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Bee- to bird-pollination shifts in *Penstemon*: effects of floral-lip removal and corolla constriction on the preferences of free-foraging bumble bees

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Abstract Plants might be under selection for both attracting efficient pollinators and deterring wasteful visitors. Particular floral traits can act as exploitation barriers by discouraging the unwelcome visitors. In the genus *Penstemon*, evolutionary shifts from insect pollination to more efficient hummingbird pollination have occurred repeatedly, resulting in the convergent evolution of floral traits commonly present in hummingbird-pollinated flowers. Two of these traits, a reduced or reflexed lower petal lip and a narrow corolla, were found in a previous flight-cage study to affect floral handling time by bumble bees, therefore potentially acting as "anti-bee" traits affecting preference. To test whether these traits do reduce bumble bee visitation in natural populations, we manipulated these two traits in flowers of bee-pollinated *Penstemon strictus* to resemble hummingbird-adapted close relatives and measured the preferences of free-foraging bees. Constricted corollas strongly deterred bee visitation in general, and particularly reduced visits by small bumble bees, resulting in immediate specialization to larger, longer-tongued bumble bees. Bees were also deterred—albeit less strongly—by lipless flowers. However, we found no evidence that lip removal and corolla constriction interact to further affect bee preference. We conclude that narrow corolla tubes and reduced lips in hummingbird-pollinated penstemons function as exploitation barriers that reduce bee access to nectaries or increase handling time.

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Introduction

Selection to minimize pollen wastage by an unwelcome visitor may favour traits that
decrease pollen removal during a flower visit or selectively discourage visitation alto-
gether. Characteristics that accomplish the latter may involve changes in nectar, colour, or
morphology (Johnson et al. 2006; Muchhala 2006; Moré et al. 2007; Lunau et al. 2011;
Owen and Bradshaw 2011). Such traits act as exploitation barriers, playing an important
role in pollination-syndrome shifts.

In *Penstemon* and its sister genera (Plantaginaceae), phylogenetic analyses estimate at
least ten separate origins of hummingbird pollination from bee pollination (Wilson et al.
2007), and the bee-pollination syndrome and the bird-pollination syndrome are clearly
evident and distinguishable. The frequent occurrence of bee- to hummingbird-pollination
shifts in this clade may arise from the relative pollen-transfer efficiencies of bees and birds.
Effective and regular grooming by corbiculate bees rapidly removes many pollen grains
from circulation (Thomson 1986; Holmquist et al. 2012). Unlike bees, however, birds do
not collect pollen to feed their brood. Several comparisons between bees and birds indicate
that the latter tend to be less wasteful pollen vectors (Castellanos et al. 2003; Schmid et al.
2011; Etcheverry et al. 2012). In particular, Castellanos et al. (2003) documented a steeper
pollen carryover curve by bumble bees than hummingbirds on bee-pollinated *Penstemon
strictus* Benth.

If birds are indeed more effective pollinators of penstemons than bees are, bees may
become conditional parasites in the presence of birds, because they deplete attractive
nectar rewards and remove pollen that would otherwise have been transferred by birds
(Thomson 2003). When hummingbird visitation is sufficiently reliable, we would expect
bee-syndrome flowers to experience selection not only "toward" birds but also "away"
from unwelcome bee visitors, i.e., selection not only for traits that tend to attract or
increase the efficiency of birds but also for those that deter or decrease pollen removal by
bees (Castellanos et al. 2004).

Castellanos et al. (2004) previously studied pollen removal, deposition, and handling
times by bumble bees and hummingbirds on *P. strictus* flowers that had been surgically
modified to conform to the bird-pollination syndrome in four traits: stigma and anther
exsertion, absence of a lower corolla lip, corolla-tube constriction, and flower inclination.
These traits influence the physical interaction between flower and pollinator; modifications
were therefore predicted to improve pollen transfer by birds by providing a better fit
between flower and bird, decrease bee performance by reducing or thwarting access to
nectar, or both. The pollen-transfer data of Castellanos et al. (2004) matched predictions
partly but not completely, possibly due to interactive effects between the singly manipu-
lated traits. Lipless and pendent flowers did make landing difficult for bumble bees and
increased handling time, therefore probably acting as "anti-bee" traits affecting prefer-
ence. Castellanos et al. (2004) inferred that longer handling times would deter bees, but did
not study preference directly.

