'Anti-bee' and 'pro-bird' changes during the evolution of hummingbird pollination in *Penstemon* flowers

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Abstract

Floral phenotypes may be as much the result of selection for avoidance of some animal visitors as selection for improving the interaction with better pollinators. When specializing on hummingbird-pollination, *Penstemon* flowers may have evolved to improve the morphological fit between bird and flower, or to exclude less-efficient bees, or both. We hypothesized how such selection might work on four floral characters that affect the mechanics of pollen transfer: anther/stigma exsertion, presence of a lower corolla lip, width of the corolla tube, and angle of flower inclination. We surgically modified bee-pollinated *P. strictus* flowers changing one trait at a time to make them resemble hummingbird-pollinated *P. barbatus* flowers, and measured pollen transfer by bumblebees and hummingbirds. Results suggest that, apart from 'pro-bird' adaptations, specific 'anti-bee' adaptations have been important in shaping hummingbird-flowers. Moreover, some trait changes may have been selected for only if changing in concert with other traits.

Introduction

Floral phenotypes are likely to be the result of a variety of selective pressures. Pollinators can impose selection on floral characteristics (e.g. Campbell *et al.*, 1991; Fulton & Hodges, 1999), yet the simplest view – that flowers are predominantly adapted to their *principal* pollinators – has been questioned by studies that consider other interacting animals (Herrera, 1993; Mayfield *et al.*, 2001). For example, antagonistic folivores and ovule predators may be attracted to large floral displays, causing selection against floral showiness (Brody, 1992; Strauss, 1997; Galen & Cuba, 2001). But without even extending consideration to herbivores, some pollinators may be conditional antagonists in the presence of better pollinators (Thomson, 2003). Most flowers have a mixed set of visitors, and plants may adapt not only to increase attendance or pollen transport by more effective pollinators but also to reduce the attendance or pollen interception of inferior ones (Thomson & Thomson,

1992). Such specialization would be advantageous for the plant in cases where the relative contributions to pollination of the flower visitors differ, and the presence of a less efficient pollinator interacts negatively with a better one. For instance, massive pollen removal by a pollen consumer (such as a bee that provisions its brood with pollen) will prevent those pollen grains from being picked up by a subsequent pollinator that would be more likely to deposit them on stigmas (such as a hummingbird with longer pollen carryover). Similarly, nectar consumption by a mediocre pollinator might reduce the attractiveness of a plant to a better pollinator (Thomson, 1988; R. Cartar, pers. comm.). Floral characters that deter less efficient pollinators can be favoured as long as the benefits from specializing outweigh the losses of such a pollinator, even if it is a frequent visitor (Aigner, 2001).

The possibility that floral characters can evolve 'away' from a less effective pollinator and not just 'towards' superior ones has been discussed before (Straw, 1956; Grant & Grant, 1968; Raven, 1972; Brink, 1980; Sutherland & Vickery, 1993; Campbell *et al.*, 1996; Schemske & Bradshaw, 1999), but not investigated with parallel data on two types of pollinators visiting varying flowers. Adaptation away and towards are not mutually exclusive; they can proceed simultaneously on the same trait.

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For example, when bird-pollination is favoured, an increase in the exsertion of anthers could be favoured by selection because it keeps pollen from being placed on bees (adaptation away from bee pollination) and/or because it places more pollen on hummingbirds (towards bird pollination). Similarly, a reduction in corolla width could restrict visitation by a large-bodied bee and at the same time improve morphological fit around the head of a long-beaked bird, increasing the precision of pollen deposition on the head. The resulting characters can thus be as much the result of selection for avoidance of some animals as attraction or fit to others (Pellmyr, 2002). Testing the relative importance of the two selective forces, away and towards, is complicated by the fact that they could select for floral changes in the same direction, as in the previous examples. In addition, floral characters can be functionally or genetically linked to each other, making it hard to disentangle effects on single characters. In spite of these difficulties, our understanding of floral evolution should recognize the possibility of both selection modes.

We have studied shifts from bee- to hummingbird-pollination among *Penstemon*, a group in which the transition has evolved independently at least 13 times (Wilson *et al.*, in press). Floral characters predict the visitor spectra following classical pollination syndromes (Straw, 1956; Crosswhite, 1967; Crosswhite & Crosswhite, 1981; Wilson *et al.*, 2004). A typical contrast is *Penstemon strictus* Benth. vs. *P. barbatus* (Cav.) Roth. *Penstemon strictus* is pollinated at high rates by a variety of bees (*Bombus*, *Anthophora*, *Osmia*) and the wasp *Pseudomasaris vespoides* (Williams & Thomson, 1998), whereas hummingbirds visit only occasionally. The flowers are purple, produce small volumes of concentrated nectar, have broad corolla tubes that allow large Hymenoptera to reach the nectaries, lower lips that extend as a landing platform, anthers and stigmas nearly included, and a stiff pedicel that holds the flowers horizontal. *Penstemon barbatus* is pollinated almost exclusively by hummingbirds (Brown & Kodric-Brown, 1979; Wilson *et al.*, 2004); the flowers are red, produce large volumes of dilute nectar, have long and narrow corolla tubes, reflexed lower lips, exserted anthers and stigmas, and a flexible pedicel that positions the flowers on an incline. Previously, we compared pollen transfer by hummingbirds and bumblebees on *P. strictus* and *P. barbatus* (Castellanos *et al.*, 2003). Hummingbirds moved pollen of *P. strictus* almost as efficiently as bees. Also, in the shift to bird-pollination, flower changes have resulted in higher pollen removal and deposition by hummingbirds, although they have come to be ineffectively pollinated by bees. We proposed that the shifts to more-efficient hummingbird pollination could be set in motion because Hymenoptera became parasites from the plant's perspective when hummingbirds became reliable visitors.

