechanical aspects of the flowers.

INTRODUCTION

Over recent years, much concern has been expressed about the potential harmful effects of pollinator decline. Over one third of global food production depends on animal-mediated pollination (Klein et al. 2007; Olsson et al. 2015), the most important of which are bees. While honeybees are of primary importance in agriculture, wild bees are essential to native plant pollination, with 87.5% of all flowering plant species dependent upon biotic pollinators (Breeze et al. 2011; Ollerton et al. 2001). Although pollinator decline is due to a number of reasons, floral resources play a significant role (Goulson et al. 2015; Vaudo et al. 2015). Seeking to understand the mechanisms driving pollinator foraging choices could provide insight into factors surrounding important floral resources for pollinator conservation efforts.

Bumblebees are generalist foragers, though every bumblebee species visits a unique network of floral species. It is thought that in any given habitat, bee species will divide themselves among floral resources based off of particular flower characteristics (Vaudo et al 2015). Foraging choices based on floral “interaction traits” should align with optimal foraging theory, which predicts that organisms will forage in a manner that maximizes energy gain over energy expenditure. Optimal foraging theory is often analyzed in terms of currencies such as time and energy, which ideally act as proxies for fitness (Houston and McNamara 2014; Pyke 2016). For bumblebees, perhaps the most effective currencies are efficiency and net rate of energy intake (Charlton and Houston 2010).

To date, studies of the mechanisms driving pollinator partitioning of floral resources have focused almost exclusively on the role of matching between the size of the proboscis or

tongue and the corolla lengths of the flowers they forage on. It is thought that the corolla to tongue length pairing is in an effort to reduce the handling time that bees have to spend on each flower, thereby increasing efficiency (Corbet et al. 1995; Anderson et al. 2008).

However, it is sometimes overlooked that proboscis and corolla length often covary with other traits, such as pistil and stem length or stem and pedicel strength (Conner and Sterling 1995; Galen and Cuba 2001; Geib 2010).

One relatively underexplored context from which to examine the ways floral interaction traits mediate pollinator foraging is the field of biomechanics, a study of how the physics in motion surrounding an organism influences its utility (Whitney and Federle 2013; Whitaker et al. 2007). Examples of biomechanical adaptations have been well-documented throughout the study of plant-pollinator coevolution (Whitney and Federle 2013). For example, many flowering plants have conical cells on their petals which can improve the pollinator's grip on the petal's surface (Whitney et al. 2009). When triggered, bunchberry (*Cornus canadensis*) stamens spray pollen straight upwards to maximize both the amount of pollen stuck on pollinating insects and the amount of pollen successfully dispersed by wind (Whitaker et al. 2007). However, with the exception of tongue-corolla length matching, the role that quantitative variation in floral biomechanics plays in pollinator niche partitioning is not well understood.

Here I hypothesize that quantitative differences in floral biomechanics have implications for pollinator choice while foraging. Specifically, I questioned whether stem and pedicel strength differences in the co-occurring closely related flowers *Trifolium daysphyllum* and *T. parryi* impact the frequency of visits and fidelity of a shared pollinator,

Bombus balteatus. By artificially strengthening the weaker *T. dasyphyllum* flowers, I sought to increase the preference of *Bombus balteatus* queens for them.

METHODS

Study Site and System

Experiments were conducted during June-August of 2014 and 2015 at the base of the alpine habitat on Pennsylvania Mountain in Park County, Colorado. The plant species studied, *T. dasyphyllum* and *T. parryi*, are two native, naturally co-occurring clovers. Inflorescences of the species are similar in many aspects, including floral structure and complexity, lack of UV reflection to guide visitors to nectaries, average number of florets per inflorescence, and nectar quality (17.2 vs 16.9 and 79.7 ug sugar/ml vs 77.0 ug sugar/ml for *T. dasyphyllum* and *T. parryi*, respectively; Geib 2010). The clover inflorescences differ in corolla length, with *T. parryi* corolla tubes measuring, on average, 1.4 times longer than those of *T. dasyphyllum* (21.6 mm vs 15.5 mm, respectively; Geib 2010). In addition, *T. parryi* is significantly sturdier than *T. dasyphyllum* with respect to both scape and pedicel strength (Geib 2010).

