CHAPTER THREE

Shifts between Bee and Bird Pollination in Penstemons

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This is a story about change and lack of change. The subjects are penstemons. The flowers of certain closely related penstemons differ dramatically, whereas certain distantly related species are eerily similar. This is due to the repeated evolution of hummingbird pollination from bee pollination. Our chapter explores the biology surrounding these evolutionary shifts.

We start by defining some useful shorthand. We construe "penstemons" to include the genus *Penstemon* and closely related genera, particularly *Keckiella,* a genus that has both bee- and bird-pollinated species. By "bees" we mean the superfamily Apoidea plus (improperly) the wasp *Pseudomasaris vespoides,* which forages like a bee and like a bee rears its young on pollen. We use "bee-flowers," "bird-flowers," and "pollination syndromes" to refer to species on one side or another of a pollinator shift. The degree to which a shift from bees to birds has occurred varies from case to case, and, in general, the notion of pollination syndromes tends to have unfortunate typological connotations (Armbruster et al. 2000; Thomson et al. 2000; Fenster et al. 2004; Waser, chap. 1 in this volume).

By choosing penstemons, we focus on bilaterally symmetrical, nototribically pollinated flowers with deeply recessed nectaries capable of quickly replenishing nectar after it has been removed; on flowers that often bloom late in the season, when pollinators are generally abundant and hungry; and on plants that have diversified in patchy continental habitats. No doubt, the evolutionary dynamics are quite different for other kinds of flowers in other circumstances, and comparison of evolutionary dynamics among groups of plants holds great promise.

Our chapter shall be an overview of studies done by ourselves and others on penstemon floral evolution as it pertains to shifts from bee to bird pollination. Detailed accounts of the methods and analyses have been or will be published elsewhere. The research program that we outline is intended to not merely document the extent to which plants conform to lists of syndrome characteristics. We take the opportunity here to focus on the evolutionary mechanisms that cause syndrome shifts, just as others have done for evolutionary shifts from outcrossing to selfing, from homostyly to heterostyly, from blooming early to blooming late, and from semelparity to iteroparity.

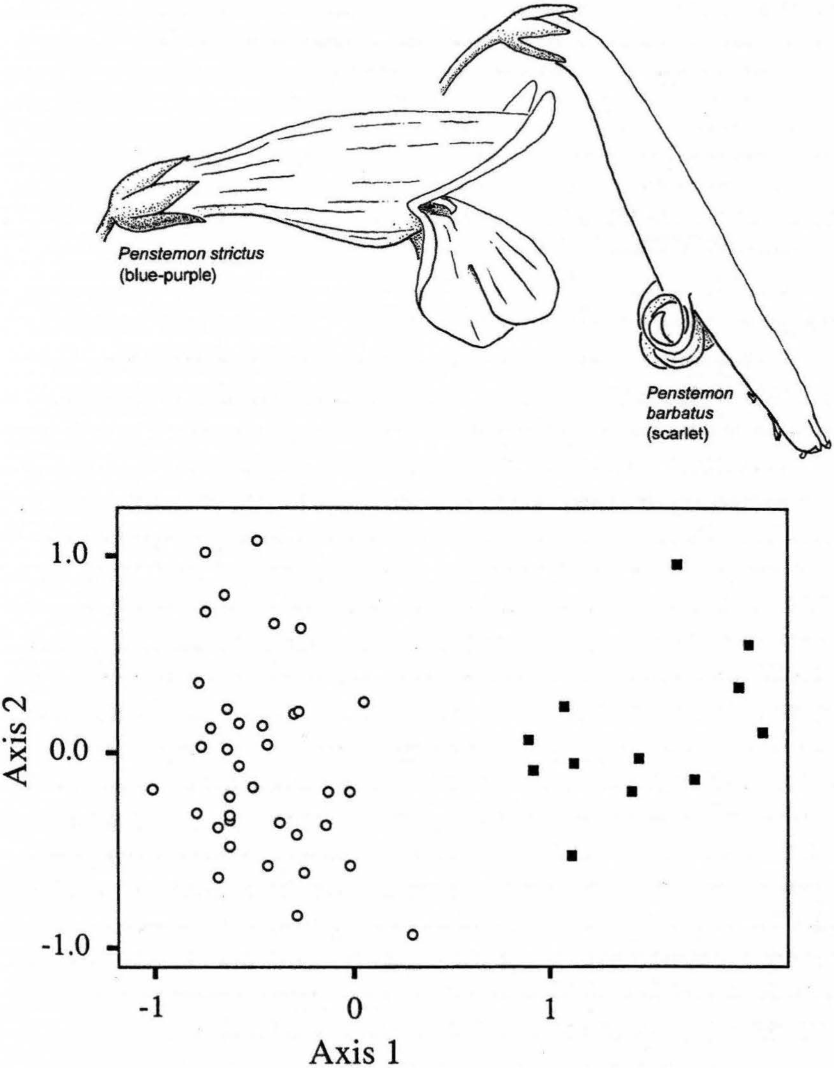
We start by describing among-species patterns of floral characters and pollinator spectra. Next, we compare bees and birds at bee- and bird-adapted flowers in terms of the amounts of pollen they move. Then, we focus on differences in the nectar rewards for the two syndromes. And then, for a series of floral characters, we speculate on the form of natural selection that might make them change during pollinator shifts. Finally, we consider macroevolutionary dynamics that could explain why bee-pollinated species greatly outnumber hummingbird-pollinated species.

The Systematic Patterns

Penstemon is the largest and most derived genus in Cheloneae (Wolfe et al. 2002), with 270 species described. The closest relatives of *Penstemon* are *Nothochelone* (1 sp.), *Chionophila* (2 spp.), *Chelone* (4 spp.), and *Keckiella* (7 spp.). Two species, in *Penstemon* section *Ambigui,* may be adapted to pollination by long-tongued flies while retaining bees (Straw 1963), and several species in the section *Penstemon* seem to be adapted to pollination by Lepidoptera while retaining bees (Clinebell and Bernhardt 1998). The vast majority of penstemons are mostly (but not exclusively) pollinated by bees and *Pseudomasaris vespoides.* Thirty-nine species show noticeable adaptations for hummingbird pollination. The extent of the shift ranges from being mostly pollinated by bees while having also taken on hummingbirds, to being nearly exclusively pollinated by hummingbirds.

Ordinations

Using a standard protocol, we tabulated data on the floral morphology and pollinator spectra of 49 bee- and bird-pollinated species (Wilson et al. 2004). We scored floral characters that have been previously implicated in pollination syndromes, such as corolla color, anther exsertion, and the narrowness of the floral tube. Pollinator visits were recorded during many 30-minute field censuses. An ordination of the penstemons based on floral characters (fig. 3.1) produces two loose clusters, which correspond well to the two syndromes. The flowers on the left of the ordination are blue-violet or yellow or purple, colors associated with bee pollination. Those on the right are red or orange in the extreme and rose or magenta toward the middle of the ordination. Axis 1 correlates positively with attendance by hummingbirds and negatively with attendance by nectar-seeking bees. Interestingly, pollen-collecting bees tend to visit the bird-syndrome flowers more than the bee-syndrome flowers, possibly because the bird-syndrome flowers present their pollen more generously on anthers that are more accessible.