In this follow-up study, we examined bumble bee preference directly for two of the
manipulated traits: lip removal and corolla constriction. Extended corolla lips serve as a

landing platform for insects (Straw 1956; Grant and Grant 1968); "lipless" flowers thus require more handling time by bumble bees and have a smaller projected area, both of which can be expected to decrease attractiveness to bees (Schemske and Bradshaw 1999; Castellanos et al. 2004). We also expected that constricted corolla tubes would deter bumble bees by decreasing nectar accessibility, especially for larger or nectar-collecting individuals. Castellanos et al. (2004) conducted experiments in a flight cage, but we conducted trials in the field so as to study the behaviour of free-foraging bees that had the opportunity to experience the handling characteristics of individual plants, learn the locations of those plants, and decide whether to continue visiting. Bumble bees are capable of this sort of associational learning and decision making (Thomson 1988; Cartar 2004; Makino and Sakai 2007), so any realistic investigation of preferences must allow scope for such behaviour. In essence, our experiments tried to assess the "popularity" to bees of rare mutant plants arising in natural populations. We hypothesized that both lip removal and corolla constriction would act as "anti-bee" traits and decrease bumble bee visitation to *P. strictus*, possibly also interacting with each other to produce an even stronger negative effect.

Materials and methods

Study plant

Penstemon strictus Benth. has typical "bee-syndrome" flowers—blue-purple and vestibular, with a prominent lower lip. It is visited primarily by Hymenoptera (*Bombus*, *Osmia*, and *Anthophora* bees, as well as the wasp *Pseudomasaris vespoides*) and occasionally by hummingbirds (Castellanos et al. 2004; Wilson et al. 2004). At our sites near Mt. Crested Butte, Colorado, USA, bumble bees were by far the most frequent visitors. Pollen-collecting bumble bees were abundant early in the season, whereas nectar-collecting bumble bees predominated later.

Manipulations

We chose two of the manipulations previously applied by Castellanos et al. (2004): removal of the lower lip and constriction of the corolla tube. Both manipulations were intended to increase morphological resemblance to typically hummingbird-pollinated *Penstemon* spp. such as closely related *P. barbatus* (Cav.) Roth., which has a strongly reflexed lower lip and a narrow corolla tube (Wilson et al. 2004). The other two manipulations applied by Castellanos et al. were impractical here because they required time-consuming surgery, and the severed flower parts would have wilted quickly under field conditions.

To create lipless flowers, we used scissors to cut off the three lower corolla lobes that extend as a landing platform for insects (Fig. 1b, d). For narrow flowers, we used 6.4 mm-diameter orthodontic elastics (Model no. 407-041S; Ortho Organizers, Inc., Carlsbad, California, USA) to constrict corolla tubes, reducing corolla diameters from an average of 8.9 to 6.1 mm (Fig. 1c, d). This constriction produced pleats in the corolla, which we flattened as much as possible. Castellanos et al. (2004) also clipped out three of the four filaments to reduce crowding within the corolla tube; we clipped out only one pair to maintain the symmetry preferred by bumble bees (Møller 1995).
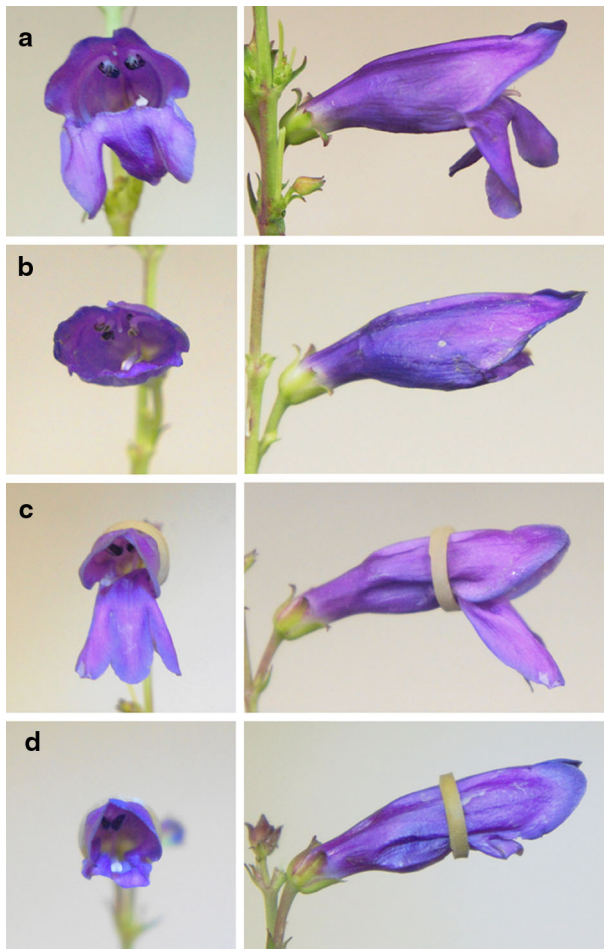
Fig. 1 Front and side views of *P. strictus* flowers from the four treatment groups: **a** control, **b** cut lip, **c** constricted tube, and **d** both cut lip and constricted tube

