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POLLEN TRANSFER BY HUMMINGBIRDS AND BUMBLEBEES, AND THE DIVERGENCE OF POLLINATION MODES IN *PENSTEMON*

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Abstract.—We compared pollen removal and deposition by hummingbirds and bumblebees visiting bird-syndrome *Penstemon barbatus* and bee-syndrome *P. strictus* flowers. One model for evolutionary shifts from bee pollination to bird pollination has assumed that, mostly due to grooming, pollen on bee bodies quickly becomes unavailable for transfer to stigmas, whereas pollen on hummingbirds has greater carryover. Comparing bumblebees and hummingbirds seeking nectar in *P. strictus*, we confirmed that bees had a steeper pollen carryover curve than birds but, surprisingly, bees and birds removed similar amounts of pollen and had similar per-visit pollen transfer efficiencies. Comparing *P. barbatus* and *P. strictus* visited by hummingbirds, the bird-syndrome flowers had more pollen removed, more pollen deposited, and a higher transfer efficiency than the bee-syndrome flowers. In addition, *P. barbatus* flowers have evolved such that their anthers and stigmas would not easily come into contact with bumblebees if they were to forage on them. We discuss the role that differences in pollination efficiency between bees and hummingbirds may have played in the repeated evolution of hummingbird pollination in *Penstemon*.

Key words.—Bumblebee, floral evolution, hummingbird, *Penstemon*, pollen removal and deposition, pollination, pollinator shifts.

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The premise that flowers are adapted to their pollinators is supported by suites of covarying floral characters that are associated with major pollinator types. These sets of characters have been traditionally called pollination syndromes (Faegri and van der Pijl 1979), and although their explanatory power can be limited (Waser et al. 1996), syndromes describe patterns of floral adaptation in some plant groups (Fenster et al. 2004). In the genus *Penstemon* (Scrophulariaceae), for example, floral characters do predict pollinator spectra (Wilson et al. 2003). Species primarily pollinated by hummingbirds tend to differ systematically from bee-pollinated relatives in corolla color, morphology, nectar characteristics, and degree of anther dehiscence (Straw 1956; Crosswhite 1967; Thomson et al. 2000). Most of these differences appear to be adaptations to disparities between the pollinators in size, shape, sensory physiology, foraging energetics, and behavior. Transitions from bee pollination to hummingbird pollination have occurred in at least 14 independent lineages in *Penstemon* (Wilson et al., in press).

Anther dehiscence, and more generally the presentation of pollen by a plant, is thought to be under selection through male function, that is, it affects the capacity of a plant to sire seed (Percival 1955; Lloyd 1984; Harder and Thomson 1989; Klinkhamer et al. 1994). As with other syndrome traits, the optimal strategy for presenting pollen can be associated with particular types of pollinators (Thomson and Thomson 1992; Thomson et al. 2000). When the pollinators are apid bees and visitation rates are high, flowers such as *Penstemon* are thought to be under selection for presenting their pollen gradually, in numerous small doses. Heavy dosing would be wasteful from the plant's perspective, because such bees frequently groom pollen off the zones on their bodies that contact stigmas and into corbiculae, where it is unlikely to be available for pollination (Thomson 1986; Thorp 2000). Rademaker et al. (1997), for instance, showed that grooming accounts for the largest fraction of pollen that is lost after being picked up by a bumblebee. Furthermore, such grooming seems to be stimulated by large loads of pollen (Harder 1990a). In contrast to bees, hummingbirds do not collect pollen and only groom it off their bills, if at all, and so are thought to waste less pollen and transfer it without a rapid decay in pollen carryover. When hummingbirds are the main pollinators, theory predicts that pollen should be presented less restrictively, in fewer, larger doses (Thomson et al. 2000). Presenting more pollen at once should also be a better strategy for a hummingbird-pollinated plant because hummingbird visitation rates are usually lower. Anthers of *Penstemon* species adapted to different pollinators dehisce in ways consistent with the theory. Those of bee-pollinated species dehisce more narrowly or more gradually than those of bird-pollinated species (Thomson et al. 2000).

Aside from pollen presentation, pollinators may differ in two properties that affect pollen transfer in a particular type of flower. The first property, ''fit,'' depends on how the interaction of morphology and behavior dictates the contact between the animal and the flower's sexual parts. Contacts between animal and anthers create and replenish the active pool of pollen on the pollinator's body, whereas stigma contacts diminish the pool. The second property, ''turnover,'' governs the persistence of the pollen pool on a pollinator's body. Although stigma contacts play a role in turnover, that role may be minor compared to the action of grooming. When animals groom thoroughly and frequently, pollen grains that get deposited on stigmas may have had a short residence time in the active pollen pool on the pollinator's body.

For the theory outlined above, the most important difference

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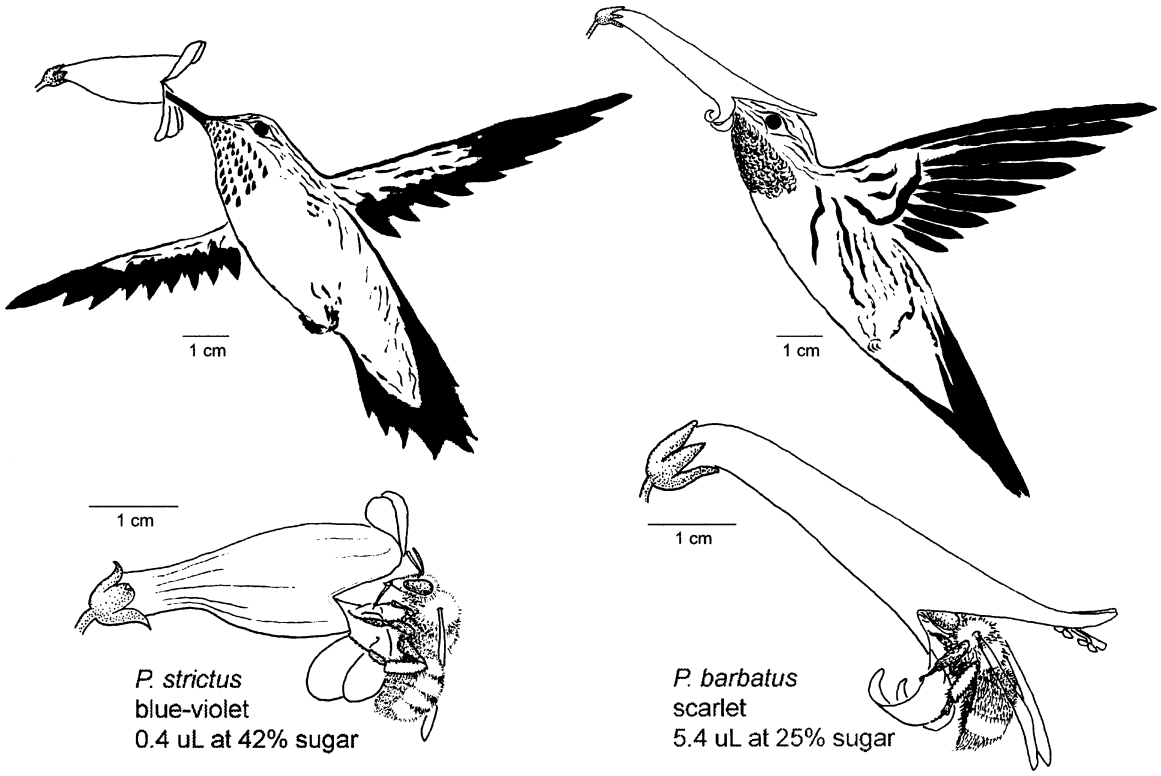