Correlations with Floral Characters

	Axis 1	Axis 2
Color	0.941	0.117
Lower lip reflexion	0.842	-0.148
Functional exsertion	0.918	0.107
Measured exsertion	0.519	0.498
Inclination	-0.640	0.128
Circumference	-0.044	0.712
Staminode length	-0.213	0.518
Tube length	0.419	0.315
Anther-floor distance	0.492	0.853

Correlations with Floral Visitors

	Axis 1	Axis 2
Hummingbirds	0.823	0.152
Pseudomasaris wasps	-0.225	-0.087
Osmia bees	-0.421	-0.482
Nectaring *Bombus*	-0.344	0.091
Pollen-collecting *Bombus*	0.114	0.054
Xylocopa bees	-0.205	0.526
Anthophora-sized bees	-0.293	0.091
Pollen-collecting *Lasioglossum*	0.358	0.107
Other pollen-collecting bees	0.179	0.042
Large hovering flies	-0.280	-0.195
Oligodranes flies	-0.140	-0.074
Small nectaring bees	-0.132	-0.124

Figure 3.1 Multidimensional scaling ordination of penstemon species based on floral characters. Circles represent species of the bee-pollination syndrome; squares represent the bird-pollination syndrome. These 49 species are those for which we have censused floral visitors. Axis 1 is positively correlated with hummingbird visitation at $r = 0.823$ and with many other types of animals. Above the ordination, a typical bee-pollinated species is shown on the left and a typical bird-pollinated species is shown on the right. Details are given by Wilson et al. (2004).

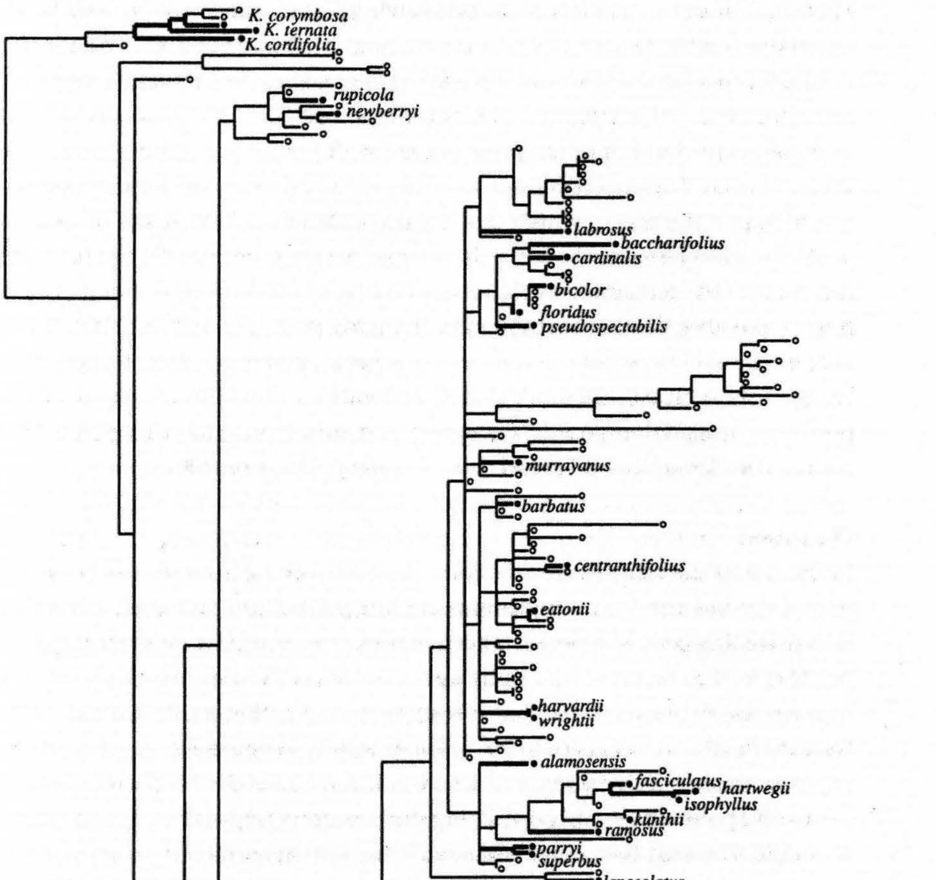
Figure 3.2 One of many parsimonious phylogenies of 194 species above the common ancestor of *Penstemon* and *Keckiella* (A. Wolfe, unpublished data), based on sequences of the internal transcribed spacer region. Outgroups have been pruned to save space. Open circles indicate species that are of the bee-pollination syndrome; solid circles, which are labeled, indicate those that are toward the hummingbird-pollination syndrome. There are 29 such hummingbird-pollinated species included in this phylogeny. Thickened bars represent hummingbird-pollinated lineages. As with other equally parsimonious phylogenies, this requires 23 shifts. Slightly longer trees allow for fewer shifts in pollination syndrome. Phylogenies based on chloroplast DNA sequences and traditional taxonomy also imply a large amount of homoplasy in pollination syndrome.

Phylogeny

The bird–bee contrasts become sharper if we consider a species' pollination system relative to that of its close relatives. For instance, *Penstemon newberryi* is visited by bees more than by hummingbirds, but its flowers show more hummingbird characteristics than those of its close relative *P. davidsonii,* which is nearly exclusively visited by bees. Given that *P. davidsonii* has the ancestral floral characters, we would say that there has been a shift toward hummingbird pollination in the lineage leading up to *P. newberryi* (Datwyler and Wolfe 2004). A phylogeny of *Keckiella* that shows two originations of hummingbird pollination is given by Freeman et al. (2003). A general phylogeny of penstemons is shown in figure 3.2 (A. Wolfe, unpublished data). The consensus trees from nuclear and plastid DNA data are not completely resolved, but all our analyses are robust to the conclusion that a remarkable amount of convergent evolution in the pollination system has occurred. Usually, if not always, the shifts appear to have been from bee to bird pollination. We have labeled the species on the phylogeny that have any tendency toward hummingbird-syndrome characters. Taking the phylogeny at face value, there might be as many as two dozen shifts between bee and hummingbird pollination, and, unless one is willing to accept a tree that is much longer than the shortest trees found in our analysis, one must believe there were at least 13 shifts. These shifts are not all equally extreme (Reid et al. 1988). Only about half of them stand out as excluding large nectaring bees.

Conservatism of Pollination System

Although the convergence is dramatic, the ordination and phylogeny also serve to highlight a subtle sort of "stasis" or maintenance of a clade's pollination system. There is nowhere near as much floral specialization as there could be. There are 576 branch segments above the common ancestor of *Penstemon* and *Keckiella* and 25 shifts between bee and bird pollination. Shifts between bees and birds are far rarer than they could be. Moreover, there is no reason to think that very many of the remaining branch segments are associated with shifts between different types of insects. We see one shift toward long-tongued-fly pollination, another toward butterfly pollination, and at most a half-dozen shifts toward specialization onto such large-bodied bees as *Xylocopa* or such small-bodied bees as *Osmia.*


K. corymbosa
K. ternata
K. cordifolia
rupicola
newberryi
labrosus
baccharifolius
cardinalis
bicolor
floridus
pseudospectabilis
murrayanus
barbatus
centranthifolius
eatonii
harvardii
wrightii
alamosensis
fasciculatus
hartwegii
isophyllus
kunthii
ramosus
parryi
superbus
lanceolatus
rostriflorus
utahensis
pinifolius

There are one or two additional shifts involving pollen placement on bees' heads rather than on their backs. Even the most liberal attribution of floral evolution to pollinator specialization would still leave (1) a great number of branch segments not associated with changes in pollinator type and (2) many species that differ in floral details but not in the principal mode of pollination. This is not taxon stasis, nor is it strictly character stasis; instead, it is stasis in the kind of pollinators used and the way in which they are used. Details change while the way of doing business remains the same. This brings up the question, "What has been the cause of the remaining evolutionary changes in the details of floral characters?"—possibly, fleeting adaptation to changing pollinator regimes that do not change the exclusivity of the flowers (Dilley et al. 2000); possibly, responses to antagonists, such as adaptations that deter floral parasites (Thomson et al. 2000); possibly, correlated responses to changes that are adaptive to other parts of the phenotype (Schemske and Bierzychudek 2001; Armbruster 2002).