Trials

Trials were conducted by JLZ at three weedy, roadside sites (Whetstone Road: 38°54′ 7.6″N 106°57′ 52.8″W, Hunter Hill: 38°53′ 22.0″N 106°57′ 58.3″W, and Wildhorse Trail: 38°54′ 58.2″N 106°57′ 7.6″W) in the vicinity of Mt. Crested Butte, Colorado, USA, from 1 July to 12 August, 2013. Over a period of several days for each trial, we first observed and recorded bee visitation to unmanipulated flowers, then applied manipulations, allowed time for bees to respond, and finally recorded visitation again in a post-manipulation period. On most days, we conducted observations between 09:00 and 18:00, although frequent rain and overcast conditions made afternoon visitation variable.

For trials at Whetstone Road (WR; 1–14 July) and Hunter Hill (HH; 17–20 July), we set out a spatially blocked design using "quads" of cut inflorescences in water bottles. One quad comprised four bunches of cut stems, set at the corners of an 80 cm × 80 cm square.

Members of a quad were matched exactly for number of open flowers (usually 20–40, depending on local availability). Quads were near a blooming *P. strictus* patch, but no closer than 80 cm to any *P. strictus* individual. We observed bumble bee visits to a quad over a 2-h period, recording species, caste, number of flowers visited, and type of visit (i.e., pollen- or nectar-collecting). We then manipulated each quad member (bunch, or "plant") according to one of four randomly assigned factorial treatment groups: unmanipulated control, cut lip, constricted tube, or both cut lip and constricted tube (Fig. 1). After manipulation, quads were left for 1 day before a 2-h post-manipulation observation period. Any newly opened flowers were manipulated before the second observation period. Data from these two sites were combined for analysis as the "cut-stem" data set.

Penstemon strictus flower abundance and bumble bee visitation were much higher at later-blooming Wildhorse Trail (WT; 21 July–12 August), prompting a change in protocol. Members of a quad at this site were whole plants within a few metres of each other and at least 50 cm from any other flowering plant. We matched members approximately for number of inflorescences and number of open flowers (usually 20–80). For each observation period, we watched one member at a time for 5 min, rotating three times among the four members of a quad. As before, we recorded species, caste, number of flowers visited, and type of visit for each bumble bee visitor. Because casual observations in earlier trials suggested different responses to treatments by bees of different sizes, we also estimated bee size for several replicates, subjectively assigning bees to one of five size classes. After one pre-manipulation observation period, plants were randomly assigned to one of the four treatment groups and manipulated. Using whole plants at this site instead of cut inflorescences allowed three to four observation periods on consecutive days following manipulation. We manipulated newly opened flowers each morning before observations. Our estimations of bee size classes were later calibrated by measurement of specimens from the Rocky Mountain Biological Laboratory insect collection.

Statistical methods

All response variables were ratios of post-manipulation to pre-manipulation values for a given quad member ("response ratios"), log-transformed (or log[x + 0.1]-transformed when data included zeroes) to achieve normality. All analyses were conducted as linear mixed-effects models (with "quad" as a random factor) using the lme4 package (Bates et al. 2012) in R v. 2.15.2 (R Core Team 2012). Significance of each model term was tested by a likelihood-ratio test of nested models (Zuur et al. 2009).

For the WT ("whole-plant") data, we examined the effect of day of observation (i.e., days 1–4 post-manipulation) using a repeated-measures analysis with "day" as the within-subjects factor and the two treatments (cut lip and constricted tube) as fixed between-subjects factors. Response variables were log-response ratios of numbers of visits to a plant and flowers per plant visit.

Effects of the two treatments on response ratios in the cut-stem experiment were tested using similar mixed models, without repeated measures. We excluded replicates (i.e., quads) if any quad received fewer than ten visits in one observation period. Because the repeated-measures analysis indicated no significant day × treatment interactions for the whole-plant data, we averaged response ratios across all four post-manipulation days so that these data could be analysed in the same way as the cut-stem data. We computed overall probabilities for the two experiments using Fisher's method for combining probabilities (Quinn and Keough 2002).