FIG. 1. Bumblebees and hummingbirds visiting bee- and bird-syndrome flowers. The animals are shown just as they would be upon arriving at flowers and before fully reaching into the corolla, except for the bird visiting *Penstemon barbatus*, which is shown with its beak fully inserted. The anthers and stigma of *P. strictus* are held just inside the upper lips. The bumblebees depicted were workers of the small-bodied *Bombus flavifrons* (left) and *B. bifarius* (right). Drawn by P. Wilson from photographs by M. C. Castellanos and P. Wilson.

between bees and birds is the assumption that a pollen grain placed on a hummingbird has a higher probability of reaching a recipient stigma than one placed on a bee. In addition, Thomson et al. (2000) suggested that bees may remove more pollen than birds per visit. This seemed to be the case when Mitchell and Waser (1992) compared their results with hummingbirds to results with bumblebees, reviewed by Harder (1990b). Bees get their protein from pollen, and have adaptations that aid in thorough removal of pollen from anthers, such as bristly body parts, pollen-harvesting behaviors, and electrostatic attraction of pollen grains (Thorp 2000). Thomson et al. suggested that some bee-adapted flowers may have restricted pollen presentation to the point that hummingbirds cannot pollinate them effectively, simply because they do not remove enough pollen, whereas flowers adapted to birds may have such open pollen presentation that bees become conditional parasites, because they waste large proportions of pollen that would be otherwise transferred by birds (Thomson 2003).

Under a pollination regime that includes both Hymenoptera and hummingbirds, flower species might adapt to use one or the other. If it were possible for the same floral phenotype to use both efficiently, generalization would be expected to evolve, whereas specialization could result if the fitness benefits of adaptation to one kind of pollinator exceed the loss by concomitant maladaptation to the other kind (Aigner 2001). Diversification via specialization is often thought to be constrained by such trade-offs (Wilson and Thomson 1996). We were, therefore, interested in contrasting plant species adapted to use birds versus bees as pollinators, thus examining the results of evolutionary specialization. Specifically, we expected that as hummingbird pollination evolved the flowers would adapt to fit well around hummingbirds while possibly fitting poorly around bees, and that this would improve the efficiency of pollen transfer by hummingbirds while lessening the capacity of bees to transfer pollen. To study such interactions, it is best to study both types of pollinators visiting the two types of flowers.

We compared pollen transport by hummingbirds and bumblebees visiting two closely related species of *Penstemon* that have flowers of different syndromes: hymenopteran-pollinated *Penstemon strictus* Benth. and hummingbird-pollinated *P. barbatus* (Cav.) Roth. These two species differ in flower morphology and also in the way they fit their respective pollinators (Fig. 1). We quantified pollen removal and deposition, and the relationship between them, when both pollinator species visited each species of *Penstemon*, in a crossed design. First we compared bees to birds, both visiting a bee-syndrome flower, and predicted that (1) bees would remove more pollen from anthers than birds, but (2) the pollen carryover curves for bees would decline more steeply than for birds (Thomson 1986), and so (3) on the whole, bees would deposit a lower proportion of the grains that they remove than birds. Next we compared hummingbirds on bee-syndrome flowers to hummingbirds on bird-syndrome flowers, and predicted that (4) they would remove less pollen per visit for *P. strictus* than for *P. barbatus*, (5) they would deposit

less pollen on *P. strictus*, and so (6) they would be more efficient pollinators of the bird-syndrome species. Bees do not normally visit *P. barbatus*, but by training bees to visit flowers filled with artificial nectar we were able to see if indeed (7) there was a poor fit between them and the bird-syndrome flowers. We discuss how differences in fit and turnover between bees and hummingbirds could cause divergence during evolutionary shifts between pollinators in *Penstemon*.

Materials and Methods

During the summers of 2001 and 2002, in the Colorado Rocky Mountains, we studied pollen removal and deposition by hummingbirds and bumblebees visiting *Penstemon barbatus* and *P. strictus*. These species are closely related, but their floral morphology and pollinators differ (Thomson et al. 2000; Wilson et al. 2003). Outgroup comparisons imply that the common ancestor of the two species was of the hymenopteran-pollination syndrome (based on molecular phylogenetics; A. D. Wolfe, pers. comm. 2003). *Penstemon strictus* flowers have broad purple corolla tubes that allow large Hymenoptera, such as bumblebees (*Bombus,* Apidae), to enter fully and reach the nectaries. They produce small volumes of concentrated nectar; the extended lower lips of the corolla tube are used as a landing platform by hymenopteran visitors. Various bees and the wasp *Pseudomasaris vespoides* (Masaridae) visit the flowers in the field at high rates (Williams and Thomson 1998). Most bumblebees primarily visit to collect nectar, but some individuals turn upside down at the flowers and actively collect pollen. Hummingbirds visit *P. strictus* only occasionally. *Penstemon barbatus* represents one of the 14 or more cases of independent shifts to hummingbird pollination in the group (Wilson et al., in press). The flowers have long narrow red corolla tubes, exserted anthers, reflexed lower lips, and produce large volumes of dilute nectar (Castellanos et al. 2002). The flowers are visited almost exclusively by hummingbirds (Brown and Kodric-Brown 1979; Wilson et al. 2003). When *P. strictus* and *P. barbatus* are grown side by side in a garden, bees and birds largely restrict their visits to the ''appropriate'' species.

The plants used in our experiments were kept indoors to prevent visitation by pollinators. Either they were potted plants kept in a screened tent, or they were cut inflorescences collected from roadside populations in the study area. We kept cut inflorescences in vases with water, where they last for three days without any sign of wilting.

For experiments with birds, we used male broad-tailed hummingbirds (*Selasphorus platycercus*) and occasionally male rufous hummingbirds (*S. rufus*). The birds were accustomed to enter a flight cage (1.5 × 2.5 × 2 m) through a hinged door, and drink sugar-water solution from a hummingbird feeder. When the flowers were ready for each experiment, we took them into the cage, waited for a hummingbird to enter, closed the door behind it, and presented the flowers. Most hummingbirds visited all the flowers within a few minutes, after which time we opened the door and let the bird exit freely. A bird was used for a single experiment at a time.

