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ORIGINAL PAPER

Division of labor of anthers in heterantherous plants: flexibility of bee pollen collection behavior may serve to keep plants honest

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Abstract Heteranthery is thought to reflect a division of labor, with some anthers serving a pollinator-feeding function and others serving a pollinating function. Mutualism theory predicts that each participant should try to maximize the benefit it receives from its partner: plants should allocate more pollen to pollination, and pollinators should collect more pollen. Accordingly, plant and pollinator may engage in a 'tug of war' with respect to pollen from each anther type, resulting in incomplete division of labor. Here, we explored this idea by conducting a fully factorial manipulation of the availability of pollen in long and short anthers of staminate flowers of *Solanum houstonii*. We found the following: (1) Bumble bees (*Bombus impatiens*) preferred to sonicate (collect pollen from) short anthers over long anthers, consistent with a role as feeding and pollinating anthers, respectively; (2) Blocking short anther pores alone increased sonication of long anthers and resulted in collection of pollen from long anthers; (3) Blocking long anther pores alone did not influence sonication of short anthers; (4) The increase in sonication of long anthers, when short anther pores are blocked, was greater when pollen was available in long anthers; (5) Despite shifting sonication effort to long anthers, bees do not move their bodies closer to long anther pores where pollen could be collected more effectively; and (6) analysis of the growth of corbicular loads over time spent buzzing indicates that significant amounts of pollen are collected from long anthers as well as short anthers. We conclude that bees can flexibly increase pollen collection from pollinating anthers, but are constrained from fully exploiting this pollen. This results in checks and balances between plant and bee that may help maintain heteranthery.

Keywords Heteranthery · Pollen collection · Floral sonication · Behavioral plasticity · Buzz pollination · Bumble bee

Introduction

Theory suggests that mutualisms are best viewed as reciprocal exploitation in which each partner achieves a net benefit (Maynard Smith and Szathmáry 1995; Leigh and Rowell 1995; Doebeli and Knowlton 1998). For example, plants that offer only pollen as a floral reward are faced with a 'pollen dilemma,' involving the need to give up pollen to a pollen consumer in order to have pollen transferred by that consumer (Westerkamp 1996; Lunau et al. 2015; Paulino et al. 2016). This conflict is thought to have led to the evolution of mechanisms to constrain pollen collection while maintaining pollen transfer by the pollinator (Hargreaves et al. 2009; Lunau et al. 2015). One such strategy is heteranthery. Hetheranthery, defined as the occurrence of multiple types of stamens within a flower, was first proposed by Muller (1883) to constitute a division of labor among the stamens, in which anthers of some stamens serve a

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pollinator-feeding function while anthers of others serve a pollen-transfer function. Typically associated with plants offering only pollen as the floral reward for bees, heteranthery is found in over 20 families of 12 orders, being especially common in the Fabaceae-Cesalpinioidea, Melastomataceae, Pontederiaceae, and Solanaceae (Buchmann 1983; Barrett 2010; Vallejo-Marín et al. 2010). Support for the division of labor hypothesis was provided by Bowers (1975) who found that fluorescent dye placed on the longer presumptive pollinating anthers of *Solanum rostratum* Dunal was more likely to be transferred by bees to stigmas than dye on the shorter presumptive feeding anthers. Vallejo-Marín et al. (2009) found that bees engaging in floral sonication behavior ("buzzing") on *S. rostratum* preferentially buzzed the feeding anthers and that the pollinating anthers transferred more pollen than the feeding anthers to stigmas (see also Luo et al. 2008).

These important studies have resolved much about the function of heteranthery in angiosperms. Nevertheless, questions remain about the evolution of this floral trait in the context of the division of labor hypothesis. Applying the perspective of reciprocal exploitation to heteranthery, we might expect that bees and plants should each try to use more of the pollen in each anther type for themselves than is in the other partner's evolutionary interests. This expectation leads us to predict that division of labor between the anther types will not be absolute, a prediction which some studies support (e.g., Gross and Kukuk 2001). We were interested here in features of the bee–plant interaction that might keep the evolution of one or the other partner in check with respect to how pollen from each anther type is used. How do bees prevent the plant from allocating more or all of the pollen to the pollinating anthers and thus giving little or nothing to the bee as a food reward in exchange for pollination services? How do plants prevent bees from collecting most or all of the pollen in the pollinating anthers as well as the feeding anthers, leaving little for pollination?