The study bee species, *Bombus balteatus*, is a native long-tongued alpine bumblebee that, unlike its subalpine counterparts, forages throughout the blooming season from mid-June to mid-August. *B. balteatus* is relatively large compared to other species that comprise the community of bumble bees on Pennsylvania Mountain. *B. balteatus* readily forages on both alpine clovers, but demonstrates preference for *T. parryi* despite the fact that *T. dasyphyllum* inflorescences are more abundant in mixed patches (86% vs 14%, respectively for *T. dasyphyllum* and *T. parryi*; Geib 2010).

Classic studies of optimal foraging in pollinators would argue that preference for *T. parryi* is reflective of matching *B. balteatus* long tongue length to the longer corolla length of *T. parryi*, resulting in efficient foraging associated with faster flower handling speeds. However, Geib (2010) showed that floret handling times of *B. balteatus* queens did not differ among the clovers. Instead, Geib (2010) examined the covarying trait of flower sturdiness, and found that handling effort of foraging bees varied with stem and pedicel strength. When presented with a flower, a bee has the option to either perch on top of it, hang on to the side of the flower by her legs, or hover in the air over the flower. Each method of foraging should be more energetically costly than the previous, because each new option requires the bee to use more of its own energy and rely less on the flower for support. Hanging off of the side of a flower will be more costly to the bee than landing on it because it has to not only use its own weight for support, but also has to deal with tension in its legs. Hanging can especially costly if the flower is “floppy” and bends around from the bee’s weight, because movement of the flower will only add to this tension. Hover foraging should be the most costly of all choices, because the bee has to support its entire weight on its own without any support from the flower (Geib 2010).

Experimental design

I built on previous foraging mechanic studies to investigate whether I could alter *B. balteatus* preference by artificially strengthening the weaker stems and pedicels of *T. dasyphyllum*. I conducted all foraging trials in bottomless mesh tent enclosures (either tan 1.25 m x 1.95 m x 1.25 m or black 2.2 m x 2.2 m x 1.25 m); similar experiments in the past were shown to produce the same results as those recorded in the field (Geib 2010).

I used only the queen caste of *B. balteatus* in foraging trials because their greater mass compared to workers should exaggerate the natural effect flower strength plays on handling effort. I captured *B. balteatus* queens in the field, then cooled them on ice for transport to the mesh enclosure. After being allowed to awaken from torpor, each individual bee (N = 25) was randomly assigned to forage on one of two arrays: a control array intended to verify previously documented preference of *B. balteatus* queens and an experimental array to test for a change in preference with *T. dasyphyllum* artificially strengthened (N = 12 and N = 13 independent trials, respectively).

All arrays consisted of a square grid of interspersed *T. dasyphyllum* and *T. parryi* inflorescences (N = 4 virgin inflorescences per clover species; Fig. 1) placed in water piks. Inflorescences were haphazardly selected from blooming specimens in the field that had been previously covered with wire screen mesh while in the bud stage. The center of the array was occupied by a “starter” waterpik containing one virgin inflorescence of each clover. Water pik centers were spaced 10 cm apart. In control arrays, all inflorescences were left completely unsupported in 2.5 cm tall water piks. In experimental arrays, *T. parryi* was unsupported, but *T. dasyphyllum* was artificially supported using 7.5 cm tall water piks around the scape and white labeling tape around the pedicel. In both treatments, all flowers were cut to match the height of the water pik-supported starter.

Each queen was released and allowed to forage openly on the grid of inflorescences for one bout, which consisted of 10 minutes of foraging time off of the starter. The number of inflorescence visits and dips into the florets of each clover were recorded using Sony IC (ICD – UX200) and Olympus (WS – 200S) digital voice recorders. Bees were marked with non-toxic Sharpie paint markers before release to prevent the use of recaptures.