To test differences between pollen collectors and nectar collectors in response ratios of number of visits and flowers visited, we ran mixed models for the cut-stem data with the two treatments and type of visit (i.e., either pollen or nectar) as fixed predictors, and quad as a random term. Replicates were excluded if a quad received fewer than ten visits of a given type in one observation period. We did not include the whole-plant data in this analysis, because pollen collectors late in the season (i.e., in the data from WT) were rare.

For the subset of whole-plant data that included bee size, we tested the effect of the two treatments and their interaction on the response ratio of mean visitor size class. We also tested whether responses to floral manipulations were influenced by bee size. For the latter analysis, the five bee size classes were reduced to two to ensure sufficient numbers of visits for each size class. Bee size class and the two treatments were used as fixed predictor variables in a linear mixed model. Response ratios of number of plant visits and flowers per plant visit were the response variables. One quad was excluded from this analysis because it received no visits by bees in the larger size class during one observation period. Inclusion of this quad did not qualitatively change results.

Results

Bees of all species and sizes were able to visit manipulated flowers; treatments did not entirely prevent any bee from landing or accessing nectaries. Any reductions in visitation appeared to be the result of decreased preference, rather than outright exclusion from a narrowed flower or inability to handle a lipless flower. Many bees were able to feed while inserting only their heads and part of their thoraxes into constricted flowers. Most bees did not appear to have difficulty landing on lipless flowers; however, bees who did not take flight between flowers on an inflorescence occasionally scrabbled unsuccessfully with their front legs in an apparent attempt to crawl up to the next flower. Pollen- and nectar-collecting behaviours were easily distinguished in the field; nectar collectors delved into flowers right side up, but bumble bees actively collecting pollen stayed near the mouth of the corolla, landing right side up and turning upside down to grasp the anthers on the roof of the corolla.

Bombus workers were the primary visitors to our arrays at all sites. At WR and HH, 11 % of 1,448 recorded visits to "plants" (in 256 plant-hours of observation) were made by queens; no males were present. *Bombus flavifrons* Cresson (43 %), *B. bifarius* Cresson (36 %), and *B. appositus* Cresson (12 %) were the primary visiting species; *B. nevadensis* Cresson and *B. californicus* Smith also visited infrequently. Almost all *B. bifarius* and most *B. flavifrons* (70 %) were exclusively collecting pollen; a small minority of bees of these species collected both pollen and nectar. No other species were recorded actively collecting pollen. On average, bees visited 5.2 flowers per "plant" visit at this site, with a range of 1–38. At WT, approximately 4 % of 5,694 recorded visits to plants (in 76 plant-hours of observation) were made by males, and <1 % by queens. *Bombus appositus* (46 %) and *B. flavifrons* (40 %) made the majority of visits at this site, while *B. californicus*, *B. bifarius*, and *B. nevadensis* were occasional visitors. Most *B. bifarius* and a small number of *B. flavifrons* workers were actively collecting pollen; the vast majority (96 %) of visits at WT were made by nectar collectors. Bees visited an average of 5.6 flowers per plant at this site, with a range of 1–58.

Visiting bumble bees ranged in size from *B. bifarius* or *B. flavifrons* workers (typically size classes 1–2) to *B. appositus* workers (typically sizes 3–4) to *Bombus* queens (typically sizes 4–5). Smaller bees entered the corolla tube almost entirely, while larger bees often

inserted only their heads and thoraxes, clinging to the outside of the corolla tube with their mid legs and to the lower lip with their hind legs. Constricted flowers had diameters smaller than the thoraxes of the largest bees (mean thorax width of size class five specimens: 8.0 mm; mean diameter of constricted flowers: 6.1 mm), but these bees were clearly still able to access the nectaries.

In the cut-stem experiment, the number of bumble bee visits to quad members with constricted tubes declined by 25 % more after manipulation than did the number of visits to "plants" with unconstricted corollas (mixed models of treatment effects on log-response ratios, $\chi^2 = 12.6$, $df = 1$, $p = 0.00038$, Fig. 2a). Tube constriction also reduced the number of flowers per visit by 15 % (22 % reduction in flowers per visit after manipulation for constricted "plants", vs. 7 % reduction for unconstricted "plants"; $\chi^2 = 4.1$, $df = 1$, $p = 0.044$, Fig. 2b). Lip removal had no effect on visits or flowers per visit, nor was there any interaction between lip removal and tube constriction (all $\chi^2 < 2.5$, $p > 0.1$, Fig. 2a, b).