In this study, we addressed these questions by experimentally manipulating the availability of pollen in the two anther types. In particular, we asked whether individual bumble bees adjusted to the loss of pollen from the feeding anthers by sonicating the pollinating anthers more and potentially collecting pollen from them. We also asked if bees adjusted their position on the long anthers so as to collect pollen more effectively. Such flexible behavioral responses would presumably deter cheating by the plant. If on the other hand, bees showed an innate and inflexible tendency to sonicate the feeding anthers, cheating by the plant could presumably arise and be maintained. A similar manipulation of pollen availability was conducted by Vallejo-Marín et al. (2009) and Buchmann and Cane (1989) on another plant species, save for the treatment in which all anthers were blocked, which these authors did not conduct. This fourth treatment is a key feature of our experiment, because it results in a fully factorial design that allows us to assess the interaction between pollen availability of the different anther types on bee behavior. For example, the design enables us to ask a question that could not be addressed by previous studies: "Does an effect of the availability of pollen within the feeding anthers on bee behavior depend on its availability within the pollinating anthers?" We predicted that the availability of pollen for one anther type would indeed interact significantly with the availability of pollen for the other anther type.

Materials and methods

Plants and bees used

Our experiment used the Sonoran nightshade, *S. houstonii* Dunal (synonym: *Solanum tridynamum*; Solanaceae) as the focal plant and the Eastern Bumble Bee, *Bombus impatiens* Cresson (Hymenoptera: Apidae), as the focal pollinator (Fig. 1). *Solanum houstonii*, a member of *Solanum* section Nycterium, is distributed throughout much of western Mexico from close to the U.S. border in the north down to the Yucatan Peninsula in the south. The species is reproductively andromonoecious, having both perfect (bisexual or hermaphroditic) flowers and male (staminate) flowers on the same plant. Staminate flowers are heterantherous, bearing two short presumptive feeding anthers and three longer presumptive pollinating anthers (Fig. 1). Anthers are poricidal (dehiscing via small apical pores) and pollen can be extracted only by bees capable of using a complex pollen-harvesting behavior termed floral sonication (Buchmann and Cane 1989). Floral sonication involves bees biting the anthers and rapidly contracting indirect flight muscles in the thorax, thereby transmitting strong vibrations to

Fig. 1 *Solanum houstonii* being visited by a tagged *Bombus impatiens* bumble bee

the anthers that cause the pollen to be rapidly ejected (De Luca and Vallejo-Marin 2013; Russell et al. 2016b). No published information on the pollinators of *S. houstonii* in nature is available; however, bumble bees are known to be frequent visitors to the most closely related *Solanum* species in this group (e.g., *S. elaeagnifolium*; Buchmann and Cane 1989). While the range of *Bombus impatiens* does not overlap with that of *S. houstonii*, it was used because it is commercially available, extensively studied, and easily maintained in the lab. Flower-naïve *B. impatiens* workers readily sonicate *S. houstonii* and successfully collect pollen from both staminate and bisexual flowers (Russell et al. 2016b). Bees were obtained from Koppert Biological Systems (Howell, MI, USA). Colonies were maintained in darkness and at room temperature (ca. 26 C). The foraging arena (where bees fed on sucrose feeders when not being tested) was kept at 14:10 L:D photoperiod and at room temperature (ca. 26 C). Workers from three colonies were used in our experiment.

Experimental protocol

Our experimental design consisted of four treatments of test staminate flowers: Both long and short anthers open; both long and short anthers blocked with glue; long anthers blocked but short anthers open and; short anthers blocked but long anthers open. All flowers used in the experiment were obtained from greenhouse-maintained plants and had not been previously visited by bees. The four treatments were systematically alternated in time to control for effects of time on foraging behavior.

We manipulated pollen release from anthers using clear acetate glue (Elmer's Glue All, Elmer's Products, Inc.; Russell et al. 2016b). A drop of glue was applied to the apical tip of each poricidal anther with a clean toothpick and allowed to dry for 5 min. The glue sealed the anther pore, preventing pollen release. We controlled for possible odor effects of the glue on bee behavior by applying drops of glue to the distal sides of each anther on the test flower (without blocking the pores) and allowed the glue to dry for 5 min.