Here we test the hypothesis that floral traits can evolve to exclude less efficient bees, in addition to, or instead of, improving pollen transfer by more efficient birds. We investigated four floral traits that are believed to play a role in pollen transfer through physical interaction with the pollinators. We modified one trait at a time in *P. strictus* flowers so that the altered flowers resembled *P. barbatus*, and measured the effect on pollen transfer and behaviours associated with the attraction of animals to flowers.

Methods

Experimental flower modifications and predictions

The four floral traits investigated were: the exsertion of anthers and stigmas, the presence of a lower corolla lip, the width of the corolla tube, and the inclination of the flower (associated with the flexibility of the pedicel). We chose these characters because they potentially affect pollen movement through physical contact with the pollinators, are among those used in recognizing pollination syndromes (Fægri & van der Pijl, 1979), and present consistently opposing states in *Penstemon* species adapted to bees or birds (Thomson *et al.*, 2000). We modified one trait at a time, i.e. we isolated individual hummingbird-flower characters in a bee-flower background. We then measured the effects on pollen movement by bumblebees and hummingbirds, as well as floral attractiveness to those pollinators.

Exserted stigmas and anthers

In *P. strictus* flowers, stigmas and anthers are included within the corolla tube (Fig. 1e). We modified the distance that these organs protruded (Fig. 1a), approximating their placement in *P. barbatus*. Female-phase flowers were made to have the stigma project about 4 mm beyond the corolla. Male-phase flowers had one of the four filaments similarly elongated. With fine scissors, we severed the filament or the style and taped it back to the inside of the corolla in the more exserted position. We changed the position of the stigmas just before the experiments and saw no sign of wilting before the flowers were visited. We imposed the anther treatment early in the morning and waited a few hours for dehiscence. Anthers dehisced normally and stayed in position.

We hypothesized that exserted organs would affect pollen transfer by both bees and birds, in opposite directions. We expected that exserted organs would be less likely to contact a bee's body, thus reducing pollen removal from anthers and deposition on stigmas, compared with nonmanipulated flowers. With hummingbirds, exserted organs might contact their forehead as opposed to the beak, and transfer more grains because the head is larger and rougher (cf. Campbell *et al.*, 1994). Organ exsertion was a minor change visually, unlikely to influence attractiveness or handling by either pollinator.
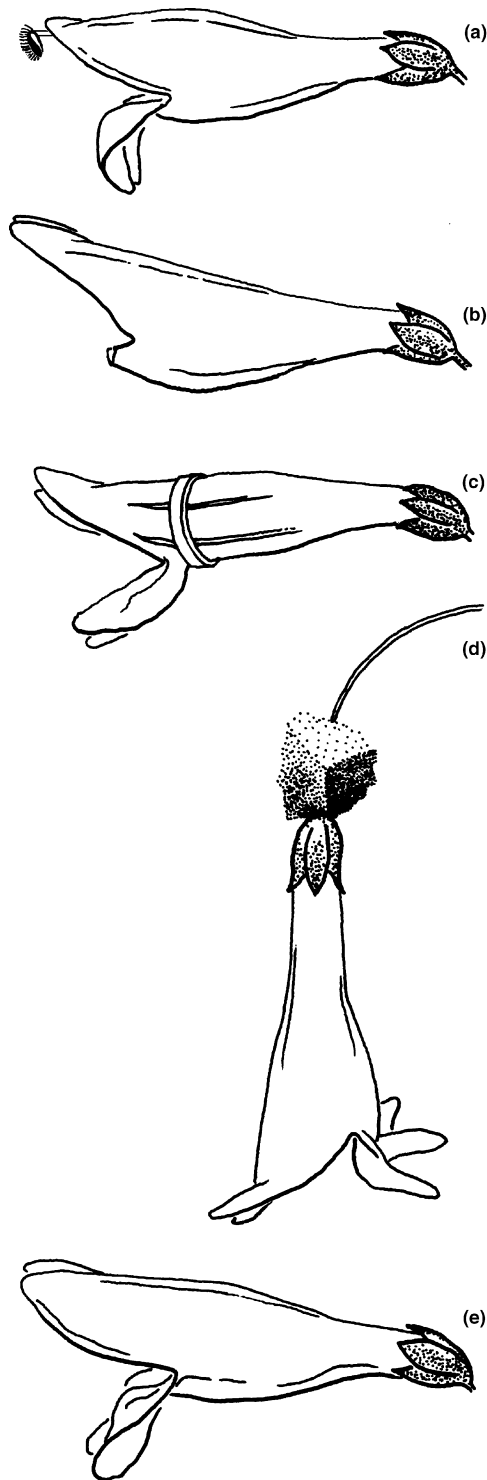
Fig. 1 Experimental trait modifications in fresh *Penstemon strictus* flowers. a, anther exserted. b, lower corolla lip removed. c, corolla narrowed. d, pendent flower (flexible pedicel). e, nonmodified flower.