For most experimental runs with bees, we used workers of *Bombus bifarius* and *B. flavifrons* that visited flowers in a flight cage. The bees were captured on *P. strictus*, allowed to groom for at least an hour, then chilled overnight in a refrigerator (>4°C). After they warmed up by feeding on emasculated flowers, they were hungry enough to visit the flowers of an experimental run. In addition to the flight-cage runs, we increased our sample size for pollen removal measurements with freely flying bees visiting prepared flowers in a garden. This was done separately for nectar-feeding and pollen-collecting bumblebees, as well as for five *Osmia* bees (Megachilidae).

We attempted to measure pollen removal and deposition in a factorial design in which both bumblebees and hummingbirds visited flowers of both *P. strictus* and *P. barbatus*. However, bumblebees would not visit *P. barbatus* for nectar, because the flowers are too long for them to reach the nectaries. By adding nectar to some flowers and using worker bees, we were able to study six visits by two bees to *P. barbatus*. In presenting our results, we focus on two comparisons: pollen movement by bumblebees versus hummingbirds visiting the bee-syndrome *P. strictus*; and pollen movement by hummingbirds visiting *P. strictus* versus the bird-syndrome *P. barbatus*.

In the field, penstemons are unlikely to be pollen-limited. Bumblebee visitation to *P. strictus* can be very high; an average flower gets 15 visits per hour in a garden (Williams and Thomson 1998). In normal conditions, fruit set approaches 100% in most species. Therefore, we focused our experiments on pollen donation success, because we believe selection through male function is likely to be stronger than selection through female function. Even though there is no reason to think that stigma loads would often be low in nature, it is perhaps worth pointing out that, in the greenhouse, a higher deposition of pollen grains results in higher seed set (Pearson correlation between number of pollen grains deposited and log seed set in *P. strictus* hand-pollination experiments; $r = 0.479$, $P < 0.001$, M. C. Castellanos, unpubl. data).

Pollen Removal

All *Penstemon* flowers have four pollen-bearing anthers. We measured pollen removal from a single anther in previously prepared flowers after a single visit by the pollinator. We presented flowers to both types of pollinators. To prepare the flowers for a visit, we removed all but one of the two front anthers before any anthers had dehisced. We presented the flower to a pollinator after the remaining anther had dehisced. Following a visit, we held a microcentrifuge tube under the anther and carefully removed it from the flower with microsurgical scissors. The visited anther and the three nonvisited anthers were separately preserved in 70% ethanol. Similar manipulations were done for control flowers that were not visited. Later in the laboratory, we used an Elzone 280-PC electronic particle counter (Micromeritics, Norcross, GA) to count the grains remaining in the visited anther and the ones present in the nonvisited anthers.

We used the mean number of grains in the three excised anthers of each flower as the estimated number of grains that were initially present in the visited anther. (There were no significant differences between the number of grains in rear

and front anthers in either *Penstemon* species; paired *t*-tests, $P > 0.4$). Occasionally, we found unvisited anthers that produced very few grains, and those were excluded from the data if the number of grains in them was less than 35% of another anther in the flower. Most of those anthers had been visibly damaged by thrips or other small herbivorous insects.

Next, the estimated initial number of grains was corrected for experimental manipulation. This correction is essential, because some grains were surely lost when we moved the flowers to the experimental cage and set them for the experiment. We estimated the loss of pollen grains by manipulation in control flowers that received the same treatment except that they were not visited by a pollinator. From those control flowers we established that, on average, 21% of the pollen grains in a *P. strictus* anther were lost due to manipulations ($n = 50$). In *P. barbatus*, the average loss by manipulation was lower, 8% ($n = 16$). (The discrepancy between 21% and 8% is curious and may be due to the difference in how the anthers push pollen to the outside as they dehisce.) To account for pollen lost by manipulation, we reduced the estimated initial number of grains in each of the visited anthers by 21% and 8% for *P. strictus* and *P. barbatus* flowers, respectively.

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. Some estimates of removal in our dataset are negative; that is, we estimated there were more grains in an anther after it had been visited than we estimated were in it before it was visited. We did not exclude the negative numbers from our averages, because even though negative removal values are nonsensical, they are the consequence of random errors in our estimation methods (e.g. in the particle counter) combined with a few cases in which very low removal coincided with below-average accidental loss during the manipulation of the flower before the visit. We believe there are as many overestimates as underestimates. Treating the error as random, there is no justification for excluding values with negative removal and not excluding an equal number of high values.

Pollen Deposition and Carryover Curves

We estimated deposition of pollen grains and the shape of deposition curves for hummingbirds and bumblebees by presenting the pollinator with 15 emasculated flowers (recipients) after it visited a single pollen-bearing flower (donor). We allowed pollinators to visit each recipient only once, and recorded the sequence and duration of the visits on videotape. We collected all the stigmas and mounted each one on a microscope slide with basic-fuchsin-tinted glycerin jelly (Beattie 1971). Later, we counted all pollen grains under a microscope.

We considered two aspects of pollen export from donor flowers. First, we conducted analyses on the total number of pollen grains deposited per run, D_{15}, on the 15 recipient flowers that followed a donor. We present the mean values as an indication of the number of pollen grains deposited per donor. For significance tests, however, we use ranks and medians because variances are not homogenous among groups.

The second aspect of deposition we considered was pollen carryover between successive flower visits. Our carryover curves are the curves resulting when plotting the number of grains deposited on each flower with the sequence of 15 recipient flowers as the horizontal axis. Because stigma contacts are literally hit-or-miss, carryover data present notable difficulties for statistical analysis. We compared the deceleration of the cumulative curve of pollen deposition in the 15 recipients for each experimental run using two different approaches. First, we calculated the slope, b, of a $\log_{10} - \log_{10}$ regression of cumulative pollen deposition on order of recipient from one to 15: log (cumulative grains + 1) = b log (flower order) + constant. This b coefficient, with the transformations, is a statistic that describes the degree to which the nontransformed data follow a curve. When $b = 1$, there is no deceleration, whereas $b < 1$ indicates degree of deceleration. Because we are considering cumulative deposition in a series of recipients, a highly decelerating relationship shows a situation in which most of the grains are deposited early in the run.

We also modeled the shape of the pollen carryover curves using maximum-likelihood methods (Morris et al. 1995). This approach allowed us to compare the fit of different models of carryover while assuming the same error distribution of the data. To describe the distribution of the error, we used the generalized Poisson distribution developed by Consul and Jain (1973; also called Lagrangian Poisson distribution; Johnson et al. 1992), which includes two parameters and allows for overdispersion of the data. This versatile distribution explained more of the error than a Poisson or a binomial distribution, and also more than other generalizations of the Poisson (e.g. stopped-sum distributions in Johnson et al. 1992). We explored a number of models of declining deposition: (1) constant, $d = af$ (used as a null model), (2) exponential, $d = a \exp(-cf)$, (3) geometric with changing carryover fraction, $d = acf \prod_{qf=1}^{f-1} (1 - cqf^b)$, a mechanistic model suggested by Morris et al. (1994), (4) two-parameter hyperbolic, $d = ac/(c + f)$, and (5) three-paramenter hyperbolic decline, $d = a/(1 + cf)^m$, a mechanistic model suggested by Rademaker et al. (1997), in which a is the initial height of the curve at first recipient, c is the decline parameter, f is the flower position in the sequence of visitation, b is a parameter of changing carryover fraction (if negative, the fraction of deposition declines with flower number), and qf is the fraction of deposition (for details on the latter parameters, see Morris et al. 1994). We chose these models because simple exponential declines have been frequently used to describe carryover (see Rademaker et al. 1997), but since our theory predicted sharper (than exponential) declines, we also tested faster declining models (models 3–5) suggested in previous studies. Maximum-likelihood values ($\hat{L}_{\max}$) were used to calculate Akaike's information criterion (AIC = $-2\hat{L}_{\max} + 2n$, where n is the number of parameters in the model), and the lower AIC (by at least 2) was chosen as the best fitting model.