Discussion

In the rest of the chapter, we envision lineages evolving from mostly bee pollination toward first including hummingbird pollination and then excluding nectar-feeding bees. In some instances, penstemons may have evolved in the opposite direction (at present, the phylogeny is not resolved sufficiently to be sure of the polarity of each shift), but it seems very likely that most if not all shifts were in the direction of bees to birds. For example, in figure 3.2, the most parsimonious tracing of character evolution would have 21 of the 23 changes be from bee to bird pollination, and postulating shifts as irreversible raises the amount of homoplasy by only two steps in details of the tree that are uncertain at this time.

Pollinator Effectiveness—the Impetus for Shifts

A possible explanation for alternative stable syndromes would be that bees have negative effects on flowers adapted to hummingbirds, while flowers adapted to bees are ill suited for hummingbird pollination. A trade-off of some sort could be involved (Aigner, chap. 2 in this volume). This was suggested by theorizing on optimal strategies for pollen presentation (Harder and Thomson 1989; Thomson and Thomson 1992; Thomson et al. 2000; Thomson 2003). When visitation rates are high, as is the case for most penstemons most of the time, flowers are thought to be under selection to place many small doses of pollen onto many individual pollinators. This is especially the case when the pollinators are bees that groom pollen into pollen-carrying structures or off of their bodies. With hummingbirds, it is reasonable to believe that pollen has greater carryover and should be presented by flowers more simultaneously and more generously. Anthers that open quickly and widely and are adapted to hummingbirds may still be visited by bees. However, in the presence of hummingbirds, the bees would be parasitic antagonists, since they would remove and waste large proportions of pollen that could otherwise be safely delivered to stigmas by the birds. Thus, flowers that are adapted to hummingbirds would be under selection to deter bees and avoid contact between anthers and bee bodies. For the other strategy, bee-syndrome flowers ought to have narrowly dehiscent anthers or pollen that is difficult to remove from anthers, making hummingbirds ineffective, we thought. These flowers would continue to be under selection to attract Hymenoptera. There would be selection against features that could make the flowers attractive to hummingbirds if it were at the expense of attracting bees. We have done a series of studies with the purple bee-syndrome *Penstemon strictus* and the red hummingbird-syndrome *Penstemon barbatus* to test some of the assumptions of the theory (Castellanos et al. 2003).

Bumblebees versus Hummingbirds on the Purple *Penstemon strictus*

We compared the numbers of pollen grains that bumblebees and hummingbirds remove and deposit per visit. A pollinator was given a male-phase donor flower followed by 15 emasculated female-phase recipient flowers. Our first comparison was between bees and birds visiting *P. strictus*. Hummingbirds were surprisingly good at removing pollen from the anthers of this bee-syndrome flower. On average, bumblebees removed 4507 grains, and hummingbirds removed 3148 grains (a nonsignificant difference). On the other hand, bumblebees deposited significantly more grains than did birds on the recipient flowers, 76 grains versus 32 on the 15 recipient stigmas. This seeming superiority of the bees at pollinating bee-adapted flowers was a result of better deposition in the first few recipients. The shape of the pollen carryover curves produced by birds and bees differed as predicted: the bumblebee curve quickly plummeted (presumably due to grooming), whereas the hummingbird curve showed essentially no sign of declining over the course of 15 recipient flowers. Extrapolating beyond 15 flowers, hummingbirds may deposit more of a donor's pollen to more recipients than bumblebees. Overall, both nectaring bumblebees and hummingbirds deliver onto the first 15 recipients about 1.6% of the grains they remove. Our experiments may overestimate deposition by bees because our use of emasculated recipients may have reduced grooming activity.

Bumblebees versus Hummingbirds on the Red *Penstemon barbatus*

In the field, nectar-seeking bumblebees do not visit *P. barbatus,* presumably because the floral tube is so narrow as to make it difficult for them to reach the nectar. We added nectar to flowers and trained small bumblebees to visit them. The visits were videotaped. No bee contacted any anther or stigma. As in other hummingbird-pollinated penstemons, *P. barbatus* has strongly exserted anthers and stigmas that are out of the way of a bumblebee entering the tubular corolla. Bumblebees foraging for nectar would be very ineffective pollinators of flowers with the morphology of *P. barbatus*. In the lineage leading up to *P. barbatus,* the flowers have in effect specialized on hummingbirds, encouraging them while excluding nectaring bees and evolving a morphology that is no longer mechanically harmonious with bees.

Hummingbirds Visiting *P. barbatus* versus *P. strictus*

The red *P. barbatus* has come to be pollinated with greater efficiency by hummingbirds than the purple *P. strictus.* The birds removed 9684 grains on average from *P. barbatus* compared to the 3148 from *P. strictus.* They deposited 182 grains on 15 *P. barbatus* stigmas compared to 32 on *P. strictus* stigmas. Moreover, they transferred a higher proportion of the grains that they removed (2.5 vs. 1.6%), and there was no significant difference in the shape of pollen carryover curves. All this is consistent with the suggestion that there has been selection on certain floral traits for better mechanical fit between the pollinator and the flower. When visiting *P. strictus,* for example, the birds came into contact with stigmas much less often than when visiting *P. barbatus.* We explain this as being due to the much broader corolla tube of *P. strictus,* which allows a bird to poke its beak into the flower from the side, often missing the anthers or stigma. Birds probably remove and deposit more pollen grains on *P. barbatus* than they do on *P. strictus* because the flowers of the former species fit the birds more snugly.

Hymenoptera Other Than Nectaring Bumblebees

The measurements mentioned thus far for pollen removal and deposition by bees are for bumblebees foraging only for nectar in a flight cage. We have also measured pollen removal by pollen-collecting queen bumblebees and by smaller *Osmia* bees, which combine nectar foraging with the deliberate rubbing of their backs against anthers. These bees removed much more pollen than nectaring bumblebees or hummingbirds. The majority of the animals that visit flowers like *P. strictus* are more interested in collecting pollen than our caged nectar-collecting bumblebees. Some, like the queen bumblebees, are primarily devoted to pollen collection and turn upside-down to manipulate the anthers. Others feed on nectar but also collect pollen. Frequent visitors in Colorado include *Osmia* bees, *Anthophora* bees, and *Pseudomasaris* wasps, all of which probe for nectar but also rub against anthers and groom pollen into pollen-carrying structures on their bodies from which, we presume, the pollen is very unlikely to ever reach stigmas. Many of these animals also seem to undervisit female-phase flowers. Indeed, there is every reason to imagine that the Hymenoptera, taken as a whole, deposit less of the pollen that they remove than hummingbirds, even though nectaring bumblebees are about equal to hummingbirds.

Discussion

Our theorizing was only partially confirmed by our studies with *P. strictus* and *P. barbatus.* Hummingbirds do not remove very much less pollen from anthers of *P.*

strictus than do bumblebees. In the first few recipients, birds are not superior at depositing it either, but, as predicted, they have pollen carryover curves that are flatter and more extended than those of bees. If we are correct about the pollen-transfer efficiencies of *Osmia, Anthophora,* and *Pseudomasaris,* and, if our data from emasculated flowers really do overestimate pollen deposition by bumblebees, hummingbirds are actually better on a per-visit basis at moving pollen of *P. strictus* than Hymenoptera considered collectively. In the bird-adapted *P. barbatus,* further adaptations have enhanced pollen-transfer efficiency by birds. In evaluating these results, it is worth mentioning that the *strictus/barbatus* species pair represents only one of many shifts between pollination systems, and this particular pair has *Habroanthus*-type anther dehiscence in which the anthers crack open incompletely and present pollen grudgingly. This is true of both species, and the amount of pollen moved from a single donor visit is probably much lower for both these species than it would be for species with widely opening anthers. Nevertheless, accepting our results as they stand, it would seem that (1) hymenopteran-adapted penstemon flowers are ready to be effectively pollinated by hummingbirds if only the birds would visit them; (2) when on occasion a lineage does acquire hummingbirds as regular visitors, further improvements in pollination efficiency by hummingbirds are likely; and (3) once this happens, hymenopteran visitors probably act as pollen-wasting antagonists when mutualistic hummingbirds are abundant.