Testing was done in a foraging arena (L×W×H, 82×60×60 cm) in our laboratory at the University of Arizona, painted gray on floor and sides to provide a neutral background (Russell et al. 2016a). A single freshly clipped flower of one of the four treatments was mounted in its natural horizontal orientation on a custom-built water tube (Fig. 2a; Russell et al. 2016b) and the water tube mounted on a gray-painted foam block placed in the middle of the arena.

To obtain a bee for testing, 1–4 flower-naive individuals were introduced into the flight arena. When a bee landed on the test flower, the other bees were removed from the arena immediately and returned to their colony. In all treatments, each bee was tested only once on a fresh, previously unvisited flower of one of the four treatments. All flowers were used for only one assay with one test bee and afterwards were discarded. Similarly, a given bee was discarded after testing on the single treatment.

Visits by the bee to the test flower were recorded on a high-definition camcorder (Canon Inc.) positioned within the arena so as to provide a view of the flower in profile. Importantly, we took care to ensure that the position of the camera and flower, and the orientation of the flower in the field of view was the same in all assays. We allowed the bee to leave and return to the flower, until the bee had made 15 visits or until 5 min from its last visit had elapsed. At that time, we noted the occurrence, or not, of pollen on the bee's body and in its corbiculae (the bee's pollen-carrying apparatus on the hind leg). The bee was then removed from the foraging arena and euthanized.

To quantify the effect of anther manipulation on sonication behavior, we viewed the bee visitation videos at half-speed, recording the position of the mandibles during sonication buzzes. Bees clamp the anther with their mandibles and vibrate indirect muscles; the vibration is transmitted to the flower via the mandibles. We scored which anther type, short or long, was grasped (Mean

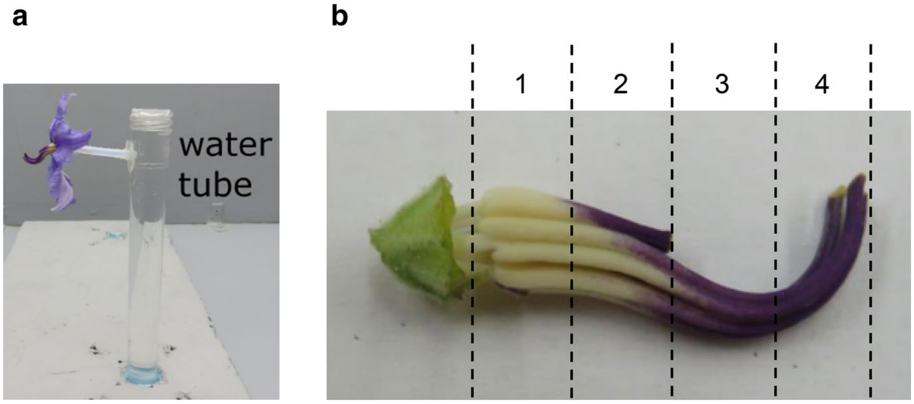

Fig. 2 **a** Staminate *Solanum houstonii* flower mounted on water tube for use in bee behavioral assays. **b** Side view of anthers and filaments dissected from an intact flower, indicating zones used to assess mandible bite positions by test bumble bees

number of grasps per bee across all visits = 338.11, SE = 37.05; $N = 35$ bees). We also recorded the duration of grasping by the mandibles. These data gave us measures of the overall percentage of time that a bee sonicated one anther type or another (percent time buzzing) and the overall percentage of sonication events that were on one anther type or another (percent sonication events). Results for both variables were identical, with only slight changes in p values in tests of significance (unpublished data); therefore, only percent time buzzing results are reported here. We also recorded where the anther was grasped along its length (zones used for scoring mandible position are shown in Fig. 2b) to assess how bees were positioning their bodies relative to the pores of each anther type, and if this position depended upon treatment.

Finally, we estimated the rate at which pollen was collected in each treatment combination by measuring the area of corbicular loads at the end of a given trial and dividing that area by the total time spent buzzing during the trial. Area was estimated as the number of pixels encompassed by the corbicular load of one of the legs in an image of the bee grabbed from video of its pollen foraging; estimates were made using the area function of ImageJ software (National Institutes of Health, Bethesda, MD, http://imagej.nih.gov/ij/). Efforts were made to select video frames in which the bee's leg was fully in profile, reducing problems of parallax.