Fitting the best model to each combination of pollinator and plant species allowed us to characterize the curves but did not allow us to compare them statistically. Therefore, we also fit a series of exponential decline models to a dataset combining bee and bird runs on *P. strictus*, using the type

of run as an independent variable. We did a similar analysis of a dataset combining hummingbird runs on *P. strictus* and *P. barbatus*. We chose the exponential decline over other models because it provided a better fit to the *combined* datasets. These models sequentially included more terms, using a stepwise approach, to find the ways in which types of runs differ (see Tables 2 and 3 for equations). First, we calculated the likelihood of a simple exponential model that did not account for birds versus bees at all. We then calculated the likelihood of a model that allowed for separate estimates of one of three different coefficients: a was the initial height of the curve at recipient 0, c was the decline parameter, and D was the sum of grains in the first 10 recipients of each run. These were allowed to vary independently for birds and bees (pollinator effect), or for each experimental run. Because each model was nested within the previous one, we were able to test the significance of adding new parameters by comparing each model to the previous one using a likelihood-ratio test. Results using AIC were identical; we present log-likelihood ratios because they allow us to provide probability values and render Tables 2 and 3 easy to follow. The order in which the parameters were included was determined by their relative effects when tested independently.

As a follow-up explanatory analysis, we explored the possible relationship between the duration of a pollinator visit to a recipient flower and the number of pollen grains deposited. We calculated a Spearman's rank correlation between the duration of the visit and the number of grains deposited for every experimental run. We then calculated an overall rank correlation coefficient averaged among runs for each combination of pollinator and plant species (Sokal and Rohlf 1995, box 15.5). Because this correlation might be affected by the position of each flower in the sequence of flowers that constitute an experiment, we report a partial correlation coefficient of visit duration with the number of grains deposited, correcting for the recipient's position in the run, with all calculations done on ranks.

We also compared the number of stigmas that received zero pollen grains after being visited by a pollinator, which reflects the number of times that a pollinator missed the stigma.

Compound Indices of Pollination Performance

After analyzing pollen removal and pollen deposition separately, we considered several ways of combining removal and deposition data into measures of pollination performance. We calculated what we call ''efficiency'' as the mean D_{15} divided by the mean removal, considering all our experiments together. It is the proportion of grains removed that were deposited on the following 15 recipients.

We present two other measures of performance: total pollen movement and pollen transfer rate. For both, we used only experimental runs for which we had paired removal and deposition data. These estimates are averages of the performance of the pollinator in each experimental run. We scaled values of removal and deposition (separately) from 0 to 1 to eliminate negative removal values and to set removal and deposition values to a similar scale. We scaled removal by subtracting the minimum value of pollen removal from each datapoint, and dividing the resulting quantity by the range of R values in the dataset (R scaled = $[R_i - \min R]$/range in R values). We scaled deposition by first log-transforming the number of grains deposited in every run, and then subtracting the minimum and dividing by the range. Total pollen movement was the sum of scaled values of removal and deposition across experimental runs. It indexes the total number of pollen grains that were moved by the pollinators, whether off of anthers or on to stigmas. Pollen transfer rate was the difference between the same two scaled values; a higher value indicates a higher capacity of the pollinator to deposit the grains that it removes. Roughly speaking, differences in transfer rate indicate differences in efficiency.

Statistical Analysis

Comparisons of means, medians, and correlations were calculated using Systat for Windows (available via www.systat.com). For variables that were not normally distributed, we compared medians using the Mann-Whitney U-test. Otherwise we compared means with t-tests. Maximum-likelihood analysis was programmed in Mathematica (Wolfram Research 1999).

RESULTS

Comparing Bumblebees versus Hummingbirds on Penstemon strictus

Nectaring bumblebees and hummingbirds did not differ in the number of pollen grains they removed in a single visit to the bee-syndrome *P. strictus* (Mann-Whitney U-test, $P = 0.932$; Table 1). Bumblebees did, however, deposit more grains on the first 15 recipients than birds (Mann-Whitney U-test, $P = 0.011$; Table 1). Hummingbird deposition curves tended to decelerate more slowly than bee curves. The three-parameter hyperbolic decline was the best model for the bee data, $d = a/(1 + cf)^m$ (the mechanistic model suggested by Rademaker et al. 1997), but an exponential decline, $d = a \exp(cf)$, fit the hummingbird data better (Fig. 2). The pollen carryover curve for birds was comparatively flat, whereas that for bees showed a steeper initial decrease. In other words, hummingbirds deposited pollen grains more evenly across the 15 recipient flowers, whereas bees tended to deposit most of the grains in the first few flowers they visited, indicated by the steeper-than-exponential decline of the hyperbolic model. This is also shown by the comparison of the mean b-values (Table 1, t-test for unequal variances $t = 2.45$, df = 59.6, $P = 0.017$).

Even though the hyperbolic model was a better fit to bee runs, overall the bees deposited more grains in all recipients, so that over 15 recipients the bee curve is higher than the hummingbird curve (cf. Figs. 2A and B). This was confirmed by the stepwise analysis (Table 2), which showed a strong significant effect of adding the between-pollinator parameter a_p (initial height of curve at first recipient; see Table 2 for P-values). In addition, there was a significantly better fit when accounting for the total number of grains deposited per run, D_r. In this analysis, we found no difference in fit when we added a parameter accounting for the between-pollinator variation in the decline parameter c_p (Table 2). Additional ex-

TABLE 1. Statistics and sample sizes of the different variables measured for pollen removal, pollen deposition on 15 flowers, and combinations of both, for bumblebees and hummingbirds visiting *Penstemon strictus* and *P. barbatus* flowers. The last two columns show the probabilities of the planned comparisons between bumblebees and hummingbirds on *P. strictus*, and hummingbirds on *P. strictus* and *P. barbatus*. Bold probabilities are significant at $\alpha = 0.05$. *R* is the number of pollen grains removed per visit and D_{15} is the total number of pollen grains deposited in 15 recipient flowers.

