Constrained lability in floral evolution: counting convergent origins of hummingbird pollination in *Penstemon* and *Keckiella*

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Summary

• In the clade of *Penstemon* and segregate genera, pollination syndromes are well defined among the 284 species. Most display combinations of floral characters associated with pollination by Hymenoptera, the ancestral mode of pollination for this clade. Forty-one species present characters associated with hummingbird pollination, although some of these ornithophiles are also visited by insects.
• The ornithophiles are scattered throughout the traditional taxonomy and across phylogenies estimated from nuclear (internal transcribed spacer (ITS)) and chloroplast DNA (*trnCD/TL*) sequence data. Here, the number of separate origins of ornithophily is estimated, using bootstrap phylogenies and constrained parsimony searches.
• Analyses suggest 21 separate origins, with overwhelming support for 10 of these. Because species sampling was incomplete, this is probably an underestimate.
• Penstemons therefore show great evolutionary lability with respect to acquiring hummingbird pollination; this syndrome acts as an attractor to which species with large sympetalous nectar-rich flowers have frequently been drawn. By contrast, penstemons have not undergone evolutionary shifts backwards or to other pollination syndromes. Thus, they are an example of both striking evolutionary lability and constrained evolution.

Key words: conservatism, constraint, homoplasy, lability, parallelism, *Penstemon*, pollination, speciational drive.

Introduction

Gould (2002, Chapter 10) wrote of constraint as not only limiting evolution but also leaving the opportunity open for evolution in some directions as opposed to all directions. In other words, he viewed constraint as having a positive as well as a negative role. Here we report on an example of what appears to be constrained lability. Among penstemons, many lineages have separately evolved from bee pollination toward hummingbird pollination, while transitions toward pollination by other types of animal are at most very limited. Penstemons therefore constitute a case in which species interactions seem to evolve with ease, but in one direction much more than in others.

Convergent evolution is the separate origin of similarities across two or more lineages. We use the word 'separate' rather than 'independent' because a consideration important to us is the possibility that a group of organisms may start with pre-aptations that predispose its various sublineages to repeatedly evolve a particular way of doing business. It is convenient although perhaps overly typological to refer to 'pollination syndromes' of floral characters (Fenster *et al.*, 2004), which are posited to represent powerful adaptive attractors that flowers converge upon from various twigs of the phylogeny of angiosperms (Thomson & Wilson, in press). Although strong convergence is implicit in the notion of pollination syndromes, patterns of convergent pollinator shifts have seldom been investigated by formal phylogenetic analysis. As part of a research program on shifts between hymenopteran and hummingbird pollination in the penstemon clade, we have wanted to know how many of these shifts are evolutionarily separate (Thomson, 2003; Wilson *et al.*, 2006). Phylogenetics promises to enable us to distinguish convergent evolution from conserved homology. This ability would in turn pave the way for understanding particular adaptations (such as changes in the biochemical pathway of pigment production), the order of character change in the 'construction' of pollination syndromes (such as whether nectar might change before flower color), and biases in diversification (such as how hummingbird pollination might inhibit cladogenesis compared with bee pollination). Despite such tantalizing promises, phylogenies with sufficient resolution are still rare in groups with many species of varying pollination syndromes, and the phylogenies offered thus far in pollination biology have supported few firm conclusions about convergence along the various pollination syndrome axes.

A clarification of terms is in order before going further. By 'penstemons' we mean members not only of the genus *Penstemon* (*c.* 270 species) but also of *Keckiella* (seven species), *Nothochelone* (one species), *Chelone* (four species), and *Chionophila* (two species) (Lodewick & Lodewick, 1999; Wolfe *et al.*, 2002). We use as shorthand 'bee' to include true bees but also the pollen-collecting wasp *Pseudomasaris vespoides*, which interacts with flowers much as bees do. We dichotomize species as having bee-pollination syndrome characters (melittophilous) or hummingbird-pollination syndrome characters (ornithophilous). These two syndromes form two clusters when species are ordinated by floral morphology, nectar characteristics, and color (Wilson *et al.*, 2004), although some intermediate species have taken on hummingbirds while still retaining bees. Generally speaking, ornithophilous morphologies tend to be associated with red colors and copious but dilute nectars, while melittophilous morphologies occur with blue-violet or whitish corollas and concentrated nectars. Details on pollinator visitation have been presented elsewhere (Wilson *et al.*, 2004) for 49 species, and we now have field data on 84 species. In what follows we refer to 41 species of penstemons that more or less conform to the ornithophily

syndrome. Generally, these have taken on reddish colors, narrowed their corollas while not shortening them, and increased their nectar volume, and are observed in nature to be frequented by hummingbirds. However, we do not have field data for all species, and when we do not, we ascribe pollination syndrome based simply on color and morphology. All ornithophiles for which we have field data are visited heavily by hummingbirds, although sometimes by bees also, and some melittophiles are visited by birds as well as bees to a modest degree. In the current paper we do not attempt to tease apart the several pollination syndrome traits from the animals' responses to those traits. We cannot improve much upon the nonhistorical 2004 analysis in that respect. The current paper attempts to use a categorization of species into syndromes in order to examine the inequalities in types of transitions between syndromes.