Statistical analysis

All statistical analyses were performed using JMP Pro statistical software (version 12; SAS Institute, Cary, NC). To test the effect of the two treatments and their interaction on percent time buzzing, we utilized two-way analyses of variance testing for main effects and the interaction. Tukey's tests were employed in pairwise contrasts. To evaluate the bees' overall preference for long versus short anthers, we subtracted the mean percent time buzzing long anthers from 50% (50% being the null expectation of non-preference) and evaluated whether the intercept of a two-way ANOVA on this difference variable was significantly greater than zero. To evaluate the preferences in particular treatments, we conducted one-sample t tests in which mean percent time buzzing was tested against the a priori null expectation of 50%. To test the effect of the two treatments and their interaction on our pollen collection rate measure, we utilized two-way analyses of variance testing for main effects and the interaction. Tukey's tests were employed in pairwise contrasts.

Results

Pollen availability affected pollen collection but not flower visitation or attempts to collect pollen

Pollen availability did not prevent bees from attempting to collect pollen. All bees in all treatments, including the 'both anther types blocked' treatment, repeatedly sonicated the test flower (total $N = 35$ bees). Anther treatment did not influence the average duration of a visit; bees tended to make shorter visits when long anthers were blocked, but the effect was not significant (Online Resource 1, Fig. S1, Table S1; Two-way ANOVAs, main effects and interaction, ns). In particular, bees did not have shorter visits on flowers with all anthers sealed than flowers in other treatments (Two-way ANOVA, Tukey's test, ns). Pollen availability did, however, influence whether pollen was actually collected. No bees in the treatment in which all anthers were sealed with glue ($N = 8$) collected pollen, as indicated by the absence of pollen on their corbiculae, even though they sonicated such flowers vigorously. This result confirms that blocking anther pores successfully prevented pollen release under our experimental conditions. In contrast, all bees in each of the three treatments in which at least one anther type was open (total $N = 27$) collected pollen, as indicated by the presence of corbicular pollen. Importantly, this includes the treatment in which pollen was available from only the long 'pollinating' anthers ($N = 9$).

Short anthers were preferred for sonication

We first assessed the overall preference for short versus long anthers. Both short and long anthers were sonicated by all bees in all treatments (total $N = 35$). However, bees preferred to sonicate the short anthers: Overall, bees spent significantly less time buzzing the long anthers than the short ones (Fig. 3; Mean % time buzzing long anthers = 36.83, SE = 1.50), despite the fact that long anthers are larger and outnumbered short ones by 3–2. We tested the significance of the short anther preference by subtracting the mean percent time buzzing long anthers from 50% (50% being the null expectation of non-preference) and evaluating the intercept of a two-way ANOVA on this difference variable. The model intercept was positive in sign and significantly different from zero, indicating a preference for short anthers (two-way ANOVA, test of model intercept against 50%, $F_{3,31} = 8.80$, $p < 0.0001$). Bees on unmanipulated flowers, i.e., flowers with all anthers open, allocated over 70% of their time sonicating the short anthers (Fig. 3; Mean % time buzzing short anthers = 71.62, SE = 2.22; one-sample t test against 50%, $t = 9.76$, df = 8, $p < 0.0001$). The only treatment in which a bias is not apparent is the treatment in which short anther pores are blocked and long

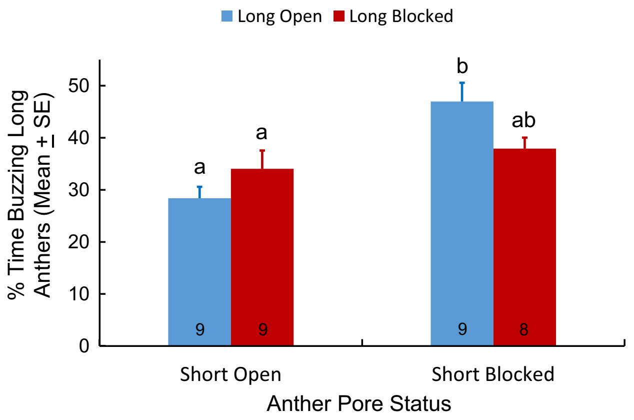

Fig. 3 The mean percentage time sonicating long anthers (± SE) in relation to the pollen-releasing status of long and short anther pores. *Blue bars* indicate treatments in which long anther pores are open; *red bars* indicate treatments in which long anther pores are blocked bars that share the same letters are not significantly different according to a Tukey's test at $p < 0.05$ in a two-way ANOVA. N's *within bars* indicate the number of test bees for the different treatments. (Color figure online)

anther pores are open; even in that case, bees buzzed long anthers slightly less than short ones although the difference is not significant (Fig. 3; Mean %time buzzing long anthers = 46.98, SE = 3.58; one-sample t test against 50%, $t = 0.84$, df = 8, $p = 0.43$).