The Primacy of Nectar Changes

All penstemons that have been tested replenish their nectar after it has been removed. They start replenishment soon after draining and complete it in 2 to 3 hours (Cruden et al. 1983; Castellanos et al. 2002). Consequently, a sizable patch of penstemon flowers is a rich resource that tends to attract heavy pollinator traffic. We postulate that penstemons cannot make hummingbirds ardently pollinate without providing high nectar rewards. The birds will investigate any penstemon, trying out a few flowers every now and then, but they will not work a patch unless the nectar is about as rewarding as that of other hummingbird flowers in the community. One circumstance that might make a bee-adapted penstemon attractive to birds would be the absence of other bird-adapted flowers or a drought that has made those bird-adapted flowers stop secreting nectar. For instance, in the Colorado Rockies during the drought of 2002, we observed more hummingbird visits at *P. strictus* than in previous years, possibly owing to a reduction in nectar offerings by *Ipomopsis aggregata.* At any rate, for a shift toward hummingbird pollination to proceed, we believe that there has to be evolution in the nectar characteristics of the plants. For birds, the economy of nectar is the bottom line.

Sugar Quantity

What hummingbirds presumably care about is how much energy can be consumed per unit time. The more nectar the better, assuming the concentration does not change. If the concentration does change—from, say, 40 to 20% sugar—then the quantity must also change in a more than compensatory way—from 2 μL to more than 4 μL. Comparing bee- and bird-syndrome flowers of *Mimulus,* which all secrete nectar at low concentrations of around 15% sugar, birds prefer species that produce more nectar, and they prefer F_2 hybrid individuals that produce more nectar when one holds other characters constant (Sutherland and Vickery 1993; Schemske and Bradshaw 1999). The nectar is hidden deep in the flower, so the birds cannot evaluate nectar rewards without visiting, but they quickly learn to associate high rewards with visible characters, such as the degree to which the corolla lobes are reflexed. We set up experimental arrays of the hummingbird-adapted *P. centranthifolius,* the hymenopteran-adapted *P. spectabilis,* F_1 hybrids, and backcrosses in both directions. Hummingbirds prefered *P. centranthifolius* and the backcrosses to *P. centranthifolius.* They visited plants of other parentages at a lower rate and probed fewer flowers on those plants before departing. After establishing the preferences, we added 5 μL of 19% sucrose solution hourly to the flowers of *P. spectabilis* and the backcross to *P. spectabilis.* Before nectar augmentation, the birds preferred the bird-adapted *P. centranthifolius* over *P. spectabilis* 3.7:1; after augmentation, the preference dropped to 1.7:1 (Jordan 2004).

Nectar Concentration

Hummingbird flowers tend to have nectar that is more watery than that of bee flowers (Pyke and Waser 1981). In penstemons bagged overnight, 21 bee-adapted species offered a median of 0.69 μL at 36%, whereas 14 bird-adapted species offered 7.76 μL at 26%. The bird-adapted *P. barbatus* brought its nectar level to about 5.4 μL at 25%, whereas the bee-adapted *P. strictus* brought its nectar level to about 0.4 μL at 42%. We believe that hummingbirds seldom visit *P. strictus* because it offers less nectar than other co-flowering plants. *Penstemon speciosus,* a species that is visually very similar to *P. strictus,* is aberrant for a bee-syndrome penstemon in bringing its nectar offerings to 2.7 μL at 13%, and it is attractive to hummingbirds. That hummingbird flowers have dilute nectar is most curious. At feeders, hummingbirds prefer concentrated nectar (Roberts 1996). The systematic pattern might have something to do with the capillary capacity of hummingbird tongues to take up less viscous, dilute nectar more quickly than concentrated nectar (Kingsolver and Daniel 1983; Roberts 1995; but see Roberts 1996). Hummingbirds may simply not mind dilute nectar as long as the total sugar content is high because they have extraordinarily good renal systems that allows them to excrete the unneeded water (McWhorter and Martínez del Rio 1999). It may also be that it is easier for the nectaries to produce copious nectar if it is watery. Bees probably prefer the concentrated nectars because dilute nectar is heavy to carry and inefficient to store in the nest. In the case of bees, it is not so costly for them to take their time drinking viscous syrup, since they do so while at rest inside the flower. At arrays of mechanical flowers when caloric return per unit time was held constant, bumblebees paid less than 10% of their visits to flowers with dilute nectar (13% sugar) and over 90% to flowers with concentrated nectar (40% sugar; J. Cnaani personal communication).

Sucrose-to-Hexose Ratio

There is a further difference in the nectar of bee- and hummingbird-pollinated penstemons; namely, the sucrose-to-hexose ratio (S:H) of bee nectars is lower than that of hummingbird nectars (Baker and Baker 1983). From our survey, bee-pollinated penstemons had a S:H of 0.315, on average, whereas bird-pollinated species had a S:H of 0.950. Hummingbirds do not mind sucrose because they have sucrase in the membranes of their intestines, and this allows them to break the sucrose down into hexoses (Martínez del Rio 1990a). It is not clear why hummingbirds prefer sucrose (it is energetically equivalent to hexoses), but, in fact, at feeders they do (Martínez del Rio 1990b; Martínez del Rio et al. 1992). One possibility is because a sucrose solution is less viscous than a hexose solution of the same caloric value. Bees do not seem to care whether their sugar is hexose or sucrose (Wells et al. 1992). Another fact worth considering is that, for the same amount of sugar, hexoses have almost double the osmotic potential of sucrose. Sucrose is the predominant sugar in phloem, so plants that offer hexose nectars must have nectaries that hydrolyze sucrose into hexoses. This could conceivably have something to do with getting the water to follow the sugar into the nectar or keeping the sugar from returning into the plant. By not hydrolyzing their sucrose, plants ought to be making their nectar more concentrated, not less, if it were only a matter of osmotic potentials (Nicolson 1998, 2002). The sucrose-rich composition of hummingbird-pollinated penstemons and their dilute sugar concentrations, therefore, are probably not merely different aspects of the physiology of secretion.

Discussion

We will consider nectar in comparison with other traits in the next section. For now, we emphasize that a penstemon must offer voluminous nectar, probably dilute and sucrose rich, to entrain hummingbird visitors. Without this evolutionary transition, hummingbirds will visit penstemons only casually unless ecological circumstances put the birds in dire need. Because of the primacy of nectar quantity and concentration in making hummingbirds frequent penstemon flowers, we believe it is the first character to change during a shift from bee to bird pollination. Evolution in other characters, including S:H, may or may not follow. This point of view arises from considering hummingbird preferences, not from a rigorous analysis of patterns of floral character states in our survey. Our conclusion here contrasts with the suggestion of Bradshaw and Schemske (2003), who were studying bee- and bird-pollinated *Mimulus*. Through backcrossing, they made *Mimulus lewisii* flowers yellow-orange instead of pink; this increased hummingbird visitation by a factor of 68 and decreased bumblebee visitation by a factor of 0.17 compared to control lines. Thus, perhaps in such a *Mimulus* a change in color could kick off a shift toward hummingbirds. However, the contrast in our conclusions is not as contradictory as it at first seems. Bradshaw and Schemske's control line of *M. lewisii* produced 2.3 μL on average, which, in our experience, is enough to get birds to visit penstemons (Castellanos et al. 2002). Their yellow-orange *M. lewisii* produced 5.1 μL, which is as much as many hummingbird-syndrome penstemons. And, although their yellow-orange *M. lewisii* attracted 68 times more hummingbird visits than the control *M. lewisii,* it was still only 0.008 times as much as *M. cardinalis* whether it had the yellow-orange allele or lacked it. This is presumably because *M. cardinalis* control lines offered 67 μL and lines without the yellow-orange allele offered 60 μL (T. Bradshaw, personal communication).