Evolutionary transitions from one pollinator type to another have been examined in a few other groups. A close reading of previous studies reveals many of the hurdles to be surmounted in fulfilling the promise of phylogenetics in ecology. In the well-studied *Mimulus* section *Erythranthe*, the most parsimonious interpretation is that hummingbird pollination arose twice; however, that conclusion and the sequence of character changes inferred would be different if only one species had gone extinct or if one fewer species had been included in the study (Beardsley *et al.*, 2003). For *Disa* orchids, which have undergone numerous shifts toward a variety of pollination syndromes, the phylogeny of Johnson *et al.* (1998) was inferred from a small number of morphological characters that are not necessarily independent of the pollination mechanism, and the study included only a minority of the species in the genus. Early studies of *Dalechampia* suggested several origins of pollination by male euglossine bees, but the number of convergences was small, fewer than half the species were studied, and again the phylogeny was based on morphological characters (Armbruster, 1993). Later molecular-based studies still detected only limited convergence (Armbruster & Baldwin, 1998). In *Erythrina*, Bruneau (1997) showed how hummingbird pollination arose four times out of passerine pollination. In *Costus*, Kay *et al.* (2005) found seven origins of ornithophily from melittophily. Regarding the number of convergences, our study goes beyond previous ones in the sheer volume of documented convergences in pollinator type. Our main problem – which the following analysis attempts to overcome – is that our phylogeny is poorly resolved. Nevertheless, we shall demonstrate that there is enough phylogenetic signal to conclude conservatively that there have been many shifts from melittophily to ornithophily, that there have been few if any reversals, and that there are few if any shifts to any other pollination syndrome. Thus, penstemon flowers are labile in the sense of being prone to evolve from bee to bird pollination and constrained in the sense that they do not evolve backwards or into other adaptive zones.

Materials and Methods

Of the 284 species in the penstemon clade, 189 were surveyed for nuclear DNA of the internal transcribed spacer (ITS) region, and 139 for chloroplast DNA of the spacers between transfer RNA *C* and *D* genes, and *T* and *L* genes (*trnCD/TL*). Details of the molecular methods and cladistic results are presented by Wolfe *et al.* (2006), who noted the convergence in pollination syndrome but did not do any of the following analyses or discuss the macroevolutionary dynamics suggested by the phenomenon. The ITS and cpDNA data sets both yielded many equally parsimonious trees, and only a few nodes of the consensus trees were well supported as monophyletic by bootstraps. Disturbingly, the solutions for ITS and cpDNA were significantly different from one another, possibly because of introgression, so combining data at the outset was not appropriate. Even if it were, for the purposes of counting convergences, it is more conservative to not constrain the answer to fit a tree that resulted from combined data but to instead explore freely the range of possible results. Although hybridization could be complicating the problem, the low amount of molecular variation suggests that the main reason for poor phylogenetic resolution is merely that *Penstemon* has undergone a recent evolutionary radiation. We sampled many species. This is more informative than sampling fewer species would have been, but dense taxon sampling inherently makes it harder to resolve all the details of the phylogeny. In what follows, we demonstrate that, even though we have not arrived at a well-resolved phylogeny, the ITS and cpDNA data contain considerable phylogenetic signal, enough signal to support some firm conclusions regarding the multiple origins of ornithophily. For merely illustrative purposes, representative ITS and *trnCD/TL* phylogenies are pictured in the Supplementary Material, but note that no single cladogram captures our conclusions.

Results

Using PAUP* (Swofford, 2002), a heuristic search with tree bisection/reconnection branch swapping was used to find 2000 equally parsimonious trees. Using MacClade (Maddison & Maddison, 2001), polytomies were resolved at random and the number of changes in pollination syndrome (melittophily = 0, ornithophily = 1) was counted for each tree. For the ITS data set, which included 25 ornithophiles, 0.6% of the trees had 19 shifts between insect and bird pollination, 5.8% had 20 or fewer, and the remaining trees had 21 or more shifts, yielding an average of 21.6 shifts. The amount of homoplasy can be measured as the retention index, which would take on a value of 1.00 if all ornithophiles formed a monophyletic clade and a value of 0.00 if each ornithophile had a melittophile as a sister species (Archie, 1996). Averaging across the 2000 ITS trees, the retention index for syndrome as a binary character was 0.32 (SD = 0.016). For the cpDNA data set, which included 20 ornithophiles, 0.15% had 17 shifts between bee and bird pollination, and the rest indicated 18 changes. Averaging across the 2000 cpDNA trees, the retention index for syndrome was 0.11 (SD = 0.002). If all of the 41 ornithophilous species had been included, it is very likely that the number of origins would have been even higher. In particular, preliminary studies suggest three additional origins that resulted in *Penstemon utahensis*, *Penstemon murrayanus*, and *Penstemon cardinalis* (Wilson *et al.*, 2006). One could say that it is most parsimonious to postulate *at least* 21 shifts from bee pollination toward hummingbird pollination or back.