Blocking of short anthers affected sonication of long anthers, dependent on blocking of long anthers

We next assessed the extent to which the pollen availability of particular anther types affected the relative percentage of time that long anthers were sonicated (Fig. 3). A two-way ANOVA was used to test the extent to which manipulation of each anther type affected sonication of the long anthers, as well as the interaction between anther types. In this analysis, there was a strongly significant treatment effect of blocking the short anther pores and no significant treatment effect of blocking the long anther pores (Table 1). There was also a significant two-way interaction between long anther status and short anther status (Table 1). This interaction indicates that the effect of blocking short anther pores on sonication of long anthers depended significantly on whether long anther pores were blocked or not. Although blocking the short feeding anther pores consistently increased the percent time sonicating the long anthers, the effect was greater and significant only when long anther pores were open (Fig. 3; Tukey's tests, $p < 0.05$). Importantly, the percent time sonicating long anthers was lowest when all anther pores were open, and highest when short anther pores were blocked but long anther pores were open, a difference that was significant (Fig. 3; Tukey's tests, $p < 0.05$). Blocking long anther pores when short anther pores were blocked resulted in an intermediate level of sonication (Fig. 3).

Table 1 Two-way ANOVA of % time spent sonicating long anthers as a function of the availability of pollen in long and short anthers Type III sums of squares reported

Source	df	Sum of squares	F ratio	Prob > F
Long anther pore status	1	25.55	0.33	0.57
Short anther pore status	1	1102.23	14.10	**0.0007**
Long status × short status	1	472.39	6.04	**0.019**
Error	31	2423.37		
Total	34	4072.34		

p values in bold face indicate a level of significance less than 0.05

$R^2 = 40.5\%$

Blocking of short anthers did not affect position of sonication along the long anthers

Finally, we assessed where on the anthers bees were biting and sonicating, and whether treatment influenced the position of the bee relative to the pores of the short versus long anthers. Overall, bees sonicating the short anthers bite within zone 1 and zone 2 approximately equally (Fig. 4). Bees tended to bite and sonicate long anthers in those same zones (zones 1 and 2; Fig. 4). Nevertheless, an average of 16.21% (±2.22 SE) of the bites occur closer

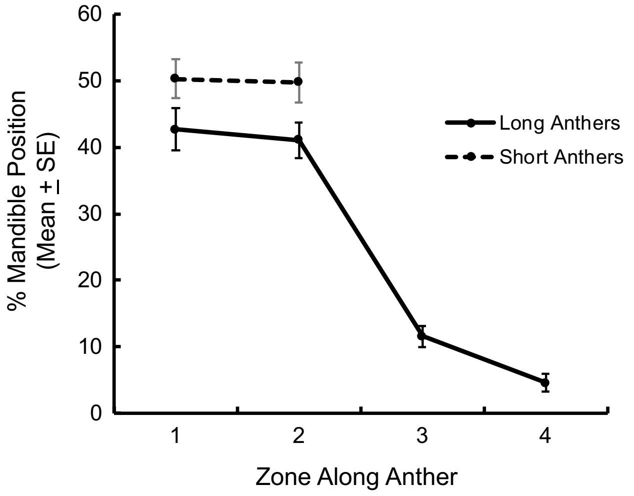

Fig. 4 The mean percentage time in which mandible bites during sonication occurred in the different zones along the length of the long and short anthers (± SE). *Dashed line* indicates data for short anthers; *solid line* indicates data for long anthers. As indicated in Fig. 2b, Zone 1 is most proximal to the filament; Zone 4 is most distal to the filament, closest to the anther pore. Short anthers have only Zones 1 and 2 drawn. Data pooled over all treatments ($N = 35$ bees)

to the long anther's pore (i.e., in zones 3 and 4), which might place the bee's body closer to the exiting pollen.

We found no significant effects of anther pore manipulation treatment on where the bees bit and sonicated along the length of the anthers. We tested the effect of treatment by assessing the difference among treatments in the percentage of time mandible bites occurred in the third and fourth zones (that is, the two zones closest to the anther pore). We then conducted a two-way ANOVA on this difference variable with long anther pore status (open vs. blocked) as one factor and short anther pore status (open vs. blocked) as the other factor. Neither the main effects nor the interaction were significant. Thus, although as reported above, bees sonicated long anthers more when short anther pores were blocked, they did not show a tendency to shift their bites closer to the long anther pores under those conditions.