The Cascade of Changes

How might selection change a flower like that of *P. strictus* to a flower like *P. barbatus*? On the principle that every intermediate condition from one mode of life to another must function well, we are inclined to think that hymenopteran-pollinated lineages must have undergone "despecialization" in the sense of the flowers taking on hummingbirds as pollinators while still having the characters that allow for bee pollination (Baker 1963). Then, in some cases, there would have been subsequent "respecialization" in which Hymenoptera were no longer encouraged or were even excluded. It would not work to first evolve away from using bees and then evolve toward using birds. In imagining such shifts, there are several questions to consider. First, has a character state (red) been selected as a "positive" adaptation because it attracts hummingbirds or as a "negative" adaptation because it discourages bees (Faegri and van der Pijl 1979, 15)? Second, has a character state been selected because it attracts pollinators, because it fosters the mechanical interaction with the pollinators, or because it influences the pollinators' tendency to move on to another flower of the same species (Waser 1983; Wilson 1994, chap. 1)? Third, what is the level at which differential success is generated—among patches of kin, among individuals, or among flowers on different individuals (Goodnight et al. 1992)? We will take one syndrome character at a time and sketch out how selection most plausibly proceeded.

Nectar

Because hummingbirds seldom frequent penstemons that have bee-syndrome nectar, we expect that the evolution of copious nectar is the first stage by which despecialization comes to be genetically based rather than based on special external circumstances. We consider a volume increase to be a positive adaptation for hummingbird pollination. The dilution of nectar might also automatically reduce bee visitation, but we note that bees are happy to visit *P. speciosus* despite its unorthodox nectar of 13% sugar. The role of nectar in penstemons cannot be to advertise the flower that is producing the nectar, since pollinators cannot evaluate nectar rewards until after probing the flower. Pollinators are more likely to keep track of patches or parts of patches than flowers or individual penstemon plants in deciding on their foraging route. Increased nectar reward probably has little effect on pollen removal or deposition in a penstemon flower (cf. in *Erythronium;* Thomson 1986); instead, the effects it has are of two other sorts. First, nectar encourages the animal to continue foraging on that type of flower (i.e., to remain constant to the plant's species). This may be selected for because it ensures that pollen removed during a visit is thereafter transferred to appropriate stigmas. Second, nectar increases may be selected for because the other flowers in the immediate vicinity—on the same plant or nearby related plants—are visited by birds that have been well rewarded. Animals make choices about revisitation that are above the level of the flower and very likely above the level of the individual (Sutherland and Gass 1995). We suppose that copious nectar would have to spread by drift to the level of substantial parts of patches before hummingbirds would start discriminating among more- and less-rewarding items in a way that would cause the fixation of such alleles.

Color

Of the characters included in our ordination study, color was the one that best predicted hummingbird visitation (fig. 3.1; Wilson et al. 2004). Bee-syndrome penstemons are white, yellow, or most commonly blue-violet. Many have nectar guides that extend from the lower lip of the corolla to inside the vestibule. Some have staminodes of a contrasting color. Species of penstemon that have despecialized, acquiring hummingbirds as pollinators, are pink or magenta. This shift in color seems to have occurred in nearly all species that have begun a shift toward hummingbirds. Those species that have respecialized to deter bees are red or orange. We suppose from the distribution of characters that color evolves quickly at each stage in the transition. Evolution of magenta from blue-violet seems to be a positive adaptation for attracting hummingbirds. Further evolution to scarlet might be a positive attractant of birds, but we are inclined to think that it also discourages bees (Raven 1972). In Sutherland and Vickery's (1993) F_2 array of *Mimulus,* they found that bees responded to color but not to how reflexed the petal lobes were, whereas birds did not respond to color differences but did respond to petal reflexion. As already mentioned, Bradshaw and Schemske (2003) selectively bred *Mimulus* hybrids, producing plants with a flower pigment of one species in a genetic background near that of the other species. Both bumblebees and hummingbirds followed color by itself when the other characters of the pollination syndrome were nearly equal. Color is a character that is probably involved in initial attraction to the patch or the plant. It has also been widely implicated in affecting constancy (Gegear and Laverty 2001). We hypothesize that both Hymenoptera and hummingbirds have a statistical tendency to continue visiting penstemon flowers of precisely the same color as ones from which they have just received a satisfactory reward. This would, however, explain the maintenance of an established color more easily than the origin of a new one. The power of reds at predicting hummingbird attendance in penstemons and among western North American plants in general has mystified many biologists. Has red color been selected because it is superior at signaling to birds from a distance (Crosswhite and Crosswhite 1981)? Have associative learning and mimicry been important (Grant 1966; Brown and Kodric-Brown 1979; Bleiweiss 2001), and, if so, might the emergence of red as the dominant signal be a historical accident? Is it an outcome of physiological biases in color perception by birds versus bees? Bees have ultraviolet receptors. They can also discern red from other colors (Chittka and Waser 1997), although reds may be less strikingly distinct to bees than colors in the blue-violet range (Chittka et al. 2001). Birds have four eye pigments (maybe more) and oil droplets in their eyes that refract light—they are thought to be able to see a richer array of colors than either bees or humans (Bennett et al. 1994). Selection related to the role of red colors at promoting initial visitation seems most plausible among patches, not among individuals. By the time a bird is choosing among individuals in a patch, it can surely see all of them. As for ensuring constancy, selection could be among individuals, although the choices the animal would be making might be between penstemon flowers and co-flowering plants of no particular relation to *Penstemon.*

Floral Dimensions

Bee-pollinated and bird-pollinated species differ in many characters that seem to affect the mechanical fit of the flower around the pollinator, including exsertion of anthers and stigma, reflexion of the lower lip, narrowness of the flower tube, and inclination of the flower on its pedicel (Straw 1956). These characters may have evolved as positive adaptations to improve the efficiency of pollen transfer by hummingbirds by improving the mechanical fit or as negative adaptations to limit the impact of bees after they became antagonists. Using the bee-syndrome *P. strictus,* we altered the flower to have characteristics of the bird syndrome (Castellanos et al. 2004). We surgically extended anthers and stigmas, trimmed away lower lips, constricted the floral tube with rubber bands, and replaced the stiff horizontal pedicel with floppy fishing line. We then measured pollen removal and deposition by birds and bees. Extending the stigmas made them less likely to contact a bee and caused deposition by bees to be reduced. Trimming the lower lip caused removal by birds to be higher, although deposition was surprisingly reduced. Modestly constricting the tube increased removal by birds. Inclining the flowers surprisingly reduced pollen deposition by birds. We conclude that there is a potential for both positive and negative adaptation. We have evidence that narrow corolla tubes potentially increase pollen transfer by hummingbirds, whereas exserted organs reduce it for bees. The sequence in which these changes occur may contingently affect their value. For instance, pendant flowers may only be of value for bird-pollinated species after the flowers have a narrow tube and exserted organs; in the wide-mouthed *P. strictus,* floppy pedicels seem to let the stigma be missed by a bird entering laterally. In addition to affecting mechanical fit, floral morphology might affect attractiveness (Wilson 1995). We found no significant effect on hummingbird choices, although the flexible pedicel treatment caused the birds to take more time visiting the flowers. We have not tried to measure the effect on bee choices, although effects on bee handling time suggest that our morphological alterations might alter visitation rates. Trimming off the lip and making the pedicel flexible caused the bees to visit more slowly and might discourage visitation. Such effects would very likely be manifest among patches or parts of patches rather than among individuals in a patch. In contrast, the selection we envision via mechanical fit would be caused by differentials among individuals in their ability to disperse their pollen to stigmas.