One possibility for how that estimate might be inflated would involve random variation in the molecular data. Thus, we went on to quantify transitions using bootstrapped data. We had PAUP* make 1000 trees by resampling the data at random with replacement and searching heuristically for a tree. For the ITS data, the distribution of numbers of shifts to or from hummingbird pollination for the 1000 bootstrapped trees is shown in Fig. 1(a). Only 0.2% of them had 18 shifts between insect and bird pollination, 1.0% had 19 or fewer shifts, and 6.1% had 20 or fewer. The average was 22.1 shifts. For the cpDNA bootstraps, shown in Fig. 1(b), 1.1% had 14 or 15 steps, and the average was 17.8 shifts. Our overall conclusion survives bootstrapping: there have been many origins of hummingbird pollination or perhaps a mixture of many origins and a few reversals.

We sought further assurance of the multiplicity of shifts by quantifying the amount of data in favor of homoplasy. One can constrain a phylogenetic search to support a particular evolutionary hypothesis (e.g. one origin of ornithophily followed by a radiation into 41 species) and quantify the 'cost' in terms of extra tree length (Armbruster, 1992, 1993). Although one monophyletic group would be silly in our case, less constrained searches allowed us to identify those ornithophiles that we are confident arose separately. We considered two ornithophiles at a time. All other ornithophiles were deleted from the data set to simplify the procedure. If they had been left in, we would have had to consider whether they should be inside or outside the constrained monophyletic group. In our case, deleting the extra ornithophiles did not greatly alter the phylogenies. Table 1 shows the costs of constraining various pairs of ornithophiles. The table could have been larger, but for the sake of brevity we deal with less certain cases in the Supplementary Material. Each cell in Table 1 indicates homoplasy based on ITS, cpDNA, or both types of data. For example, in the most parsimonious trees (Supplementary Material Figs S1, S2), *Penstemon newberryi* and *Penstemon rupicola* appear to represent independent origins of hummingbird pollination, which is an unexpected result (Datwyler & Wolfe, 2004); if we constrain the tree search to force these two species together, the ITS tree would be two steps longer and the chloroplast tree would be five steps longer, as indicated on the second line in Table 1. One may choose to set an arbitrary threshold, such as five or more extra steps, for rejection of the
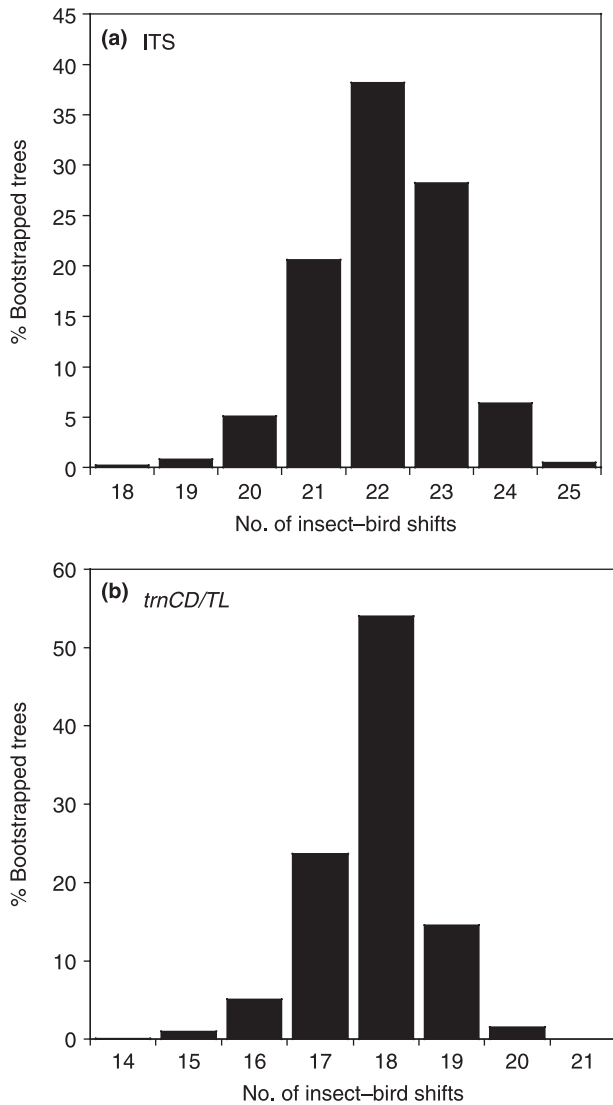
Fig. 1 Distribution of 1000 bootstrap samples for the number of changes between hymenopteran and hummingbird pollination. (a) Based on internal transcribed spacer (ITS) nuclear sequence; (b) based on chloroplast sequence from the spacers between transfer RNA genes *C* and *D*, and between genes *T* and *L* (*trnCD/TL*). Each data set included 20 of the 41 *Penstemon* species that showed at least some of the features of ornithophily.