Lower lips removed

The five petals of *P. strictus* flowers are fused into a tube of about 19 mm. Lobes extend beyond the tube separately, and the three lower ones form a lip that is used as a landing platform by insects (Straw, 1956; Grant & Grant, 1968). We excised this lip (Fig. 1b), mimicking *P. barbatus* flowers, whose lip is strongly reflexed. We cut the corollas a few minutes before presenting them to pollinators.

We expected that lipless flowers would affect both pollen transfer and attraction. A missing landing platform could discourage bee visits by increasing handling time, as observed by Clements & Long (1923) in a classic study that included manipulations of *P. alpinus* flowers. Also, a smaller projected area could render the flower less appealing to bees (Schemske & Bradshaw, 1999). When visits occurred, lipless flowers might allow the bees to drop below the reproductive organs. We did not expect this modification to affect the attraction of humming-birds. Removing the lower lip might allow them to insert their head (not just their tongue) farther into the flower and cause them to come into firmer contact with the anthers and stigma.

Narrow corollas

We constricted the corolla tubes of *P. strictus* using small orthodontic rubber bands, reducing the inside diameter of the tube to <5 mm (Fig. 1c). This treatment produced some pleats of petal tissue that could obstruct the corolla tube. To reduce this problem, we made the tube less crowded by clipping out filaments other than the one holding the donor anther.

We expected this treatment to discourage bee visitation, because access to nectar would be reduced for them (Straw, 1956). We did not predict an effect on hum-mingbird preferences, but did expect increased pollen removal and deposition by both birds and bees. As the narrowed corolla leaves less room for off-axis pollinator movements, contact with the reproductive organs would be more likely (Murcia, 1990; Smith *et al.*, 1996; Fetscher *et al.*, 2002) and at a more precise location on the pollinator's body.

Pendent flowers

This treatment changed flower inclination by changing the flexibility of the floral pedicel. Stiff *P. strictus* flowers became pendent. We attached flowers to a 4-cm piece of nylon fishing line (10 pound test) the other end of which was attached to the stem (Fig. 1d). It was necessary to remove the flower from the stem, so to prevent wilting we placed a small cube of water-soaked florist's foam in contact with the cut pedicel (Fig. 1d).

We expected that bees could be deterred by the difficulties of landing on floppy, downwardly facing, large flowers. Bumblebees do visit pendent flowers (e.g. *Mertensia*), but they are usually smaller. We did not expect an effect on hummingbirds, which visit many

flowers that face down. With respect to pollen transfer, we anticipated opposite effects for bees and birds. Because bees might enter the flowers from varying angles, we expected pollen removal, and especially deposition, to be low in pendent flowers. Birds, on the contrary, were expected to remove and possibly deposit more pollen than in nonmodified flowers, because increased flower shaking and more consistent contact while hovering would allow more pollen to be dislodged from anthers and deposited on stigmas (Hurlbert *et al.*, 1996; Tadey & Aizen, 2001).

Experiments

We measured pollen transfer by hummingbirds and bumblebees in an outdoor flight cage, at the Rocky Mountain Biological Laboratory, Colorado, USA, during the summer of 2002. *Penstemon strictus* flowers were taken from potted plants or from cut inflorescences, all kept indoors to prevent visitation. We kept cut inflorescences in vases with water, where they produced nectar and lasted for 3 days without signs of wilting.

For experiments with birds, we used male Broad-tailed Hummingbirds (*Selasphorus platycercus*) or occasionally, male Rufous Hummingbirds (*S. rufus*). Birds were accustomed to enter a flight cage (1.5 × 2.5 × 2 m) through a trap door, and drink sugar-water from a hummingbird feeder. When the flowers were ready, we waited for a hummingbird to enter, closed the door behind it, and trained the bird on emasculated *P. strictus* flowers. We then presented the experimental flowers. Most hummingbirds visited all the flowers within minutes, after which time we opened the door and let the bird exit freely. Before every experimental run, we caught the birds with our hands and cleaned the forehead and beak with a moistened cotton swab to remove pollen grains. For bee experiments, we used workers of *Bombus bifarius*, *B. flavifrons*, and *B. mixtus*. The bees were captured on *P. strictus*, allowed to groom for at least an hour, and then chilled overnight in a refrigerator. After they were warmed up on emasculated flowers, they were hungry enough to visit the flowers of an experimental run in the flight cage.