Both anther types contributed to pollen collection

The estimated rate at which pollen was collected and packed into a corbicula by the bee was greatest when both anther types were open (Fig. 5), consistent with the idea that pollen is normally collected from both types of anthers. To assess the effect of blocking anther pores on the rate at which corbicular loads expanded, we conducted a two-way ANOVA with long anther pore status (open vs. blocked) as one factor and short anther pore status (open vs. blocked) as the other factor. Both main effects were highly significant (Table 2). There was no significant interaction between these two factors. As might be expected based on the bias for sonicating short anthers (Fig. 3), the estimated rate of pollen collection when only the short anthers were open was greater than the rate when only the long anthers were open. However, this difference was not statistically significant (Tukey's test, ns).

Table 2 Two-way ANOVA of estimated rate of expansion of corbicular load (pixel area/sec) as a function of the availability of pollen in long and short anthers Type III sums of squares reported. $R^2 = 54.7\%$

Source	df	Sum of squares	*F* ratio	Prob > *F*
Long anther pore status	1	83.18	10.18	**0.004**
Short anther pore status	1	230.99	28.27	**0.0001**
Long status × short status	1	0.34	0.04	0.84
Error	31	253.29		
Total	34	559.35		

p values in bold face indicate a level of significance less than 0.05

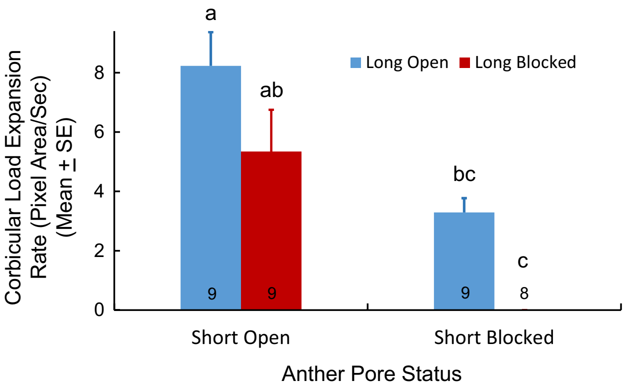

Fig. 5 The mean rate of expansion of corbicular loads (pixel area per sec (±SE) for bees in different treatments. *Blue bars* indicate treatments in which long anther pores are open; *red bars* indicate treatments in which long anther pores are blocked. Bars that share the same letters are not significantly different according to a Tukey's test at $p < 0.05$ in a two-way ANOVA. Numbers within bars indicate the number of test bees for the different treatments. (Color figure online)

Discussion

Among our findings, the following are most relevant to a division-of-labor explanation for heteranthery: (1) Short anthers were preferred for sonication by bumble bees over long ones, suggesting short anthers function as feeding anthers; (2) Blocking short anther pores alone increased sonication of long anthers and resulted in collection of pollen from long anthers; (3) The increased time spent sonicating long anthers, when short anther pores are blocked, was greater if pollen was available in the long anthers; (4) Sonicating bees tended to bite the anthers at their base, regardless of anther type or pore treatment; and (5) significant amounts of pollen from both anther types are collected.

We believe that our results taken together are consistent with the notion that checks and balances limit the extent of reciprocal exploitation in mutualisms, specifically with respect to the division of labor of anther types in bee-pollinated pollen-only plants. We found that pollen-foraging *Bombus impatiens* bumble bees shifted allocation of effort in floral sonication from the short 'feeding' anthers of *S. houstonii* to the long 'pollinating' anthers if the short ones are not releasing pollen. This finding is consistent with findings of Vallejo-Marín et al. (2009) for *S. rostratum*. In our study, bees made a greater shift if the long anthers are releasing pollen (Fig. 3). We confirmed that long-anther pollen is collected by bees under these conditions. As such, if a plant provides insufficient pollen in the short anthers, then bees will shift effort to the long anthers and the pollinating function of the long anthers may be compromised.