Anther Dehiscence and Pollen Production

The anthers of bird-adapted penstemons tend to open more widely, more rapidly, and more synchronously than those of bee-adapted ones, at least if one compares pairs of related taxa (Thomson et al. 2000; Castellanos 2003, chap. 4). More widely or more simultaneously opening anthers in hummingbird-syndrome species would be a positive adaptation to hummingbirds. We cannot construe it as a negative adaptation for avoiding bees; rather, we suppose it comes at the cost of inefficient pollen transfer by bees, should they still be frequent visitors. We imagine that the generous pollen presentation of bird-adapted penstemons would arise only after flowers had started to respecialize away from bees. A character we once hypothesized to be related to the speed of pollen presentation is the amount of pollen produced in an anther. Because we believe birds to be more efficient, we predicted that bird-syndrome species should produce less pollen than bee-syndrome species after controlling for factors such as flower size and mode of dehiscence. We also predicted that, all other things being accounted for, anthers with narrower dehiscence should produce less pollen. In our survey of penstemon species, however, we failed to find any significant relationship between pollen production and syndrome or anther dehiscence (Castellanos 2003, chap. 4). Although pollen production is correlated with various aspects of flower size, it appears relatively invariant across shifts from bee to bird pollination. The wideness of anther dehiscence is thought to affect fitness via male–male competition because of the physical interaction of anthers with pollinators, not because of attraction. We suppose this differential success acts among flowers (on different individuals) that are in effect vying for the opportunity to fertilize the limited number of ovules that are available during their life.

The Reduction of Staminodes

In addition to the four fertile stamens, penstemon flowers have a sterile staminode. Staminodes vary in color, length, and hairiness—associated with the type of pollinator. Hummingbird-pollinated species tend to have short or flimsy hairless staminodes. Among insect-pollinated penstemons there is high variability in staminode characters, but in general staminodes are enlarged and often bearded at the end. Walker-Larsen and Harder (2001) suggested that the staminode is a vestigial organ in hummingbird-pollinated penstemons, while it functions to increase pollination success when the main pollinators are insects. By removing staminodes from flowers, they found in hummingbird-pollinated species (*P. centranthifolius* and *P. rostriflorus*) that the staminode had no detectable effect on hummingbird visitation or on pollen transfer. On the other hand, the large staminode of insect-pollinated *P. ellipticus* and *P. palmeri* increased pollen receipt, and, in the latter species, the rate of pollen removal. For the bee-pollinated *P. digitalis,* Dieringer and Cabrera (2002) found an effect on pollen deposition (albeit no effect on removal, which was always almost complete over the life of a flower), and the value of the staminode was greater when the pollinators were small. Staminodes in bee flowers seem to contribute to better contact of the insect body with the reproductive organs of the flowers, but the exact mechanism varies. In certain species, the staminode might act as a lever that pushes the reproductive organs down when a large insect puts pressure on it (Torchio 1974). If the visitor is a small bee, the hairy tip of the staminode might contribute to keeping the insect in contact with the stigma (Dieringer and Cabrera 2002). In other bee-pollinated species, even when the staminode is less enlarged, it might force a nectaring bee to probe one nectary at a time, pushing its body back and forth in contact with anthers and stigmas. We expect that reduction associated with hummingbird pollination ought to occur after the changes directly involved in attracting hummingbirds, and therefore only in a subset of the clades that have shifted away from bees.

Discussion

Our studies weakly support the existence of trade-offs, but of a multitrait sort and involving interactions between the effects of the two kinds of pollinators. It is rarely as simple as changing a single character that increases hummingbird visitation concomitantly with decreasing bee visitation by similar amounts. Rather, birds probably have steeper fitness functions for nectar (following the terminology of Aigner, chap. 2 in this volume), and bees are perhaps choosier about color, given that all penstemons make enough nectar to interest bees. For penstemons, we believe what prevents the flowers from being adapted to all pollinators is that better ones make inferior ones into conditional parasites (Thomson 2003). If we are correct about the sequence of stages in the evolution of hummingbird pollination, then there ought to be a nested statistical pattern among the many shifts. If we are absolutely correct, all shifts would involve a change in nectar rewards (as we see in the purple *P. speciosus*); in a subset of those, flowers would become pinkish or reddish (*P. newberryi*); in a subset of those, the anthers would open more widely (*P. hartwegii*) or be more strongly exserted (*P. kunthii*); in a subset of those, the floral tube would narrow to the extent that it would exclude bees, with a simultaneous evolution of orange or red rather than merely pink (*P. pinifolius*); and finally, in a subset of those, the staminode would be reduced (*P. centranthifolius*). At present, we do not have complete enough character data or sufficient phylogenetic resolution to statistically evaluate this prediction.

Possible Macroevolutionary Processes

Why are there about 245 bee-pollinated species of penstemons when there are only 39 hummingbird-pollinated species? For that matter, why aren't all of them generalized to be pollinated by both bees and birds? We only have data to show that hummingbirds are nearly as good as bumblebees on *P. strictus*, but we still suspect that hummingbird-pollinated flowers are across a saddle and up-slope on an adaptive landscape compared with bee-pollinated flowers (albeit for reasons of male–male competition that do not speak to species superiority or inferiority). So, why have all clades not specialized on birds?

Speciation Rates

Waser (1998) commented that it is hard to believe that pollinator specialization is so complete as to affect reproductive isolation in and of itself, but it is possible that pollination specialization affects speciation rates through assortative mating or in some other way (Jones 2001). Hypothetically, if pollination syndrome did affect net speciation rates, the difference in species numbers could be explained. All hummingbird species may cause nearly the same selection on hummingbird-adapted penstemons, so hummingbird pollination under this view is an innovation that would tend to disfavor subsequent radiation. In contrast, the clades that are hymenopteran-pollinated move through many different pollinator regimes with different types of Hymenoptera that, under this view, would be expected to cause local floral adaptation. This greater niche diversity in hymenopteran pollination than in hummingbird pollination could explain the greater species richness among hymenopteran-pollinated penstemons. One could statistically compare the number of species in hummingbird-pollinated clades to the number of species in sister clades that are bee-pollinated; this would tell of net differences in diversification affected by changes in speciation and/or extinction rates (Farrell 1998; Mitter et al. 1988). Our phylogeny does not yet support an analysis, but it is tantalizing to note individual cases in which a monotypic hummingbird-pollinated clade is sister to a clade of many bee-pollinated species (e.g., *P. rostriflorus* and *P. pinifolius* in fig. 3.2).

Are Hummingbird-Pollinated Lineages More Prone to Extinction?

Another possible explanation for the small number of hummingbird-pollinated penstemons would be a tendency of hummingbird-pollinated clades to have higher extinction rates. Perhaps specialization on hummingbirds repeatedly arises but tends to be a dead end. Under this explanation, one would expect that the extant hummingbird-pollinated species would be on short twigs of the phylogeny and that the shifts would be concentrated in branches that are high in the tree with a paucity of shifts inferred lower down. We wish to statistically evaluate this in future work. Many of the hummingbird-pollinated clades seem to be on short twigs, but certainly not all.