In the whole-plant experiment, number of flowers per visit declined by an average of 23 % over the 4 days of post-manipulation observation, but treatment effects on number of visits and flowers per visit did not vary significantly over time (Table 1; Fig. 3). Plants experienced an average 49 % reduction in bumble bee visits following tube constriction (compared to a mean 4 % increase in visits to plants with unconstricted corollas; $\chi^2 = 117.4$, $df = 1$, $p < 0.0001$, Fig. 2c). In this experiment, lip removal also reduced the number of bumble bee visits (14 % greater reduction in visits to cut-lip plants compared to plants with intact lips; $\chi^2 = 10.6$, $df = 1$, $p = 0.0011$), but neither treatment significantly affected the number of flowers per visit ($\chi^2 < 3.0$, $df = 1$, $p > 0.09$, Fig. 2d). There were no interactions between lip removal and tube constriction ($\chi^2 < 0.3$, $p > 0.6$).

Combined p values for the cut-stem and whole-plant experiments indicate that both tube constriction and lip removal significantly reduced number of visits ($\chi^2 = 78.8$, $df = 4$, $p < 0.0001$ for tube constriction; $\chi^2 = 11.6$, $df = 4$, $p = 0.020$ for lip removal). There was no detectable lip × tube interaction ($p = 0.92$). Neither of the treatments nor their interaction significantly affected the number of flowers per visit (all $p > 0.07$).

We detected no difference between nectar- and pollen-foraging bees in their responses to either floral manipulation (mixed models of visits and flowers per visit, both log[x + 0.1] transformed; $\chi^2 < 3$ and $p > 0.09$ for all treatment × visit type interactions); however, our power for this test was low, as only three quads received enough nectar visits to be included in the analysis. Smaller bees were more deterred than larger bees by narrow corollas, showing a 77 % reduction in mean visit number to plants following corolla constriction (compared to a 5 % reduction in visits to plants with unconstricted corollas) and a 47 % decrease in flowers per visit following tube constriction (vs. a 6 % decrease without tube constriction). In contrast, large bees showed no reduction in visit number and a 21 % increase in flowers per visit to plants with constricted corollas, relative to unconstricted plants (mixed models of log-response ratios; tube × bee size $\chi^2 = 25.8$, $df = 1$, $p < 0.0001$ for visits; $\chi^2 = 21.6$, $df = 1$, $p < 0.0001$ for flowers per visit). Corolla-tube constriction therefore caused a marked increase in mean size class of bee visitors from 2.3 to 2.8 (mixed models of log-response ratio in bee size class, $\chi^2 = 25.5$, $df = 1$, $p < 0.0001$, Fig. 4). This shift in mean size class corresponds approximately to a change from 4.7 to 5.0 mm in thoracic width, or from 3.8 to 3.9 mm in intertegular distance ($N = 33$ measured specimens). There was no effect of lip removal on bee size class ($\chi^2 = 0.1$, $df = 1$, $p = 0.76$).
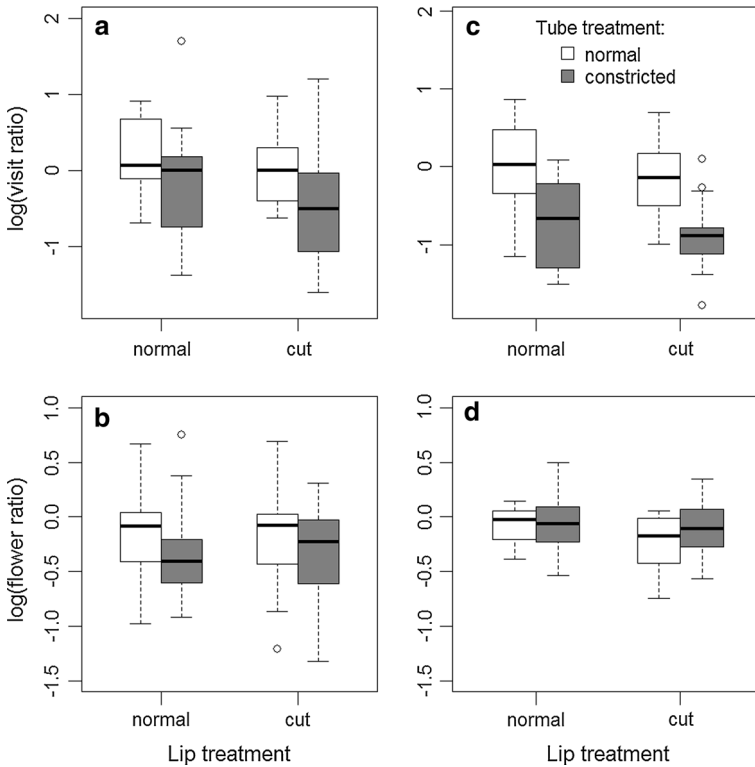
Fig. 2 Tukey *box-plots* showing treatment effects on bumble bee visits **a**, **c** and flowers per visit **b**, **d** in the cut-stem **a**, **b** and whole-plant **c**, **d** experiments. Data are shown as log-response ratios (log-transformed ratios of post- to pre-manipulation values for each quad member) and therefore represent the magnitude of change in each variable in response to the treatment. *Boxes* show medians and interquartile ranges; whiskers extend to values within $1.5\times$ the interquartile range of the upper and lower quartiles. Significant differences among treatments are described in the text. $N = 15$ cut-stem and 16 whole-plant quads