Statistical analysis

I conducted a number of analyses to assess whether I was able to experimentally alter *B. balteatus* foraging by manipulating the strength of the weaker host. I fit parametric distributions (PROC UNIVARIATE, SAS 10.0), and used Student's t test to determine whether the observed distribution mean differed from the expected value for two measures of foraging bias in a) mean proportion of floret visits to each clover species per trial ("preference") and b) mean proportion of transitions among the four transition types possible for each trial ("constancy"). Expectations for the null hypothesis were based off of probabilities of random foraging: 0.50 for mean proportion of floret visits for each clover species and 0.25 for mean proportion of each transition type. For data that failed the Shapiro-Wilk test for normality we used a sign-rank test rather than Student's t. We also conducted Analysis of Variance to assess whether mean proportions of total floret visits and mean proportion of each transition type was different between the control and experimental arrays.

RESULTS

Queen *B. balteatus* foraging behavior in control experiments reaffirmed their previously supported preference for *T. parryi*. Bees visited *T. parryi* florets at a significantly higher proportion than as if by random choice ($t=3.71$, $p < 0.01$; Fig. 2A). Additionally, bees were less constant on *T. dasycyllum* during control treatments; *T. dasycyllum* to *T. dasycyllum* movement accounted for a disproportionately small proportion of all transition types ($t = -6.00$, $p < 0.0001$; Fig. 2A).

Alternatively, *B. balteatus* preference in experimental treatments was most similar to random choice. Analysis found that overall proportion of visits in both *T. parryi* and *T. dasyphyllum* did not deviate from a 0.50 probability ($t = 0.410$, $p = 0.69$ and $t = -0.410$, $p = 0.69$, respectively). However, results showed the same patterns for constancy in the experimental trials as in the control with one exception: *T. dasyphyllum* to *T. parryi* transitions increased from random in the control ($t = 0.514$, $p = 0.62$) to higher than expected in the experimental ($t = 2.23$, $p = 0.046$; Fig. 2B).

DISCUSSION

By artificially strengthening *T. dasyphyllum*, *B. balteatus* queen choice changed from a definite preference toward *T. parryi* to an equal preference between the two clovers with respect to both constancy and proportion of visits. We therefore believe that we have presented a credible alternative hypothesis as to why *B. balteatus* queens prefer *T. parryi* over *T. dasyphyllum*. Our results suggest that floral preferences of long-tongued pollinators such as *B. balteatus* are driven by biomechanical aspects of the flowers.

While previous work on corolla to tongue length matching approached optimal foraging currency from an efficiency perspective, our biomechanical hypothesis investigates bumblebee choice from a net energy intake point of view. By preferentially foraging on *T. parryi*, *B. balteatus* queens minimize their energy loss by relying more on the flower for support than on their own strength (Geib 2010). If net energy intake and efficiency are equally valid measures of currency (Charlton and Houston 2010), we have proposed a credible hypothesis for *Trifolium* – *Bombus* association.

Our results may be especially significant considering that we conducted studies in the alpine habitat, which is characterized by a short growing season and harsh winds (Körner 1999). With less time to produce new queens and males, foraging optimally may be especially important for alpine bees. Additionally, our enclosed studies may have underrepresented the effect of biomechanics on bee choice, because experimental flowers were shielded from the wind.