We measured pollen removed per visit, pollen deposited on the stigmas of the first 10 recipient flowers following a visit to a donor, and the number of stigmas that were not contacted by the pollinator per experimental run, plus handling time per flower. These variables serve as analogues of the success of pollen donation by the visited plants. We focus on the effect of floral traits on male function because *Penstemon* flowers usually attract enough pollinators that seed set is unlikely to be pollen limited in the wild. Selection on the traits we were studying is likely stronger through pollen-transfer efficiency (Wilson *et al.*, 1994).

Each experimental run in the flight cage comprised a single visit to a pollen donor flower followed by single visits to 10 emasculated recipient flowers. These eleven flowers, plus a nonvisited control donor, were modified in the same way, or left intact in the case of the nonmodified flowers. We presented all recipient flowers at the same time, and removed them as they were visited. Visits were videotaped, and handling times determined from the tapes. Bee handling time was estimated to the nearest s, and bird visits to the nearest 30th of a s. Each run gave one data point for pollen removal, pollen deposition in 10 recipients, plus handling times in each flower.

We calculated pollen removal from a single anther in the donor flower by comparing the pollen remaining in it to unvisited anthers. In preparation for the experiment, we removed three of the four anthers in the flower, leaving one front anther before any had dehisced. After the remaining anther had dehisced, we presented the flower to a pollinator. Following a visit, we held a microcentrifuge tube under the anther and carefully removed it with scissors. The visited anther and the three unvisited anthers were separately preserved in ethanol. We did the same to control flowers, which were modified but never visited. We had a control flower in each experimental run. We used an Elzone 280-PC (Micro-meritics, Norcross, GA, USA) electronic particle counter to estimate the grains remaining in the visited anther and the grains produced by the three unvisited anthers. The mean number of grains in the three unvisited anthers was assumed to equal the grains initially present in the visited anther. This number was corrected to account for pollen losses because of handling by the experimenter. We based our estimated pollen losses on the handled control flowers, which had received the same treatment but were not visited; the mean proportion of grains lost within each flower treatment was used as a correction factor by multiplying all donor flowers by it. Finally, *pollen removal* was estimated by subtracting the number of grains remaining in the visited anther from our corrected estimate of the initial number of grains present in the anther.

After removing the anther from the visited and control flowers of each experiment, we noted whether there was none, some, or a large amount of nectar remaining in both flowers. By assuming that both flowers contained the same amount of nectar at the beginning of the experiment and comparing the control with the visited flower, we tested for differences in nectar consumption.

For estimating *pollen deposition*, we collected the stigmas of visited recipient flowers and mounted them on a microscope slide with fuchsin-tinted glycerin jelly (Beattie, 1971), and counted all pollen grains under a microscope. We also compared the number of stigmas (out of 10) that received no grains after a visit. We consider those to be stigmas missed by the pollinator.
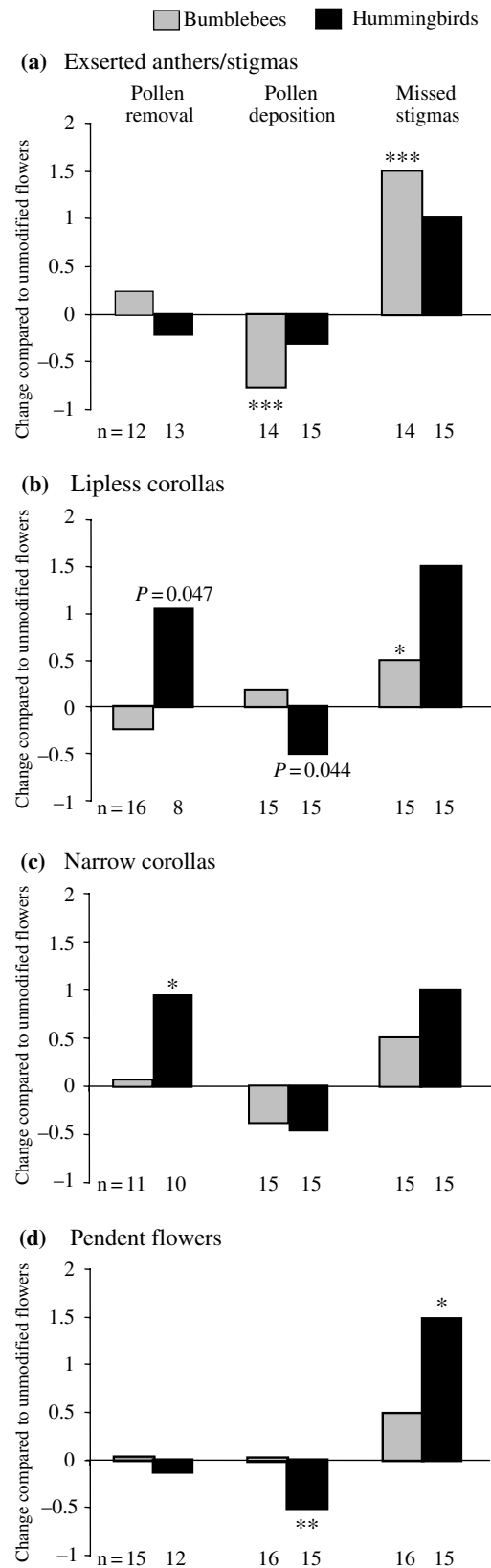
Choice experiment with hummingbirds