Discussion

We found generally strong deterrent effects of corolla-tube constriction on bumble bee visitation to *P. strictus*, and a significant but weaker deterrent effect of floral-lip removal. These results suggest that although both features affect bumble bee floral choices, access to floral rewards plays a more important role than presence of a landing platform. Below, we discuss how each aspect of our findings affects our understanding of evolutionary transitions to hummingbird pollination.

Corolla-tube constriction

Bumble bees' aversion to constricted corolla tubes is easily explained by the restricted access the nectaries. Because we observed successful visits to constricted flowers by bees of all sizes, the effect of tube constriction seems only to reflect preference for a "comfortable fit" in our experimental flowers. However, tube constriction may prevent access entirely in narrower, true bird-pollinated penstemons. Castellanos et al. (2004)

Table 1 Repeated-measures analysis of effects of experimental treatment (cut lip or constricted corolla tube), post-manipulation day of observation (the repeated-measures variable), and quad (a random blocking variable) on number of (a) bumble bee visits per plant and (b) flowers entered per visit (both transformed as log[response ratio + 0.1]) in the whole-plant experiment

	Variable	χ^2	p
(a) Visits	Tube	117.4	**<0.0001**
	Lip	10.6	**0.0011**
	Tube × lip	0.17	0.68
	Day	0.021	0.89
	Day × tube	2.0	0.16
	Day × lip	0.065	0.80
	Day × tube × lip	0.87	0.35
	Quad	96.8	**<0.0001**
(b) Flowers per visit	Tube	0.0	1.0
	Lip	2.8	0.093
	Tube × lip	0.26	0.61
	Day	5.4	**0.020**
	Day × tube	2.9	0.088
	Day × lip	0.76	0.38
	Day × tube × lip	0.39	0.53
	Quad	21.4	**<0.0001**

Significance of each term was tested by a likelihood-ratio test of nested models, with 1 *df*

p values <0.05 are in boldface

found no preference by hummingbirds for or against constricted corolla tubes in the absence of bees, and weak evidence for improved pollen transfer by hummingbirds in narrow flowers. Therefore, this trait functions partially, perhaps primarily, as an exploitation barrier, deterring bees and thereby preventing them from wasting pollen and consuming nectar that attracts birds. Several other studies also indicate that corolla-tube width is an important "anti" trait that serves to restrict access by unwanted visitors—e.g., bumble bees, solitary bees, and flies on *Polemonium* (Campbell et al. 2014), hawkmoths on *Mandevilla* (Moré et al. 2007), and bats on *Burmeistera* (Muchhala 2006).

Constriction affected visitation more strongly in the second (whole-plant) experiment than in the first (cut-stem) trial. The cause probably lies not in the different states of the plants but in the preponderance of pollen-collecting bees at the time of first trial. Pollen collectors typically do not delve for nectar at all, and constriction at the middle of the corolla tube does little to inhibit their access to the anthers to which they direct their activities. Pollen collectors usually comprise a small minority of visitors in these habitats, and the numerical dominance of pollen collectors during the cut-stem trials was unprecedented in JDT's years of experience (personal observation; also see Williams and Thomson 1998). All of the bees tested by Castellanos et al. (2004) were collecting nectar. We were unable to detect a difference in the effect of tube constriction between nectar- and pollen-collecting bees in our cut-stem trials, as our sample size for this comparison was low, but nectar collectors did tend to respond more negatively to corolla constriction than did pollen collectors (data not shown). We would expect this difference to be more pronounced if we had not clipped out two anthers from constricted flowers, as clipping anthers