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FIGURES

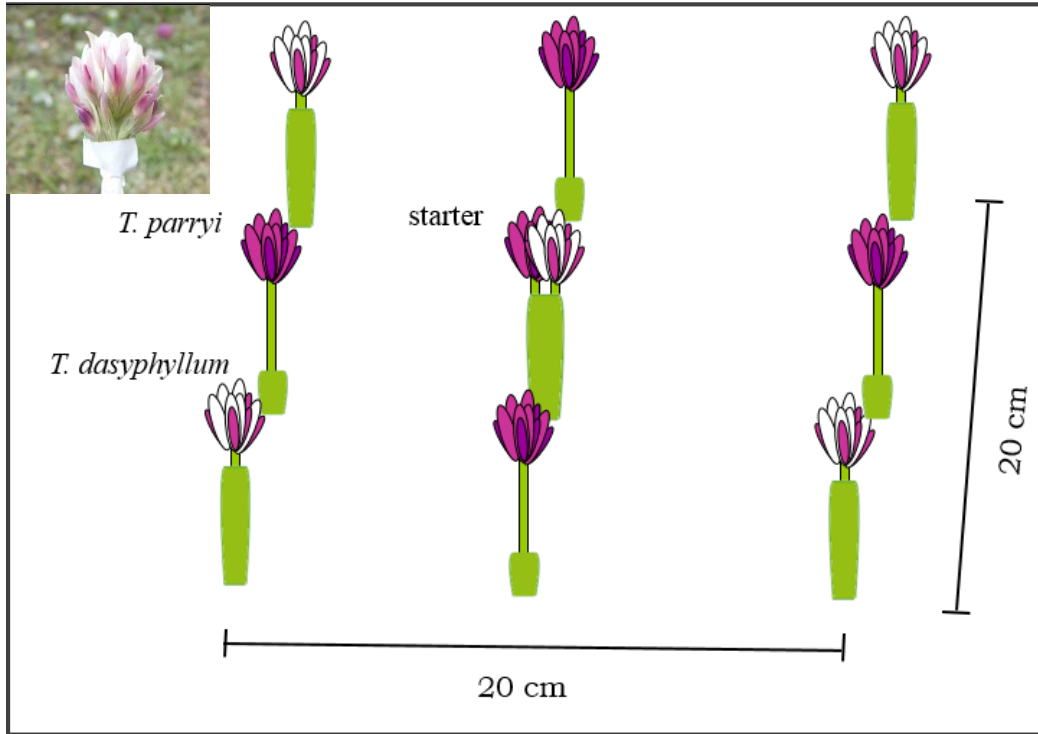


Fig 1. Experimental floral array with *T. parryi* unsupported and *T. dasyphyllum* artificially strengthened with water piks and labeling tape; four of each clover type surrounds a starter containing one inflorescence of each clover.