	Nectaring bees in *P. strictus*	Hummingbirds in *P. strictus*	Hummingbirds in *P. barbatus*	Bees vs. birds on *P. strictus*	Birds on *P. strictus* vs. *P. barbatus*
Removal					
Mean *R*	4869.2	4330.8	12010.7		
Median	4507.1	3147.8	9684.33	$P = 0.932$	$\mathbf{P < 0.001}$
	$n = 59$	$n = 96$	$n = 62$		
Deposition					
Mean D_{15}	79.6	72.1	297.9		
Median D_{15}	75.5	32	182	$\mathbf{P = 0.011}$	$\mathbf{P < 0.001}$
	$n = 15$	$n = 25$	$n = 14$		
Deceleration of carryover curve (*b*)	0.78 ± 0.069	1.05 ± 0.081	1.12 ± 0.143	$\mathbf{P = 0.017}$	$P = 0.67$
	$n = 24$	$n = 38$	$n = 27$		
Mean number of stigmas without grains in a run	4.0 ± 0.59	4.8 ± 0.50	2.9 ± 0.42	$P = 0.46$	$\mathbf{P = 0.021}$
	$n = 15$	$n = 36$	$n = 23$		
Compound indices of performance					
Efficiency:					
Mean D_{15}/mean *R*	1.63%	1.66%	2.48%		
Total pollen movement:					
D_{15} scaled + *R* scaled	0.645 ± 0.040	0.601 ± 0.037	0.953 ± 0.088	$P = 0.480$	$\mathbf{P < 0.001}$
	$n = 14$	$n = 33$	$n = 17$		
Pollen transfer rate:					
D_{15} scaled − *R* scaled	0.400 ± 0.033	0.331 ± 0.034	0.458 ± 0.059	$P = 0.228$	$\mathbf{P = 0.050}$
	$n = 14$	$n = 33$	$n = 17$		

planation was provided by allowing both a_r and c_r to vary among experimental runs.

With respect to our compound measures of performance, bees and hummingbirds had comparable efficiencies, total pollen movements, and pollen transfer rates on *P. strictus* flowers (Table 1). There were no significant differences for the latter two indices, and significance tests are not possible for efficiency per se (based on overall means).

Mean removal, mean deposition, and efficiency are parameters that are useful in calculating the number of pollen grains transferred per idealized run, but it should be recognized that they were sensitive to the values of particular runs, and that they were calculated from distributions that were not normal. In particular, one bee run on *P. strictus* had low removal and very high deposition, which greatly affected our estimates. Therefore, we excluded it from the average D_{15} value we report in Table 1. To be conservative, however, we did not exclude it from the statistical analyses.

Hummingbirds and bees did not differ in the number of stigmas they missed (i.e. the ones that had zero pollen deposited) in *P. strictus*. On average, they both missed 4–5 stigmas out of the 15 flowers in a run (Mann Whitney *U*-test, $P = 0.46$).

The correlation between duration of the visit by bumblebees and the number of grains deposited in a flower was weakly positive and significant (overall Spearman $r = 0.178$, $P < 0.05$, $k = 22$ runs). This correlation was still significant after we held constant the position of the flower in the sequence of the experiment (partial correlation $r = 0.132$, $P < 0.05$, $n = 305$). The same was true for hummingbirds visiting *P. strictus* (overall Spearman $r = 0.106$, $P < 0.05$, $k = 36$ runs; partial correlation $r = 0.105$, $P < 0.05$, $n = 450$). Occasionally bees started a deposition run slowly and sped up as they visited more flowers, as indicated by a correlation between the duration of the visit and the position of the flower in the sequence (overall Spearman $r = -0.353$, $P < 0.05$, $k = 25$ runs). The corresponding correlation was not significant for hummingbirds (overall Spearman $r = -0.007$, $P > 0.05$, $k = 36$ runs).

All the above estimates of pollen movement are comparisons of birds to nectar-feeding bumblebees. Pollen removal from single anthers by pollen-collecting bumblebee queens on *P. strictus* averaged 24,256 grains per visit (SE = 2660, $n = 37$). Pollen removal by *Osmia* bees averaged 27,961 grains (SE = 3122, $n = 5$). Thus, pollen-collecting bumblebees and *Osmia* seem to remove much more pollen than nectaring bumblebees or hummingbirds (see Table 1). Unfortunately, getting pollen-collectors or *Osmia* to behave normally in captive conditions is very difficult, and we have no estimate of deposition by these animals.

Comparing Penstemon strictus to Penstemon barbatus Visited by Hummingbirds

In general, hummingbirds moved much more pollen of the bird-syndrome *P. barbatus* than the bee-syndrome *P. strictus* (Table 1). Pollen removal was more than twice as high for the birds visiting *P. barbatus* than *P. strictus* (Mann-Whitney *U*-tests, $P < 0.001$). Note that we found no differences in the number of pollen grains per anther produced by the two