We tested whether hummingbirds preferred to visit a plant with normal flowers over altered flowers in choice experiments inside the flight cage. Each hummingbird was presented with two sets of six flowers, one with nonmodified flowers and one with all flowers modified for one of the traits. The two 'plants' (of six flowers each) were separated by 50 cm and located on either side of the bird's perch. A bird was allowed to visit all 12 flowers at least once before data recording started, so that it experienced both flower types as rewarding. We then replaced the flowers with fresh ones and recorded which set of flowers was visited first. This was repeated 10 consecutive times for each hummingbird and flower modification, each time using fresh flowers and randomizing their location with respect to the perch. We repeated such experiments with narrowed flowers (three birds), lipless flowers (three birds), and pendent flowers (four birds). In total, we used five different birds for these 10 sets of experiments over 2 days. No bird was tested on the same modification twice.

Statistical analyses

Using planned comparisons, we contrasted pollen removal, deposition, nectar consumption and handling time under each treatment with those recorded for nonmodified flowers. We used Mann–Whitney U-tests, because our data were not normally distributed. In addition, we compared the means of the treatments using randomization tests with 10 000 iterations in Rundom 1.1 (Jadwiszczak, 2003). Randomizations yielded similar results to U-tests, so we present only the latter.

In Figs 2 and 3, the horizontal axis represents the median value of the nonmodified flowers, and the bars show the change with respect to those medians. We did not adjust for multiple comparisons, because we were interested in the differences between each modification treatment and normal flowers and not among modified flowers. Bonferroni-corrected tests would use $\alpha' = 0.05/4$ comparisons $= 0.0125$.

Fig. 2 Effects of experimental floral trait modifications [(a) exserted anthers and stigmas; (b) lower corolla lip removed; (c) narrowed corolla; and (d) pendent flowers] on pollen removal, pollen deposition to 10 recipients, and the number of missed stigmas of *Penstemon strictus*, when visited by bumblebees and hummingbirds. Each result was compared with that of nonmodified flowers within pollinators (nonmodified $N = 22$–36 for bees, $n = 23$–63 for birds). The zero line on the Y-axis represents the median value found for nonmodified flowers, and the bars show the mean change, calculated as (median on modified flower – median on nonmodified)/median on nonmodified, for each trait-pollinator combination studied. Numbers under the bars are sample sizes for modified flowers, and asterisks show significance levels: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
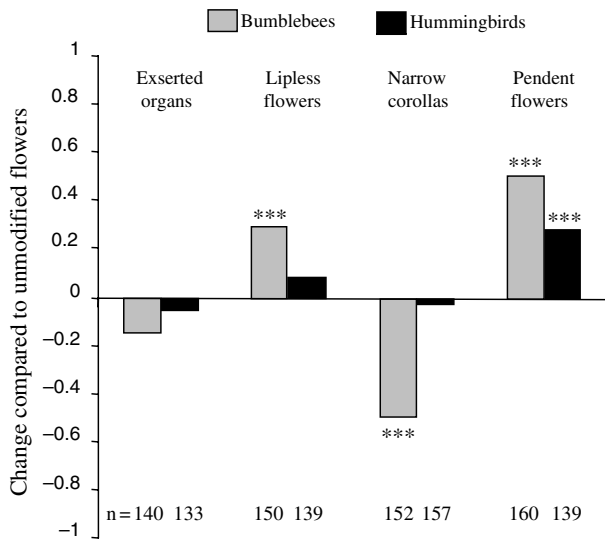
Fig. 3 Effects of experimental flower trait modifications on handling time spent by bumblebees and hummingbirds visiting *Penstemon strictus* flowers. Handling time was compared to that of nonmodified flowers within pollinator (nonmodified $n = 411$ for bees, $n = 636$ for birds). The Y-axis represents the median handling time found for nonmodified flowers and the bars show the median change (median on modified flower – median on nonmodified)/median on nonmodified, for each trait studied. Numbers under the bars are sample sizes for modified flowers, and *** show significant differences at $P < 0.001$.

For the choice experiment with hummingbirds, we pooled the 10 choices for each bird-modification combination, and calculated the probability of such outcome (or worse) under a binomial distribution where modified and normal flowers had equal probabilities of being visited.