Limits to Genetic Variation and Ecological Circumstance

A final explanation (our null explanation) would be that evolutionary shifts away from hymenopteran pollination have been stymied by the response to selection being slower than the tempo of ecological change. Genetic variation would have to arise and be present in just the right ecological circumstance, such as when bees are rare or other hummingbird-pollinated plants are offering poor nectar rewards (Stebbins 1989). Cruden (1972) suggested that this might be the situation in mountains where the morning chill allows birds to visit before bees are active. The biogeography of the hummingbird-pollinated penstemons is not merely montane but includes many desert species. All we can say about the geography of hummingbird-syndrome penstemons is that they are in regions with abundant and diverse hummingbirds—the southwesterly subset of the geographic range of penstemons as a whole (Crosswhite and Crosswhite 1981). At any rate, we suppose there are few places where ecological circumstance is sustained for long enough to initiate a shift. Mitchell and Shaw (1993) and Mitchell et al. (1998) have reported heritability for nectar production in *P. centranthifolius* at $h^2 = 0.38$. With this heritability, how steep would the selection gradient have to be to change 1 μL to 5 μL in 100 generations? On average, the selection differential would have to be only $S = 0.11$ μL per generation. But, how commonly does a penstemon population live for 100 generations, or even a tenth of that period, with either a paucity of bees or a paucity of other flowers that reward hummingbirds? Shifts between modes of life are plausibly rare because over even tiny amounts of evolutionary time the small adaptive peak that a bee-syndrome species is on is on average slightly better than the intervening saddle that would have to be crossed to reach the hummingbird-syndrome peak. That selection, we think, would have to be above the level of individuals, which might make the bee-syndrome adaptive peak all the harder to escape from.

Discussion

Our phylogenetic analyses are premature, so we will not state any results regarding asymmetries in the phylogeny that might be consistent or inconsistent with hypotheses about speciation and extinction rates. Additional phylogenetic data promise to allow those patterns to be sought. As for the third hypothesis—macroevolutionary inequalities maintained by microevolutionary stabilizing selection—it is hard to gain hard data on the sustained press of ecological conditions favoring shifts toward bird pollination, but we can report that the traffic of hymenopteran visitors at bee-syndrome penstemons is consistently high in our experience.

Conclusion

Flowers of plants in the genus *Penstemon* and in related genera such as *Keckiella* can be arrayed along a "syndrome gradient," from having characters associated with bee and wasp pollination to having characters associated with hummingbird pollination. Evolution along this gradient (pollination shifts) seems to have occurred many times to varying degrees. *Penstemon barbatus,* a hummingbird-adapted flower, has its pollen more efficiently transferred by hummingbirds than *P. strictus,* a bee-adapted flower. On *P. strictus,* birds and bees move comparable amounts of pollen in a visit. If birds were to visit frequently enough, they would immediately be nearly as good at pollination and further adaptation would be possible by simple changes in floral dimensions. However, to secure such a shift requires evolution in nectar rewards. Once nectar has evolved to be more plentiful and possibly less viscous, other adaptations to hummingbirds generally ensue to various extents. In some cases, a further step is taken in which characters that exclude bees are favored. Curiously, there are still more bee-adapted than bird-adapted penstemons.

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References

Armbruster, W. S. 2002. Can indirect selection and genetic context contribute to trait diversification? A transition-probability study of blossom-colour evolution in two genera. Journal of Evolutionary Biology 15: 468–486.

Armbruster, W. S., C. B. Fenster, and M. R. Dudash. 2000. Pollination "principles" revisited: Specialization, pollination syndromes, and the evolution of flowers. Det Norske Videnskaps—Akademi. I. Matematisk Naturvidenskapelige Klasse, Skrifter, Ny Serie 39: 179–200.

Baker, H. G. 1963. Evolutionary mechanisms in pollination biology. Science 139: 877–883.

Baker, H. G., and I. Baker. 1983. Floral nectar constituents in relation to pollinator type. Pp. 117–141 *in* C. E. Jones and R. J. Little (eds.), Handbook of experimental pollination biology. Van Nostrand Reinhold, New York.

Bennett, A. T. D., I. C. Cuthill, and K. J. Norris. 1994. Sexual selection and the mismeasure of color. American Naturalist 144: 848–860.

Bleiweiss R. 2001. Mimicry on the QT(L): Genetics of speciation in *Mimulus*. Evolution 55: 1706–1709.

Bradshaw, H. D., and D. W. Schemske. 2003. Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. Nature 426: 176–178.

Brown, J. H., and A. Kodric-Brown. 1979. Convergence, competition, and mimicry in a temperate community of hummingbird-pollinated flowers. Ecology 60: 1022–1035.

Castellanos, M. C. 2003. The evolution of transitions between pollination modes in *Penstemon*. PhD dissertation, University of Toronto.

Castellanos, M. C., P. Wilson, and J. D. Thomson. 2002. Dynamic nectar replenishment in flowers of *Penstemon* (Scrophulariaceae). American Journal of Botany 89: 111–118.

Castellanos, M. C., P. Wilson, and J. D. Thomson. 2003. Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in *Penstemon*. Evolution 57: 2742–2752.

Castellanos, M. C., P. Wilson, and J. D. Thomson. 2004. "Anti-bee" and "pro-bird" changes during the evolution of hummingbird pollination in *Penstemon* flowers. Journal of Evolutionary Biology 17: 876–885.

Chittka, L., J. Spaethe, A. Schmidt, and A. Hackelsberger. 2001. Adaptation, constraint, and chance in the evolution of flower color and pollinator color vision. Pp. 106–126 *in* L. Chittka and J. D. Thomson (eds.), Cognitive ecology of pollination. Cambridge University Press, Cambridge.

Chittka, L., and N. M. Waser 1997. Why red flowers are not invisible to bees. Israel Journal of Plant Science 45: 169–183.

Clinebell, R. R., and P. Bernhardt. 1998. The pollination ecology of five species of *Penstemon* (Scrophulariaceae) in the tallgrass prairie. Annals of the Missouri Botanical Garden 85: 126–136.

Crosswhite, F. S., and C. D. Crosswhite. 1981. Hummingbirds as pollinators of flowers in the red-yellow segment of the color spectrum, with special reference to *Penstemon* and the "open habitat." Desert Plants 3: 156–170.

Cruden R. W. 1972. Pollinators in high-elevation ecosystems: Relative effectiveness of birds and bees. Science 176: 1439–1440.

Cruden, R. W., S. M. Hermann, and S. Peterson. 1983. Patterns of nectar production and plant–pollinator coevolution. Pp. 80–125 *in* B. Bentley and T. Elias (eds.), The biology of nectaries. Columbia University Press, New York.

Datwyler, S. L., and A. D. Wolfe. 2004. Phylogenetic relationships and morphological evolution in *Penstemon* subg. *Dasanthera* (Veronicaceae). Systematic Botany 29: 165–176.

Dieringer, G., and L. Cabrera R. 2002. The interaction between pollinator size and the bristle staminode of *Penstemon digitalis* (Scrophulariaceae). American Journal of Botany 89: 991–997.

Dilley, J., P. Wilson, and M. R. Mesler. 2000. The radiation of *Calochortus:* Generalist flowers moving through a mosaic of potential pollinators. Oikos 89: 209–222.

Faegri, K., and L. van der Pijl. 1979. The principles of pollination ecology, 3rd ed. Pergamon Press, Oxford.

Farrell B. D. 1998. "Inordinate fondness" explained: why are there so many beetles? Science 281: 555–559.

Fenster, C. B., W. S. Armbruster, P. Wilson, M. R. Dudash, and J. D. Thomson. 2004. Pollination syndromes and floral specialization. Annual Review of Ecology, Evolution, and Systematics 35: 375–403.