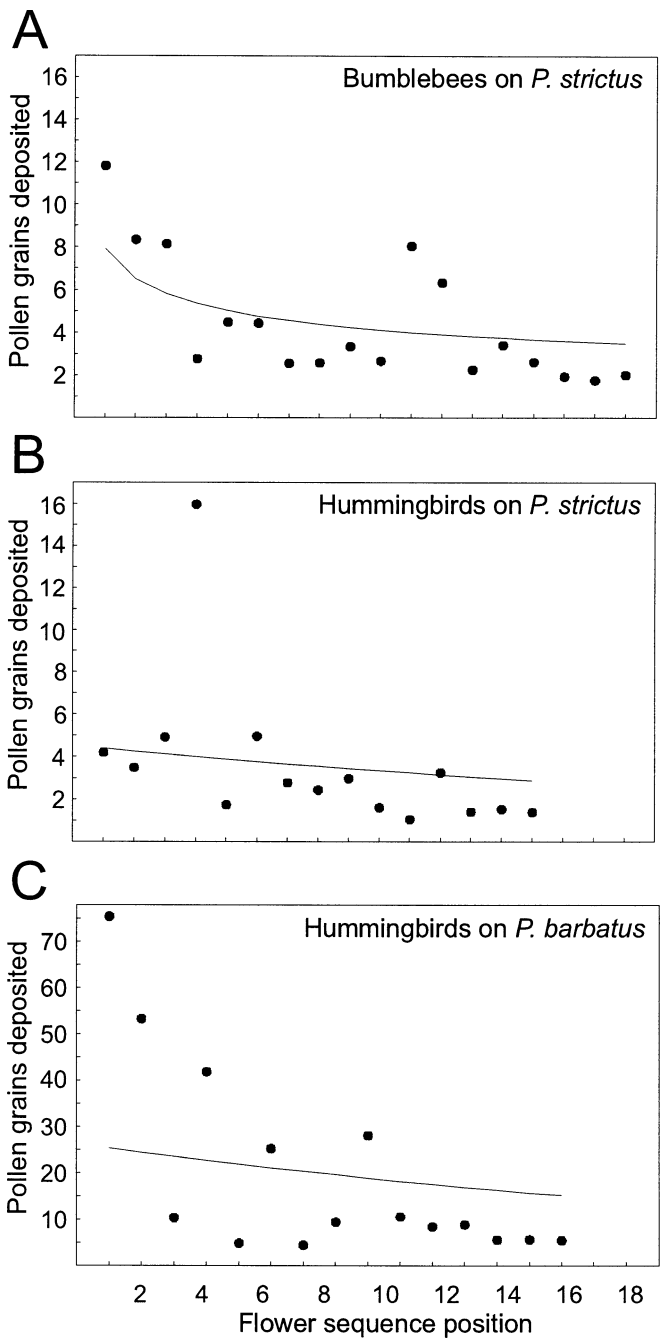

FIG. 2. Pollen carryover curves for emasculated flowers following a visit to a donor flower. The curves are the models best fit by maximum likelihood, using a generalized Poisson distribution as the underlying error distribution. Points represent mean number of grains deposited in each flower in the sequence of visitation. These means are provided as a visual aid, but the models were fit to the dataset allowing for variation among runs. In all three cases, goodness-of-fit tests between observed values and values expected from the model were not significant ($P > 0.05$). All models include an additional parameter κ, the shape parameter in the generalized Poisson distribution, used to describe the distribution of the error. (A) For 21 nectaring bumblebees visiting *Penstemon strictus*, a three-parameter hyperbolic curve: $d = [6.27/(1 + 46.37\ f)^{0.288}]/(1 - 0.74)$. (B) For 25 hummingbirds visiting *P. strictus*, an exponential curve: $d = [1.078 \exp(-0.031\ f)] / (1 - 0.0.76)$. (C) For 14 hummingbirds visiting *P. barbatus*, an exponential curve: $d = [2.061 \exp(-0.037\ f)] / (1 - 0.92)$.

species of plants (*P. strictus* produced on average 40,875 ± 11,351 grains/anther, and *P. barbatus* 39,643 ± 9381; n = 565 and 152 respectively; $t = 1.23$, $P = 0.219$). Deposition (D_{15}) was more than four times higher (Mann-Whitney *U*-tests, $P < 0.001$), and the shapes of the pollen carryover curves did not differ significantly between the two *Penstemon* species (*t*-test for the difference between mean *b*-values with unequal variances, $P = 0.67$). In both cases, the exponential model was the best fit (Fig. 2B and 2C). Table 3 shows the results of the models that best fit the combined dataset for hummingbirds in both plant species. Inclusion of parameter *a* gives a much better fit, which indicates that the curves in *P. barbatus* are much higher, that is, the birds deposit more grains in that species.

Birds were significantly more likely to miss a stigma of *P. strictus* than a stigma of *P. barbatus* (Table 1, comparison of stigmas with zero grains deposited, Mann-Whitney *U*-test, $P = 0.021$).

Our estimate of total pollen movement confirms that hummingbirds put more pollen in motion for *P. barbatus* than for *P. strictus* ($t = 4.35$, df = 48, $P < 0.001$). Hummingbirds also pollinated red *P. barbatus* more efficiently than *P. strictus*; they delivered 2.48% of the pollen they removed from *P. barbatus* and 1.66% of that removed from *P. strictus*. Their higher efficiency in *P. barbatus* was corroborated by a higher value for the pollen transfer rate ($t = 2.01$, df = 48, $P = 0.05$).

For hummingbirds visiting *P. barbatus* flowers, there was a weak correlation (but higher than for birds in *P. strictus*) between the duration of the visit to recipient flowers and the number of pollen grains deposited (common Spearman $r = 0.163$, $P < 0.05$, $k = 27$ runs; partial correlation holding constant the flower position in the sequence $r = 0.162$, $P < 0.05$, $n = 250$).

Bumblebees on Penstemon barbatus

Even though nectar-feeding bumblebees do not normally visit *P. barbatus* flowers, we trained workers of *Bombus bifarius* and *B. flavifrons* to visit *P. barbatus* flowers to which we had added sugar solution. The bees could therefore reach the nectar. We videotaped six of those visits, in which they drank the nectar by pushing themselves into the corolla. In no case did they touch the exserted anthers or stigmas at all (Fig. 1, lower right). Thus, nectar-seeking bumblebees would be negligible pollinators of *P. barbatus* flowers.

DISCUSSION

Our experiments confirm only some of the assumptions of the theory put forward by Thomson et al. (2000). There is no reason to believe in a large difference in per-visit pollination efficiency between bees and birds at *P. strictus*. Nectaring bumblebees and hummingbirds removed about the same number of pollen grains from anthers of the bee-syndrome *Penstemon strictus*, but as predicted, bee curves decline faster than bird curves. The comparison of *P. strictus* and bird-syndrome *P. barbatus* closely followed our expectations. Hummingbirds transferred a higher proportion of the grains of *P. barbatus*. Overall, our results suggest that bee-bird differences in fit and turnover combine to produce an

TABLE 2. Comparison of exponential models fit to the combined data of pollen carryover curves by bumblebees and hummingbirds visiting *Penstemon strictus*. Models were fit using maximum likelihood. Parameters were added sequentially, and each model was compared to the one in the previous row except for E and F, which were compared to C. Significance was evaluated using likelihood ratio tests. *f*, flower position in the sequence of visitation; *p*, pollinator, either bee or bird; *r*, run (see sample sizes in Fig. 2); D_r, total grains deposited in first 10 recipients of run. Unless subscripted, all other parameters were modeled identically across all runs and both pollinators. All models include an additional parameter κ, the shape parameter in the generalized Poisson distribution, used to describe the distribution of the error. In line E, κ was allowed to differ between pollinators.

Added term	Model	Number of parameters	−log-likelihood	*P* from χ^2
	a	2	1580.85	—
A. Flower position effect	$a\exp(-cf)$	3	1575.38	0.001
B. Effect of total grains deposited on 10 recipients	$(a + bD_r)\exp(-cf)$	4	1546.04	<0.001
C. Pollinator effect for initial height of curve, *a*	$(a_p + bD_r)\exp(-cf)$	5	1541.84	0.004
D. Pollinator effect for decay parameter, *c*	$(a_p + bD_r)\exp(-c_p f)$	6	1541.77	0.292
E. Pollinator effect for shape of the error distribution, κ	$(a_p + bD_r)\exp(-cf)$, κ_p	6	1541.84	0.936
F. Run effect for initial height of curve, *a*	$a_r\exp(-cf)$	48	1497.43	<0.001
G. Run effect for decay parameter, *c*	$a_r\exp(-c_r f)$	93	1468.09	0.083

interesting asymmetry: birds can pollinate bee-syndrome flowers almost as well as bees do, but bees pollinate bird-syndrome flowers very poorly. Although the birds fit the bee flowers poorly, their lower (i.e. better) turnover can compensate. The higher turnover for bees would tend to reinforce, rather than compensate, their poor fit on bird flowers.

Fit

The pollen transfer capacities of birds and bees in our experiments depend on flower morphology. Visits by nectaring bees to *P. barbatus* are impeded by narrow corollas. Better morphological fit around the hummingbird's face in *P. barbatus* as well as more exserted anthers and stigmas seem to be responsible for the difference in removal and deposition between the two types of flowers. Experimental studies have shown that birds can impose selection on such floral traits through pollen transport (corolla width: Murcia 1990; Campbell et al. 1991; stigma position: Campbell et al. 1994; corolla length: Nilsson 1988; pedicel flexibility: Hurlbert et al. 1996; spur length: Fulton and Hodges 1999). Morphology can be favored that increases the probability that more pollen will be placed on a location of the head that will subsequently touch the stigma of other flowers. In *P. barbatus* and in other hummingbird-pollinated penstemons, such as *P. centranthifolius* and *P. kunthii*, hummingbirds appear to be more precisely channeled into the flower by a narrow corolla tube, so that their foreheads come into more definite contact with stigmas and anthers (Fig. 1, upper right).