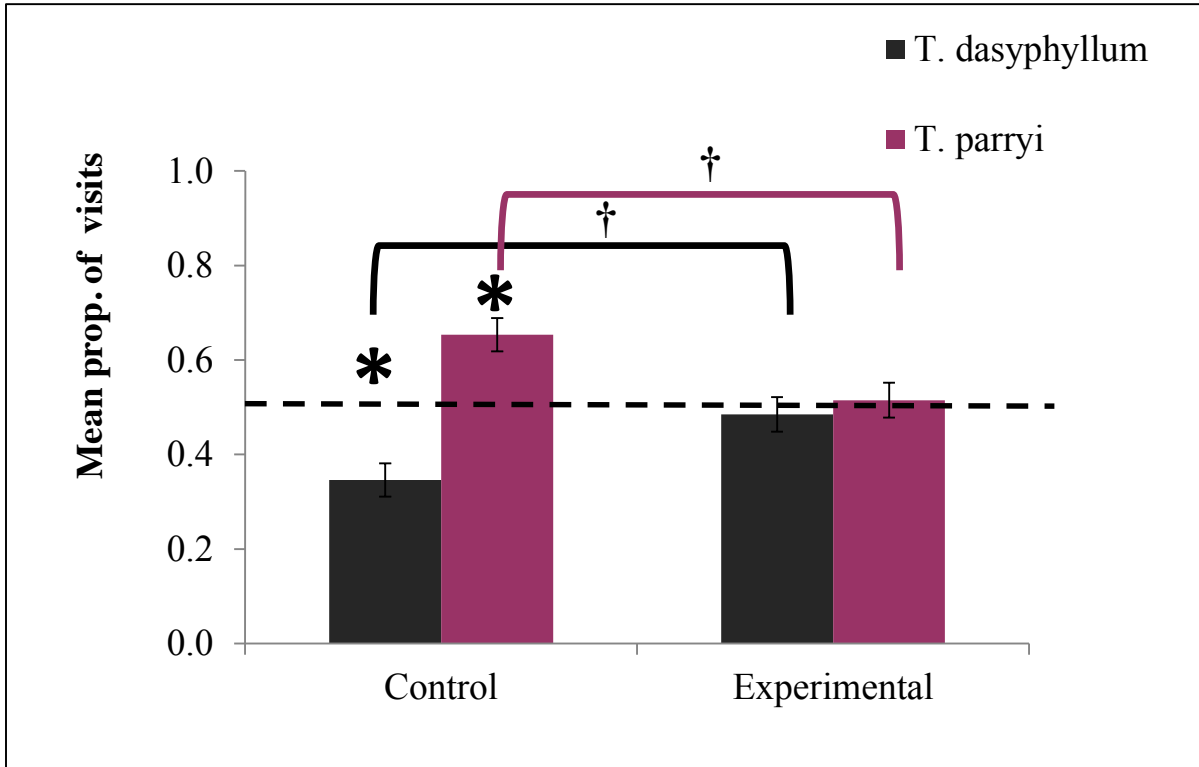


Fig 2. Mean proportion of *B. balteatus* queen visits between control (all flowers unsupported) and experimental (*T. parryi* unsupported and *T. dasyphyllum* artificially strengthened) arrays. Asterisks denote where the mean differs significantly from the expected value (0.5, signified by the dashed line), and daggers denote where two means significantly differ from each other.

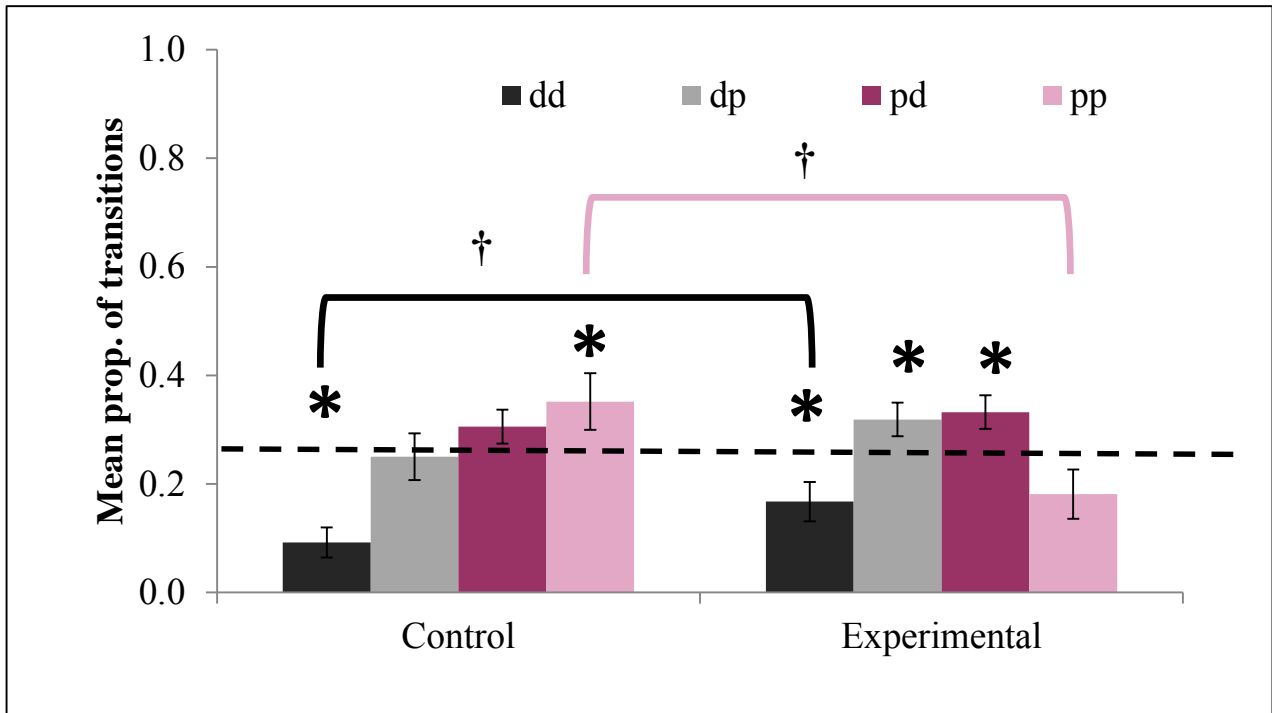


Fig 3. Constancy of *B. balteatus* queens among clovers in control (all flowers unsupported) and experimental (*T. parryi* unsupported and *T. dasyphyllum* artificially strengthened) arrays. Asterisks denote where the mean differs significantly from the expected value (0.5, signified by the dashed line), and daggers denote where two means significantly differ from each other.