Results

Effects on pollen transfer

Increasing the exsertion of anthers and stigmas (Fig. 2a) reduced pollen deposition by bees by 75% ($U_{14,22} = 51.0$, $P < 0.001$). Consistent with this result, the probability of a bee missing a stigma was 1.5 times higher in flowers with exserted anthers than in nonmodified flowers ($U_{14,24} = 272.5$, $P = 0.001$). Pollen removal by neither bees nor birds was significantly affected by this treatment ($U_{12,37} = 277.0$, $P = $ n.s. for bees; $U_{13,63} = 382.0$, $P = $ n.s. for birds). Also neither pollen deposition nor stigmas missed by hummingbirds were affected by greater exsertion of organs ($U_{15,23} = 135.0$, $P = $ n.s. deposition; $U_{15,28} = 280.5$, $P = 0.07$ stigmas missed).

In flowers with lower lips removed (Fig. 2b), bees were more likely to miss stigmas ($U_{15,24} = 254.0$, $P < 0.05$); however, the total deposition of pollen was not significantly reduced ($U_{15,22} = 189.0$, $P = $ n.s.), and neither was pollen removal ($U_{16,37} = 256.0$, $P = $ n.s.). For hummingbirds, both removal and deposition were affected; removal increased ($U_{8,63} = 361.0$, $P < 0.05$) whereas deposition decreased ($U_{15,23} = 105.0$, $P < 0.05$).

The effect of reducing the girth of the corolla tube was less consistent than we had predicted (Fig. 2c). Hummingbirds removed almost twice as many pollen grains from narrow flowers than from nonmodified flowers, as we had expected ($U_{10,63} = 459.0$, $P < 0.05$). Bees, however, removed about the same amount in both types of flowers ($U_{11,37} = 227.0$, $P = $ n.s.). Pollen deposition by bees was reduced, and the probability that they would miss a stigma was increased, but neither result was significant ($U_{15,22} = 137.0$, $P = $ n.s.; $U_{15,24} = 236.0$, $P = 0.097$). The same was true for hummingbirds ($U_{15,23} = 122.5$, $P = $ n.s. for deposition, $U_{15,28} = 269.0$, $P = $ n.s. for stigmas missed).

For bumblebees, making flowers pendent did not significantly affect pollen transfer (Fig. 2d; $U_{15,37} = 301.0$, $P = $ n.s. for removal, $U_{16,22} = 204.0$, $P = $ n.s, for deposition, $U_{16,24} = 232.5$, $P = $ n.s for stigmas missed). Pollen removal by hummingbirds was also not affected ($U_{12,63} = 341.0$, $P = $ n.s.), but deposition was significantly reduced to half the number of grains ($U_{15,23} = 84.5$, $P < 0.05$), and birds missed more than twice the number of stigmas in flowers with flexible pedicels ($U_{15,28} = 293.0$, $P < 0.05$).

Effects on flower handling time and nectar consumption

The median duration of a bumblebee visit was cut in half by narrowing the corolla tubes (Fig. 3; $U_{152,411} = 18945$, $P < 0.001$). Hummingbird visit duration was not affected by this treatment ($U_{157,636} = 46820.5$, $P = $ n.s.). The exsertion of organs, as expected, did not affect handling time by either pollinator ($U_{140,411} = 28046.0$, $P = $ n.s. for bees; $U_{133,636} = 39957.0$, $P = $ n.s. for birds). Down-facing flowers increased the handling time of bees by 50% ($U_{160,411} = 43800.0$, $P < 0.001$) and of hummingbirds by somewhat less ($U_{139,636} = 55115.5$, $P < 0.001$). Making flowers lipless increased the handling time of bees (visits were about 25% longer; $U_{150,411} = 38290.0$, $P < 0.001$), but not of hummingbirds ($U_{139,636} = 47031.0$, $P = $ n.s.).

Nectar consumption by bees was reduced by narrowing the corolla tubes. In such flowers, bees were likely to leave more nectar in the flowers than in nonmodified flowers (*t*-test with separate variances, $t_{15,23} = 2.58$, $P < 0.05$). In all the other types of flowers, both bees and hummingbirds consumed as much nectar as they did in nonmodified flowers.

Effects on hummingbird attraction

In the flight cage, hummingbirds showed no preference for or against any of the modified flowers. Birds went to

narrowed flowers 19 of 40 times ($P = 0.875$), to lipless flowers 18 of 30 times ($P = 0.362$), and to pendent flowers 14 of 30 times ($P = 0.856$). There did not appear to be any heterogeneities in how the birds chose: each bird chose both the modified and the nonmodified inflorescences at least twice.