Fig. 3 *Box-plots* showing numbers of **a** bumble bee visits and **b** flowers per visit on each day of observation for each treatment group in the whole-plant experiment. Day 0 = pre-manipulation; Days 1–4 = post-manipulation. $N = 16$ quads
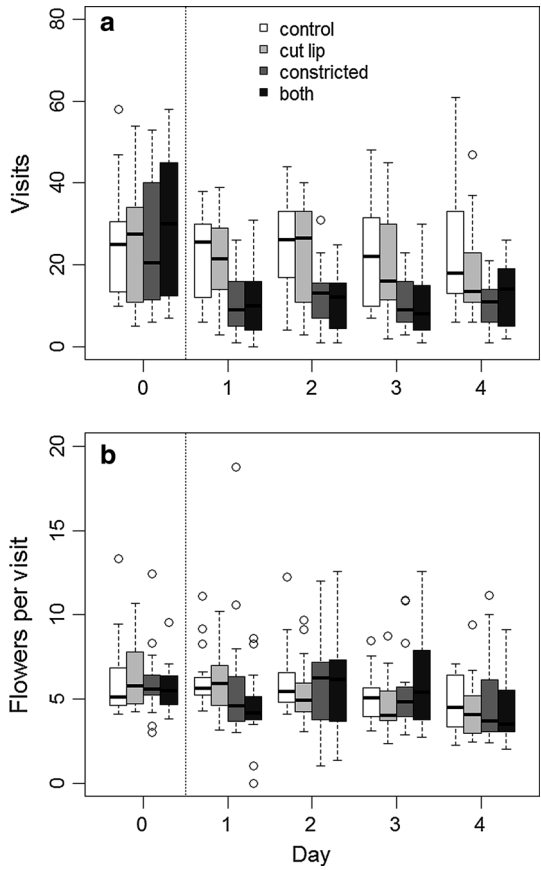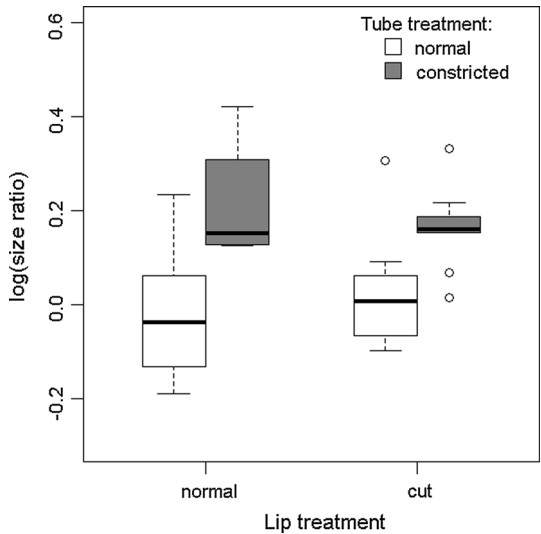

Fig. 4 *Box-plot* showing treatment effects on bee size, measured on a qualitative 1–5 scale and plotted as log-transformed ratios of post- to pre-manipulation means for each quad member. $N = 9$ quads, 199–533 bees per quad

decreased the amount of pollen available for pollen collectors but is unlikely to have affected nectar collectors' decisions. However, the removal of two anthers does not appear to have had a major confounding effect. Although we might expect pollen collectors to be dissuaded by the halved pollen reward, visits to constricted flowers dropped by roughly twice as much in the whole-plant trials (where most bees were collecting nectar) as in the cut-stem trials dominated by pollen collectors.

An increase in mean visitor size to constricted flowers was an unexpected result, as we would expect narrower corolla tubes to be particularly objectionable to larger bees. At our sites, however, larger bees were typically *B. appositus*, who have longer tongues than the other principal visitor, *B. flavifrons* (Inouye 1980). Bumble bee size also correlates positively with tongue length within a species (Harder 1982). Constricted flowers would be in fact more accessible to the larger—and hence longer-tongued—bees. Our results demonstrate how a single floral-trait change can result in a slight functional specialization onto a subset of a flower's visitors. Although a considerably less dramatic shift than bee- to bird-pollination, our finding makes it easier to envision simple floral-trait changes with large, disparate effects on visitation by various functional groups of pollinators. It must be noted, however, that corolla-tube constriction did not cause large bees to begin visiting *P. strictus*; rather, it deterred their smaller, shorter-tongued counterparts. In the course of a full-blown syndrome shift, bee-pollinated flowers must acquire hummingbirds as pollinators before excluding bees (Wilson et al. 2006; Lara and Ornelas 2008; Wilson and Jordan 2009). Once hummingbirds are reliable visitors, selection can favour a more constricted, longer tube that excludes even the longest-tongued bees but is still accessible by narrow hummingbird bills.