However, we also found that bees did not shift their position along the anthers much when they buzzed long anthers, compared to their position when buzzing the short anthers (Fig. 4). This means that the long anther pores are not as close to where the bee is sonicating as they are when the bee buzzes the short anthers. This suggests to us in turn that pollen may not be collected as efficiently from the long anthers. When bees buzz the short anthers, they commonly position their body such that pollen from the short anther pores will spray pollen directly onto the underside of the abdomen or thorax, where it is groomed by the bee into its corbiculae. In contrast, the bee's venter is commonly well away from the long anther pores when they are sonicating the long anthers, even when those are the only anthers providing pollen. Summing up, bees are able to adjust their behavior if the plant shorts them, so to speak, but they are also constrained to make inefficient use of pollen from the long 'pollinating' anthers.

We suggest that the observed preference for sonicating short anthers, despite there being fewer of them (two versus three), is the result of a difference in collection efficiency. This preference supports one of the central tenets of the division-of-labor hypothesis, namely that pollinators focus more of their effort in pollen collection on one type of anthers, i.e., the 'feeding anthers' (Vallejo-Marín et al. 2009). Further support would require an analysis of which anther type contributes more of the pollen deposited on stigmas (e.g., Luo et al. 2008; Vallejo-Marín et al. 2009). Interestingly, the magnitude of the buzzing preference shown by our test bees for our presumptive feeding anthers was considerably less than that reported by Vallejo-Marín et al. (2009) for the feeding anthers of *S. rostratum* using the same bee species (mean percent time spent buzzing feeding anthers: 94.8% on *S. rostratum* vs. 71.62% on *S. houstonii*). *Solanum houstonii* differs from *S. rostratum* in that the pollinating anther of the latter is distinctly different in size, color, and shape from the feeding anther, whereas the *S. houstonii* anther types differ primarily in length. It would be interesting to test the two plants side-by-side under controlled conditions to determine if the difference in preference is real (i.e., not due to arbitrary differences in protocol or ambient conditions) and, more generally, if heterantherous species vary in the extent to which feeding anthers are preferred over pollinating anthers. The occurrence of heteranthery has been associated with low levels of autonomous self-fertilization, which varies among members in the genus *Solanum* (Vallejo-Marín et al. 2014). Self-fertilization in *S. houstonii* has not been studied to our knowledge. Based on our results, we would predict it to have more self-fertilization than, say, *S. rostratum*, and to show corresponding changes in allocation of pollen to the two anther types (specifically, proportionately more pollen inside the pollinating anthers; Tang and Huang 2007).

The modest preference for short anthers suggests that as expected (see Introduction), the reproductive division of labor between anther types is not absolute. Indeed, although pollen loads were not quantified, bees in the treatment with short anthers blocked and long anthers open appeared to collect at least as much pollen as did bees in the treatment with long anthers blocked and short anthers open. We suspect that some long-anther pollen is always collected under normal conditions. A similar case for a less-than-absolute division of labor was made by Gross and Kukuk (2001) studying *Amegilla anomola* bees at *Melastoma affine* flowers. In evolutionary terms, this may reflect the consequences of a tug of war between plant and pollinator that results in a less-than-perfect division of labor.

The fact that blocking the long pollinating anthers did not influence sonication of the short feeding anthers is also consistent with Vallejo-Marín et al.'s (2009) results; however, its significance is diminished by our observation that the increase in sonication effort on long anthers when short anthers are blocked depends on the long anther pores being open (and thus potentially providing pollen). This is a novel finding, to our knowledge. It suggests that bees are responding not only to sensory feedback from the short anthers, in terms of pollen receipt, but also to feedback from the long anthers. Pollen from the long pollinating anthers was shown here to be successfully collected when the short feeding anther pores were blocked; such collection should thus provide direct sensory feedback to the bee and should influence which anthers the bee sonicates. We note that our inference might appear to contrast with that of Vallejo-Marín et al. (2009) who concluded that "bees modify their foraging behavior in response to the amount of pollen extracted from feeding anthers only" (p. 836). They based their conclusion on the fact that blocking the long anthers did not reduce visit duration (which they termed visitation time) and did not affect the percent time spent sonicating those anthers.