Discussion

We found evidence for both 'pro-bird' and 'anti-bee' adaptations, and 'anti-bee' traits appear to be quite important in the process of floral specialization to bird pollination. However, agreement between our predictions and results was modest. Our predictive success may have been hampered in three ways. (1) Our sample sizes provided modest statistical power to detect effects that could be evolutionarily important. Using the data of Castellanos *et al.* (2003) comparing normal *P. strictus* and *P. barbatus* flowers being pollinated by hummingbirds, we estimated that the statistical power in the present study was 58 and 60% for pollen removal and deposition respectively, given our experimental sample sizes. This power analysis assumes that we were interested in detecting the 'final' effect size that a shift to the hummingbird floral syndrome achieved. (2) Our predictions might not have been accurate because of meaningful responses that we can explain in hindsight, and misconceptions in our views of how birds and bees interact with flowers. (3) Responses to manipulation from the 'bee-syndrome' to the 'bird-syndrome' may require several simultaneous character changes. We return to this below.

In some cases we found the unanticipated result that pollen removal and pollen deposition responded discordantly (e.g. lipless flowers for birds). We expected removal and deposition to change concordantly because anthers and stigma are presented in the same location within the flower, and we assumed that both processes are based on the same positioning of the pollinator's body. However, perhaps pollen removal is not always determined by the vigour with which an animal contacts the anthers. We cannot rule out the possibility of pollen being removed by shaking of the flower without anther contact. A manipulation that causes increased fumbling, but does not channel animals against the organs, might increase removal although reducing deposition.

Exsertion

For bees, the predicted role of organ exsertion in reducing pollen deposition and stigma contacts was confirmed and is easily explained: bees entered these flowers without contacting the stigmas. Several other studies have found an effect of exsertion on pollen transfer by insects (Murcia, 1990; Harder & Barrett, 1993; Conner *et al.*, 1995). The lack of a concomitant decline in pollen removal is harder to explain. Pollen may have

been shaken out regardless of direct contact between bee and anther, and any shaking could have been amplified by the cantilevered position of the exserted anther.

We expected that exserting organs would increase contact with the heads of hummingbirds, increasing pollen removal and deposition, but neither effect was significant. Likewise, Campbell *et al.* (1996) found no selection for exserted stigmas in hummingbird-pollinated *Ipomopsis aggregata*. For *Penstemon*, it remains possible that exsertion would have produced the expected effects if it had been combined with narrowing the corolla tube. Such a tube would enforce contact between the organs and the head, as in *P. barbatus*. With an unconstricted *P. strictus* tube, birds probably enter at angles that let them reach nectar without contacting organs.

It is difficult to conclude whether exsertion serves more as an 'anti-bee' or a 'pro-bird' character. In many hummingbird-adapted penstemons, organs are exserted to the point of avoiding contact with nectaring Hymenoptera. Taking our results at face value, organ exsertion in bird-pollinated penstemons more likely evolved away from bees, rather than towards birds. Assuming that nectar is costly to the plant (cf. Pyke, 1991), one can imagine that the exsertion of organs would be most beneficial if accompanied by restricted access to bees to nectaries (e.g. through narrowing the corolla); otherwise bees would be encouraged as nectar thieves. Together with a narrow tube, exsertion could then also improve the fit to birds. A balance between the costs of replenishing nectar (Castellanos *et al.*, 2002) and of losing pollen could determine the strength of selection for exserted anthers and stigmas.

Lipless flowers

With bees, the only significant effect of removing the 'landing platform' was a reduction in the frequency of stigma contact. This probably occurred when bees landed on the side of the corolla and entered laterally, but this tendency to miss stigmas did not significantly diminish mean deposition. It did, however, significantly increase handling time. Assuming that bees in nature forage to optimize rewards per unit time, a higher handling time suggests that bees would under-visit lipless plants (Waser & Price, 1985; Kunin & Iwasa, 1996; Stout *et al.*, 1998; Ohashi, 2002).

For birds, liplessness produced marginally significant increases in removal and decreases in deposition. The reduced deposition and the large but nonsignificant increase in missed stigmas may arise from birds being better able to duck under the reproductive organs. The increased removal is ambiguous, and all of the bird effects are weak. Smith *et al.* (1996) suggested that a lip might serve as a directional cue for birds, reducing bill insertion error and increasing contact time with reproductive organs. This was confirmed by Temeles & Rankin (2000), who found that birds removed less pollen from

experimentally lipless *Monarda* flowers. They attributed this to the duration of contact with anthers, because the presence of a lip (the normal state in this hummingbird-pollinated species) reduces the speed of bill insertion and forces longer contacts. In our experiments, lip presence or absence did not affect bird-handling time. It is possible that the birds were indeed able to push their heads farther into a lipless flower and remove more pollen from an anther, but this did not improve the chances of contacting the stigma in wide-mouthed flowers. It seems that a reduction in lips would not be selected for as an adaptation to hummingbirds without other characters changing first. Liplessness seems to be chiefly an 'anti-bee' character that acts by making the flowers harder to handle.

Corolla constriction

The narrow corollas of hummingbird-pollinated pentstemons may function to improve the morphological fit of flowers to birds or to prevent visits by nectaring bees. Our results give some evidence for both. We found greater removal by birds and curtailed handling times by bees. In the absence of increased deposition by birds, the greater removal is ambiguous. It may be that the inward-bulging pleats of the narrowed tubes sometimes pushed stigmas out of position, preventing precise contact. Fetscher *et al.* (2002) showed that hummingbirds can actively change their position although entering a flower to avoid touching organs that might interfere with their vision. The crowded corolla tube in our narrow flowers might have led the birds to visit the flowers awkwardly.