Lip removal

Lip removal was also effective in reducing bumble bee visitation in our experiments, although it did not have a deterrent effect as strong or consistent as corolla-tube constriction. Castellanos et al. (2004) found that removal of the lower lip increased handling time by bumble bees on *P. strictus*, so a reduction in foraging efficiency likely explains why bumble bees avoided our lipless flowers. Lip removal could have affected foraging efficiency, and therefore preference, in several ways. Landing on a lipless flower is likely more awkward and may take more time, especially on first encounter by a naive bee. Even when bees do not take flight between visiting flowers within an inflorescence, the lower lip is often useful for footholds as bees clamber upwards along an inflorescence; its removal might force bees to fly between flowers. Larger bees who thrust only their heads and thoraxes into flowers often cling to the lower lip as they feed and might spend longer adjusting their grip on lipless flowers. Moreover, lipless flowers present a smaller projected area to approaching pollinators, which would tend to decrease the flowers' attractiveness to bees disproportionately (Schemske and Bradshaw 1999). The perceived sparseness of cut-lip inflorescences could also have contributed to the observed decrease in visitation.

We can envision some ways in which the negative effect of lip removal on visitation might have been partially mitigated. It is possible that bees show initial clumsiness on lipless flowers, accounting for the slower handling times in the flight cage, but overcome their performance deficiencies with more experience over several days in the field. A second possibility is that while lip loss did deter some individual visitors, this allowed more nectar to accumulate, making the flowers more attractive to a group of foragers specialized on lipless flowers and willing to accept slower handling times in exchange for greater rewards. Although we believe the latter is a likely scenario, testing it directly would

have required us to apply unique identifying marks to individual bees. Another possibility is that the plants at our field sites may not have been far enough apart, or provided with sufficient landmarks, to allow bees to memorize the locations of modified plants and avoid them. In this scenario, bees approaching a plant may have been better able to detect beige elastics on purple flowers (i.e., to identify stems with constricted corollas) than to detect missing lips. Inspection of the first few lipless flowers on a modified plant and a quick departure could account for the marginal reduction in number of flowers per visit (an 8 % reduction; $p = 0.09$) in the whole-plant experiment.

Loss of the lower lip has also been shown to prevent bumble bees from visiting *chinless* mutants of *Mimulus lewisii* (Owen and Bradshaw 2011), but the presence of a lower lip may have different functions in other systems. In *Chloraea* orchids visited by *Centris* bees, for instance, Cuartas-Domínguez and Medel (2010) found no effect of lip removal on pollinium removal or fruit set and no direct selection on lip size. Interestingly, Temeles and Rankin (2000) found that the presence of the lower lip of *Monarda* flowers actually improved hummingbird pollination by increasing both the precision of bill insertion and pollen removal. Our previous experiments with hummingbirds do not suggest such a role for lips in *Penstemon*. Instead, the results of the present study in combination with the convergent reduction or reflexion of lower lips in hummingbird-pollinated penstemons support the hypothesis that lipless flowers reflect selection for bee deterrence.

Lip × tube interaction

Although selection is likely to act on several traits in concert (Campbell 2009), we found no joint effect of corolla-tube constriction and lip removal on bumble bee preference. Nevertheless, it is possible that these traits work in conjunction on pollen-transfer efficiency, an aspect of pollinator-mediated selection which we did not investigate in this study. When modified by itself, a cut lip marginally increases pollen removal but decreases deposition by hummingbirds in *P. strictus* (Castellanos et al. 2004). It is possible that a narrow corolla without a lip would allow hummingbirds to insert their heads further into the flower while preventing approaches from the side, promoting firm and precise contact with the reproductive organs. Exserted anthers and stigmas could further improve the chances of precise pollen transfer by placing pollen on the bird's head as opposed to the thinner beak. However, an experimental test of this idea has yet to be conducted.

Conclusions

Narrowed corolla tubes in hummingbird-pollinated penstemons are most likely an exploitation barrier that deters bees by limiting access to nectaries, while flowers without landing platforms likely deter bees by making flowers more challenging to handle or less attractive for bees. However, we found no evidence of a synergistic effect of lip removal and corolla constriction on bee preference for *P. strictus*. Other traits that could potentially discourage bee visitation to hummingbird-pollinated penstemons include nectar dilution, floral inclination, and red colouration (Rodríguez-Gironés and Santamaría 2004; Cnaani et al. 2006; Makino and Thomson 2012). The evolution of such "anti-bee" traits is a key step for flowers undergoing bee- to bird-pollination shifts.

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