In our experiment, blocking long anthers also did not reduce visit duration or percent time sonicating those anthers (Fig. 3; Table 1; Online Resource 1; D. Papaj, S. Buchmann and A. Russell, unpubl. data). Nevertheless, our factorial design allowed us to demonstrate that the status of the long anthers affects the bee's response to the status of the short anthers, resulting in a significant interaction in the analysis (Fig. 3; Table 1). This result strongly suggests that feedback from both types of anthers regulates the degree to which bees sonicate long versus short anthers. Moreover, the length of the anthers in relation to where on the anther bees bite and sonicate (Fig. 4) dictates a difference in the degree of pollen feedback obtained from each anther type. Given that the bees sonicate near the base of the anthers (Fig. 4), the short anthers likely deliver more collectable pollen to the bee per unit time spent sonicating, and thereby give them more positive sensory feedback than does sonication of the long anthers, leading to the observed preference for the short anthers.

To the best of our knowledge, the means by which bees detect pollen on their bodies has not been characterized. Bees might conceivably detect pollen on their bodies via chemosensory (Sanchez 2011), mechanosensory (Buchmann and Cane 1989), and/or electrosensory (Sutton et al. 2016) modalities. Gustatory receptors are found on mouthparts, antennae, legs and even wings; many of the campaniform sensilla over a bee's body respond both to electrical fields (pollen is often electrostatically charged) and to mechanical stimuli.

Despite the role of pollen-associated sensory feedback in which anthers are sonicated, pollen is not required to elicit sonication. Blocking the pores of *S. houstonii* anthers did not prevent bees from sonicating the anthers, even though pollen could not be collected from them (see also Buchmann and Cane 1989; Vallejo-Marín et al. 2009; Burkart et al. 2011; Russell et al. 2016b). In fact, earlier glued anther assays in the field with another *Solanum* species showed that wild (and presumably experienced) bees even sonicate anthers that have been experimentally drained of pollen (Buchmann and Cane 1989). Consistent with such results, we have prepared solvent extracts of the anther surface that elicit sonication of artificial surrogates and have further found that adding loose pollen to extract-treated surrogates does not enhance sonication levels (Russell et al. unpubl. data). It is conceivable that the active chemicals are in higher concentration at the base of the anthers, causing bees to bite there.

Our findings and inferences suggest a number of future directions. First, we are assuming that an increase in time spent sonicating anthers results in a greater degree of pollen collected from those anthers. Second, we are assuming that long anthers furnish more of the pollen that is transferred to floral stigmas. Both assumptions seem reasonable, but should be evaluated, perhaps with the use of UV fluorescent dye particles or similar pollen marking techniques (e.g., Bowers 1975). A third assumption is that more pollen is ejected from an anther if that anther is grasped directly by the mandibles. In *S. houstonii*, the anthers are appressed closely together and vibrating one anther appears to transmit vibrations to adjacent anthers. We assume that florally transmitted vibrations are lower in intensity than direct vibrations and thereby eject less pollen (see De Luca et al. 2013; King and Buchmann 1996), but this assumption should be evaluated.

The tendency for bees to sonicate anthers at their base may reflect this position being more effective in ejecting pollen from the anthers. This could be tested by artificially vibrating the anther at different points along its length and quantifying the amount of pollen released.

Future research should also assess whether the bee's behavioral flexibility in floral sonication has advantages in terms of responding to changes in anther status and pollen standing crop in a plant population. For example, as bees forage throughout the day and deplete feeding anthers of most of their pollen, do they shift to greater effort on the pollinating anthers? Given that pollinating anthers commonly contain more pollen than feeding anthers (e.g., Gross and Kukuk 2001; Hrycan and Davis 2005; Luo et al. 2008; Paulino et al. 2016), they may deplete more slowly, thereby giving rise to a shift in foraging.

Finally, as the work of Vallejo-Marín and colleagues demonstrates, *Solanum* is an interesting and instructive genus in which to study heteranthery because the trait has independently evolved repeatedly throughout the genus, particularly in the so-called spiny *Solanums*, of which *S. houstonii* is a member (Levin et al. 2006; Bohs et al. 2007; Vallejo-Marín et al. 2009, 2010, 2014). The heterogeneous distribution of heteranthery suggests that conditions for its evolution vary among relatively closely related taxa. We suggest that this heterogeneity could partly reflect variation in characteristics of the local bee community that influence whether division of labor will be an evolutionarily stable strategy or not. These characteristics could include a capacity on the part of the bee(s) to adjust their sonication behavior in response to changes in the availability of pollen in anthers of different types. This prospect invites us to conduct further experiments similar to ours and Vallejo-Marín et al. (2009) in additional poricidal species, perhaps focusing on an array of species that vary in degree of autonomous self-fertilization or other traits often associated with heteranthery.

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