The higher number of stigmas that are left with no grains after a hummingbird visit to *P. strictus* flowers (''missed stigmas''), compared to *P. barbatus*, is another result consistent with the interpretation that the fit of the birds in *P. strictus* flowers is less tight than in *P. barbatus* flowers (Fig. 1, upper left). Because hummingbirds can probe the broader openings of *P. strictus* flowers from various angles, the pollen pools on their heads might be less localized, and the probability of contacting a stigma reduced. This is consistent with the predictions of Lertzman and Gass (1983), who simulated pollen carryover under different conditions of pollen pick-up and delivery. They suggested that increased variability in orientation of the pollinator and flower contact would lead to a patchy distribution of pollen on the pollinator. A simulation of those circumstances resulted in a higher proportion of stigmas missed in the deposition curve, and at the same time an increase in carryover distance, compared to the outcome of models in which a united pollen pool was formed on the pollinator.

As the theory we have outlined would predict, removal

TABLE 3. Comparison of exponential models fit to the combined data of pollen carryover curves by hummingbirds visiting *Penstemon strictus* and *P. barbatus*. Models were fit using maximum likelihood. Parameters were added sequentially, and each model was compared to the one in the previous row except for F, which was compared to D. Significance was evaluated using likelihood ratio tests. *f*, flower position in the sequence of visitation; *s*, plant species, either *P. strictus* or *P. barbatus*; *r*, run (see sample sizes in Fig. 2); D_r, total grains deposited in first 10 recipients of run. Unless subscripted, all other parameters were modeled identically across all runs and both pollinators. All models include an additional parameter κ, the shape parameter in the generalized Poisson distribution, used to describe the distribution of the error.

Added term	Model	Number of parameters	−log-likelihood	*P* from χ^2
	a	2	1542.32	—
A. Flower position effect	$a\exp(-cf)$	3	1539.11	0.011
B. Plant species effect for initial height of curve, *a*	$a_s\exp(-cf)$	4	1516.67	<0.001
C. Plant species effect for shape of the error distribution, κ	$a_s\exp(-cf)$, κ_s	5	1502.77	<0.001
D. Effect of total grains deposited on 10 recipients	$(a_s + bD_r)\exp(-cf)$, κ_s	6	1495.18	<0.001
E. Plant species effect for decay parameter, *c*	$(a_s + bD_r)\exp(-c_s f)$, κ_s	7	1493.94	0.115
F. Run effect for initial height of curve, *a*	$a_r\exp(-cf)$, κ_s	42	1440.88	<0.001

might also be affected by pollen presentation at the anther level (Harder and Thomson 1989; Thomson et al. 2000). The similarity of average removal by birds and bees in *P. strictus* suggests that the number of grains that can be removed from such flowers is, at least to a certain extent, controlled by the anther's dehiscence schedule. In *P. strictus*, anthers dehisce slowly, and pubescence along the dehiscence line probably prevents large numbers of grains from being shaken out by muffling the movements of a hummingbird or a nectaring bee visiting the flower. It is possible that if it were not for the narrowness of anther dehiscence, visitors would have the potential for removing more pollen. In *P. barbatus*, the anthers open more widely than in *P. strictus*, and have no hairs to restrict pollen removal.

Turnover

Our bee pollen-carryover curves declined sharply. In other plants, bee curves are also best described by rapidly declining models (Morris et al. 1994, 1995). Rademaker et al. (1997) argued that a better fit to a hyperbolic decline (rather than an exponential decline) could be an effect of pooling experimental runs for analysis, but we found that the three-parameter hyperbolic was also a better fit when accounting for experimental runs. We believe that grooming is responsible for the steepness in the decline. We observed bees grooming during our experiments, often in flight and also in pauses between flowers. In a short period of time, bees can redistribute pollen on their bodies and store it or discard it. In our hummingbird carryover curves, there was no steep decline. The same pattern of shallow decline over the first few flowers has been found for hummingbirds visiting *Ipomopsis aggregata* (Price and Waser 1982). Our stepwise exercise of adding parameters to exponential models casts some doubt on this conclusion, in that fitting separate c_p coefficients for bees and birds did not add significantly to the model's fit ($P = 0.292$ in Table 2). This is presumably because the stepwise analysis forced an exponential decline on the bee data, even though our other analysis had shown that a three-parameter hyperbolic was a significantly better model. In 15 flowers, birds deposited fewer grains than bees, but because hummingbirds waste less pollen and therefore deposit grains on an extended number of flowers, they are likely better at dispersing grains beyond the plant from which they were collected (i.e. beyond geitonogamous pollination).

Waser (1988), studying *Delphinium nelsonii*, also compared pollen carryover by hummingbirds and bumblebees. Like *P. strictus*, *D. nelsonii* has a morphology that suggests adaptation to bees, although hummingbirds frequent it at the beginning of the flowering season. Several of our findings in *Penstemon* were consistent with Waser's results. Bumblebees deposited more pollen grains in the first five flowers, and carryover curves declined more sharply than hummingbird curves. Pollen deposition on stigmas by hummingbirds also fluctuated greatly, a variation that Waser attributed to hummingbirds missing some stigmas. Waser estimated the transfer distance of the mean and median pollen grain in each carryover run, that is, the sequence number of the flower where such grains were deposited. The estimates of pollen transfer distances by hummingbirds that he presented are not directly comparable with our carryover data, because our runs were much shorter (15 recipient flowers vs. 31 on average), and observed carryover is sensitive to the length of the experimental run (Lertzman and Gass 1983). However, for bumblebees, the average run length with *Delphinium* was 16.1 flowers, so we can compare Waser's results to our bumblebee data on *P. strictus*. Our findings were again similar; mean transfer distance for bees in *P. strictus* was 6.7 versus 6.1 in *Delphinium*, and median transfer distance was 6.3 in *P. strictus* versus 5.5 in *Delphinium*. Bumblebees deposit grains of both species soon after pick-up, or never.

We successfully detected differences in the shape of the carryover curves for the first flowers visited, but it is important to point out that using only 15 emasculated recipient flowers is not ideal for measuring pollen carryover. Unfortunately, wild-caught bumblebee workers would visit only a limited number of flowers in our experimental cage. Another obstacle to measuring pollen carryover is that pollen from the donor can seldom be distinguished from other grains picked up at recipients. Using emasculated recipients, as we did in this study, is commonplace but imperfect. It lowers the potential for interference between pollen from newly visited anthers and the grains we were tracking (Price and Waser 1982; Lloyd and Webb 1986). On the one hand, re-emergence of pollen buried under pollen from other flowers (pollen layering) may produce long tails on the pollen carryover curve (Lertzman and Gass 1983). On the other hand, Harder (1990a) showed that, as bees get more pollen on their bodies, they are more likely to groom. Lertzman and Gass were specifically considering carryover by hummingbirds, and they did not consider grooming to be important. The dynamics of deposition are probably more complicated than our results show (Lertzman and Gass 1983; Harder and Wilson 1998), but it is possible that our experiments underestimate the initial decline of the deposition curve for bees.