The short bee handling times are easily explained. Bees were often unable to reach the nectaries at the flower base, so they tended to depart quickly. Indeed, we found more nectar left in the constricted flowers after visits than in normal wide flowers. Although bees still contacted anthers and stigmas, producing normal pollen transfer, we imagine that they would learn the locations of narrow-tubed plants and avoid them in favour of others where they could obtain nectar more efficiently (Thomson, 1988; R. Cartar pers. comm.). In *Polemonium viscosum*, visitation rate by bumblebees increased strongly with corolla width (Galen & Cuba, 2001).

The handling time of hummingbirds was not affected by corolla constriction. Studying other plants, Temeles (1996) and Grant & Temeles (1992) found that narrower corollas increased hummingbird's handling time; in wider flowers, birds could insert their heads farther into the flower and spend less time extending their tongues. In contrast, Campbell *et al.* (1991) found no effect of corolla width on handling time or visitation rates by birds on *Ipomopsis aggregata*, and they did find an increase in pollen export with increasing corolla width, apparently contradicting our prediction. Campbell *et al.* were comparing *I. aggregata* with the even narrower flowers of the moth-pollinated *Ipomopsis tenuituba*. Their results com-

bined with ours suggest that an intermediate corolla width is optimal for pollen transfer by birds.

Pendent flowers

This manipulation significantly increased handling times for both pollinators. In addition, birds deposited less pollen and missed stigmas more often. This pattern, which seems to be both 'anti-bee' and 'anti-bird', is puzzling. We suspect that the principal effect is 'anti-bee': bee-handling times increased, which could reduce visitation. Bees are frequent visitors of flowers that face down, but such flowers are usually smaller than *P. strictus*, and the bees need not crawl into them. For larger, walk-in flowers, bees seem to prefer rigidly horizontal flowers that provide a firm landing.

Birds also prolonged their visit duration, but the relative increase was smaller and the absolute duration very short. Also, birds did not reject pendent flowers in our choice experiment. The decreases in stigma contacts and deposition by birds are probably attributable to the wide-mouthed corolla of *P. strictus*, which permits off-axis approaches. Approaching a pendent flower, hovering hummingbirds may enter from an angle that dodges the reproductive organs.

If a flexible pedicel is an adaptation to improve pollen transfer by birds, it likely needs to be preceded by a narrowing of the floral tube. Studying *Impatiens capensis*, Hurlbert *et al.* (1996) experimentally changed the flexibility of flower pedicels and found no evidence of hummingbird preference for or against floppy flowers. They also saw an increase in flower handling time. In contrast to our results, they showed that flexible pedicels led to higher pollen removal. Alternative hypotheses proposed for explaining the frequency of flexible pedicels in hummingbird-pollinated flowers include protection against flooding during rainstorms. Tadey & Aizen (2001) did not find evidence for a negative effect of rain on pollen transfer or nectar characteristics in *Tristerix corymbosus*.

Concluding remarks

Taken together, our results show that altering one floral character whereas leaving others unchanged does not always have the predicted effects. Floral parts may well function in an integrated manner and are likely to be selected as a group (Armbruster, 1991; Campbell *et al.*, 1994; Campbell *et al.*, 1996; Herrera, 2001; Hodges *et al.*, 2002). Perhaps as one character changes, its altered state sets the context for subsequent changes. For example, it may be that narrow flower tubes become beneficial only after reproductive organs are exserted. Alternatively, several characters may be under selection to change in concert. For instance, increasingly narrow tubes might be adaptively coordinated with organs that are increasingly exserted. Experiments that consider the functional integ-

ration of the flower as a whole could complement conclusions derived from altering single traits (see Smith *et al.*, 1996; Temeles, 1996; Fenster *et al.*, 2004). We limited this study to single-characters, because experiments that modify floral features in realistic ways require meticulous work that also constrains sample sizes. Natural variation in pollen removal and deposition is inherently large, because pollination is a messy process. Other studies also have found that studying how floral morphology affects pollen transfer is rather difficult (Wilson, 1995; Fulton & Hodges, 1999; Galen & Cuba, 2001).

Here we concentrated on biomechanical characters that might have evolved at an advanced state of a pollinator shift and that have been little studied. Nevertheless, attraction characters like flower nectar (Stiles, 1976; Meléndez-Ackerman *et al.*, 1997) and colour (Raven, 1972; Sutherland & Vickery, 1993; Schemske & Bradshaw, 1999) are also candidates for differential selection by diverse pollinators and adaptation 'away' from inferior pollinators and 'towards' superior ones. Both nectar and colour are relevant in the shifts of *Penstemon* species from bee- to hummingbird-pollination.

Acknowledgments

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