Freeman, C. E., J. S. Harrison, J. P. Janovec, and R. Scogin. 2003. Inferred phylogeny in *Keckiella* (Scrophulariaceae) based on noncoding chloroplast and nuclear ribosomal DNA sequences. Systematic Botany 28: 782–790.

Gegear, R. J., and T. M. Laverty. 2001. The effect of variation among floral traits on the flower constancy of pollinators. Pp. 1–20 *in* L. Chittka and J. D. Thomson (eds.), Cognitive ecology of pollination. Cambridge University Press, Cambridge.

Goodnight, C. J., J. M. Schwartz, and L. Stevens. 1992. Contextual analysis of models of group selection, soft selection, hard selection, and the evolution of altruism. American Naturalist 140: 743–761.

Grant, K. A. 1966. A hypothesis concerning the prevalence of red coloration in California hummingbird flowers. American Naturalist 100: 85–97.

Harder, L. D., and J. D. Thomson. 1989. Evolutionary options of maximizing pollen dispersal of animal-pollinated plants. American Naturalist 133: 323–244.

Jones, K. N. 2001. Pollinator-mediated assortative mating: Causes and consequences. Pp. 259–273 *in* L. Chittka and J. D. Thomson (eds.), Cognitive ecology of pollination. Cambridge University Press, Cambridge.

Jordan, E. 2004. Inheritance patterns in floral characters of *Penstemon* and pollinator preference. MS thesis, California State University, Northridge, CA.

Kingsolver, J. G., and T. L. Daniel. 1983. Mechanical determinants of nectar-feeding strategy in hummingbirds: Energetics, tongue morphology, and licking behavior. Oecologia 60: 214–226.

Martínez del Rio, C. 1990a. Dietary, phylogenetic, and ecological correlates of intestinal sucrase and maltase activity in birds. Physiological Zoology 63: 987–1011.

Martínez del Rio, C. 1990b. Sugar preferences in hummingbirds: The influence of subtle chemical differences on food choice. Condor 92: 1022–1030.

Martínez del Rio, C., H. G. Baker, and I. Baker. 1992. Ecological and evolutionary implications of digestive processes: Bird preferences and the sugar constituents of floral nectar and fruit pulp. Experientia 48: 544–551.

McWhorter, T. J., and C. Martínez del Rio. 1999. Food ingestion and water turnover in hummingbirds: How much dietary water is absorbed? Journal of Experimental Biology 202: 2851–2858.

Mitchell, R. J., and R. G. Shaw. 1993. Heritability of floral traits for the perennial wild flower *Penstemon centranthifolius* (Scrophulariaceae): Clones and crosses. Heredity 71: 185–192.

Mitchell, R. J., R. G. Shaw, and N. M. Waser. 1998. Pollinator selection, quantative genetics and predicted evolutionary responses of floral traits in *Penstemon centranthifolius* (Scrophulariaceae). International Journal of Plant Sciences 159: 331–337.

Mitter, C., B. Farrell, and B. Wiegmann. 1988. The phylogenetic study of adaptive zones: Has phytophagy promoted insect diversification? American Naturalist 132: 107–128.

Nicolson, S. W. 1998. The importance of osmosis in nectar secretion and its consumption by insects. American Zoologist 38: 418–425.

Nicolson, S. W. 2002. Pollination by passarine birds: Why are the nectars so dilute? Comparative Biochemistry and Physiology B 131: 645–652.

Pyke, G. H., and N. M. Waser. 1981. The production of dilute nectars by hummingbird and honeyeater flowers. Biotropica 13: 260–270.

Raven, P. H. 1972. Why are bird-visited flowers predominantly red? Evolution 26: 674.

Reid, W. H., P. Sensiba, and C. E. Freeman. 1988. A mixed pollination system in *Penstemon pseudospectabilis* M. E. Jones (Scrophulariaceae). Great Basin Naturalist 48: 489–494.

Roberts, W. M. 1995. Hummingbird licking behavior and the energetics of nectar feeding. Auk 112: 456–463.

Roberts, W. M. 1996. Humingbirds' nectar concentration preferences at low volume: The importance of time scale. Animal Behavior 52: 361–370.

Schemske, D. W., and P. Bierzychudek. 2001. Evolution of flower color in the desert annual *Linanthus parryae:* Wright revisited. Evolution 55: 1269–1282.

Schemske, D. W., and H. D. Bradshaw. 1999. Pollinator preference and the evolution of floral traits in monkey flowers (*Mimulus*). Proceedings of the National Academy of Sciences (USA) 96: 11910–11915.

Stebbins, G. L. 1989. Adaptive shifts toward hummingbird pollination. Pp. 39–60 *in* J. H. Bock and Y. B. Linhart (eds.), The evolutionary ecology of plants. Westview Press, Boulder, CO.

Straw, R. M. 1956. Adaptive morphology of the *Penstemon* flower. Phytomorphology 6: 112–118.

Straw, R. M. 1963. Bee-fly pollination of *Penstemon ambiguus*. Ecology 44: 818–819.

Sutherland, G. D., and C. L. Gass. 1995. Learning and remembering of spatial patterns by hummingbirds. Animal Behavior 50: 1273–1286.

Sutherland, S. D., and R. K. Vickery. 1993. On the relative importance of flower color, shape, and nectar rewards in attracting pollinators to *Mimulus*. Great Basin Naturalist 56: 282–282.

Thomson, J. D. 1986. Pollen transport and deposition by bumble bees in *Erythronium:* Influences of floral nectar and bee grooming. Journal of Ecology 74: 329–341.

Thomson, J. D. 2003. When is it mutualism? American Naturalist 162: S1–S9.

Thomson, J. D., and B. A. Thomson. 1992. Pollen presentation and viability schedules in animal-pollinated plants: Consequences for reproductive success. Pp. 1–24 *in* R. Wyatt (ed.), Ecology and evolution of plant reproduction. Chapman and Hall, New York.

Thomson, J. D., P. Wilson, M. Valenzuela, and M. Malzone. 2000. Pollen presentation and pollination syndromes, with special reference to *Penstemon*. Plant Species Biology 15: 11–29.

Torchio, P. F. 1974. Mechanisms involved in the pollination of *Penstemon* visited by the masarid wasp, *Pseudomasaris vespoides* (Cresson). Pan Pacific Entomologist 50: 226–234.

Walker-Larson, J., and L. D. Harder. 2001. Vestigial organs as opportunities for functional innovation: The example of the *Penstemon* staminode. Evolution 55: 477–487.

Waser, N. M. 1983. The adaptive nature of floral traits: Ideas and evidence. Pp. 241–285 *in* L. Real (ed.), Pollination biology. Academic Press, Orlando, FL.

Waser, N. M. 1998. Pollination, angiosperm speciation, and the nature of species boundaries. Oikos 81: 198–201.

Wells, H., P. S. Hill, and P. H. Wells. 1992. Nectarivore foraging ecology: Rewards differing in sugar type. Ecological Entomology 17: 280–288.

Wilson, P. 1994. The habits of selection for pollination success. PhD dissertation, State University of New York, Stony Brook, NY.

Wilson, P. 1995. Selection for pollination success and the mechanical fit of *Impatiens* flowers around bumble bee bodies. Biological Journal of the Linnean Society 55: 355–383.

Wilson, P., M. C. Castellanos, J. N. Hogue, J. D. Thomson, and W. S. Armbruster. 2004. A multivariate search for pollination syndromes among penstemons. Oikos 104: 345–361.

Wolfe, A. D., S. L. Datwyler, and C. P. Randle. 2002. A phylogenetic and biogeographic analysis of the Cheloneae (Scrophulariaceae) based on ITS and matK sequence data. Systematic Botany 27: 138–148.