We detected weak correlations between the number of grains deposited on a stigma and the handling time spent in the flower by both types of pollinators. For bumblebees, and not for hummingbirds, the handling time decreased slightly as the run progressed; that is, occasionally bees sped up. This effect might have reinforced a sharp decline in the bee carryover curves and led us to overestimate the effect of grooming. However, the correlation between time and sequence in the run was weak. Moreover, freely foraging bumblebees often shorten their visits toward the end of a bout, possibly as their crops fill up, their body temperature increases, or they become faster at handling the flowers (Keasar et al. 1996; Gegear and Laverty 1998). The change in speed by the bees might then reflect normal behavioral differences between bees and birds.

The Evolution of Hummingbird Pollination and the Maintenance of Hymenopteran Pollination

There seem to be two alternative adaptive pollination strategies for *Penstemon*: hymenopteran and hummingbird pollination. Hummingbirds are very efficient pollinators of *P. barbatus*, nectaring bees have been practically excluded from the flowers, and pollen collectors are plausibly parasites when birds are abundant. The hymenopteran syndrome represents

another strategy, but not necessarily to the exclusion of hummingbirds. The bird's main inferiority is that they visit the bee-syndrome flowers so infrequently, presumably because the nectar is not rewarding enough to attract high visitation rates by hummingbirds (Wilson et al., in press). That hummingbirds discriminate against flowers with low nectar rewards has been shown by Schemske and Bradshaw (1999) in bird-pollinated *Mimulus cardinalis* and bee-pollinated *M. lewisii*.

The fact that hummingbirds can be almost as efficient as bees on *P. strictus* suggests that hummingbird pollination could invade hymenopteran pollination under certain circumstances. Once *Penstemon* plants start using hummingbirds as pollinators by offering high nectar rewards, the interaction between the contributions of the two pollinators to fitness can be negative—relatively less efficient bees remove pollen that could be more efficiently delivered by birds (Thomson and Thomson 1992; Thomson et al. 2000). Using Aigner's (2001) terminology, this leads to a trade-off in which specialization occurs if the marginal gain from specializing on birds surpasses the fitness loss of excluding comparatively less efficient bees. This would help explain the frequent shifts from bee to hummingbird pollination in the group (''evolutionary specialization,'' sensu Fenster et al. 2004), since the shift to bird pollination would have resulted in an overall increase in pollination efficiency. We discuss below the conditions in which hummingbird pollination could arise.

The relative efficiency of hummingbirds as pollinators of a bee-syndrome *Penstemon* could be even higher if we consider hymenopteran visitors other than nectaring bumblebees. We found that bees actively collecting pollen can remove six times as many grains as nectaring bees, and most of those grains are probably quickly groomed off. Unfortunately, it is very hard to study pollen deposition by bees that are collecting pollen in a 15-flower sequence of emasculated flowers. Rademaker et al. (1997) did not find a difference in pollen carryover between pollen-collecting and nectaring bumblebees on *Echium vulgare*, but their sample size for pollen-collecting bees was only two. In the field, about 61% of the visits to *P. strictus* are by visitors that are more interested in pollen than *Bombus*, including *Osmia*, *Anthophora*, *Pseudomasaris* wasps, and several smaller bees that turn upside down and actively remove pollen (Wilson et al. 2003). If all those bees and wasps consume a disproportionate amount of the pollen they remove (e.g., Wilson and Thomson 1991; Conner et al. 1995; but see Dieringer and Cabrera 2002), the presence of hummingbirds would render Hymenoptera collectively as pollen parasites.

However, hummingbirds only occasionally visit *P. strictus* in the field. During 54 30-min visitor censuses on various *P. strictus* populations throughout the flowering seasons, no hummingbirds were observed (Wilson et al. 2003). At other times, we have seen hummingbirds visiting flowers of this and other bee-adapted penstemons. In *P. strictus*, the occasional bird visit may be more frequent early in the morning, before bees begin visiting, as has been reported for *P. pseudospectabilis* (Lange and Scott 1999). (Similarly, rare bird visits to bee-type *Mimulus* phenotypes occur primarily in the early morning; D. W. Schemske, pers. comm. 2003). In our experience, bird visits to bee-syndrome penstemons are also favored by high densities of penstemons (e.g., *Penstemon gentianoides* in Mexico) and/or low densities of competing nectar sources (e.g., *P. speciosus* in California). Hummingbirds will certainly often investigate patches of purple flowers. Whether they return to them likely depends on the amount of nectar they provide and the availability of alternative nectar plants in the community.

Under circumstances that encourage higher visitation rates over a number of generations, hummingbird pollination could favor mutations for increased nectar rewards. For *P. centranthifolius*, Mitchell and Shaw (1993) found that nectar production rate was genetically variable both within and among populations. Higher rewards in turn can lead to higher attractiveness: the experimental augmentation of nectar can bring hummingbird visitation rates at hymenopteran-adapted plants nearly up to the rates observed for hummingbird-adapted penstemons (E. Jordan, unpubl. data).

After hummingbirds are acquired as reliable pollinators, continuing selection may favor other traits that make the flowers more specialized for hummingbird pollination. Natural populations manifest ample variation in corolla length, corolla width, and flower color. If bees were absent for some generations, hummingbird visitation could select for hummingbird-adapted flowers. The absolute absence of bees is implausible, even though there might be areas where bee abundance or flight capabilities are reduced. Cruden (1972) suggested that hummingbird pollination is more common at higher altitudes, where bee flight abilities are reduced. The present distribution of *Penstemon* species does not give support to that idea: hummingbird-pollinated species tend to be present or extend into lower altitudes than their close bee-pollinated relatives (*P. barbatus*' range is lower than *P. strictus*, *P. hartwegii* is lower than *P. gentianoides*, etc.). Of course, present distributions could reflect range expansions of hummingbird-adapted species into habitats other than where they evolved.

Of some 284 species of *Penstemon* and relatives, only about 39 show signs of adaptation to hummingbird pollination, and not all of those are specialized on hummingbirds to the exclusion of Hymenoptera. Pollination by bees likely represents the ancestral mode of pollination (Wilson et al. in press). We suggest that it is maintained in many species because penstemons are exceedingly rewarding to Hymenoptera and tend to bloom late in the growing season, so they usually receive abundant bee and wasp visits. The shift to the alternative adaptive peak requires just the right prolonged ecological circumstances coinciding with appropriate genetic variation. Further work, including computer simulations of the effect of multiple visits by bees and birds on pollen transfer, would help us understand the conditions under which pollinator shifts can occur.

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