possibility of a single pollinator shift. By this criterion, 10 of the ornithophiles would signal separate origins (or conceivably reversals). An additional five shown in Table 1 are supported as separate with less (although not zero) support. Note that, for each of the 15 focal species in Table 1, the importance of hummingbirds in pollination has been confirmed with field data (Wilson *et al.*, 2004 and unpublished; Lange *et al.*, 2000).

In the Supplementary Material, we examine all the other sampled ornithophiles that may or may not be homologously ornithophilous with one of the 15 focal species. Although it

Table 1 The number of extra DNA steps in the phylogenies that would have to be added to force two ornithophilous species together

	ternata	newberryi	rupicola	pinifolius	rostriflorus	centranthifolius	lanceolatus	superbus	kunthii	hartwegii	baccharifolius	havardii	labrosus	barbatus
newberryi	17 55													
rupicola	17 60	2 5												
pinifolius	26 56	14 42	16 48											
rostriflorus	25 53	10 38	15 43	7 6										
centranthifolius	25 53	13 40	15 45	8 5	6 6									
lanceolatus	27 52	15 39	16 44	11 7	11 3	7 5								
superbus	23 –	9 –	14 –	5 –	4 –	3 –	6 –							
kunthii	32 55	19 44	21 49	13 18	9 12	10 15	14 9	10 –						
hartwegii	26 52	14 36	18 41	11 2	11 0	7 3	8 2	4 –	4 10					
baccharifolius	24 52	12 38	15 44	8 2	6 5	1 5	5 1	2 –	10 11	6 1				
havardii	26 48	14 36	14 41	6 4	8 4	3 5	4 2	3 –	13 11	10 1	1 3			
labrosus	23 50	12 36	14 41	8 4	6 12	3 5	5 3	4 –	12 12	9 5	1 1	2 0		
barbatus	26 46	13 37	11 42	7 7	9 11	4 5	8 2	4 –	12 12	8 2	2 6	1 1	3 4	
floridus	26 –	13 –	16 –	10 –	9 –	4 –	6 –	3 –	11 –	6 –	1 –	3 –	3 –	4 –

All other ornithophiles were deleted from the data sets, and heuristic searches were performed with and without constraining the two focal ornithophiles to be monophyletic; the numbers shown are differences in the lengths of the two trees. In a cell, the first number refers to the internal transcribed spacer (ITS) data; the second number refers to the data from the spacers between transfer RNA genes *C* and *D*, and between genes *T* and *L* (*trnCD/TL*) from the chloroplast (there were no *trn* data for *P. superbus* or *P. floridus*).

is hard to be confident that any one of the others is separate, on the whole there is considerable evidence for homoplasy beyond the 15 origins. There is no compelling evidence for reversals after taking into account Freeman *et al.*'s (2003) data that indicate multiple origins in *Keckiella*, but reversals are fairly hard to tell from convergences, and there are several instances where reversals are conceivable. The Supplementary Material also details the cases of species that are pollinated by a mixture of birds and bees. Although some of these could be transitional to a hummingbird specialist, the data are usually at least as well explained by postulating the separate origin of ornithophiles that differ in the characters that make them ornithophilious (see Supplementary Material).

Amid these many transitions to hummingbird pollination, other transitions to pollination by anything other than bees and *Pseudomasaris* are rare if they exist at all. The most unusual of penstemons is *Penstemon ambiguus*, which offers essentially no nectar, and has narrow long corolla tubes and large white landing platforms. Straw (1963) observed small long-tongued bombyliid flies pollinating *P. ambiguus*. We have found these flies, namely *Oligodranes*, to be fairly common visitors of many penstemons. They rest in the flowers and are seen feeding only sporadically. In observations made on species other than *P. ambiguus*, they have no noticeable pollen on their bodies. Our field data indicate that *P. ambiguus* and its less radical relative *Penstemon thurberi* are pollinated mostly by pollen-collecting bees, although it is possible that their peculiar floral traits are adaptations to the bombyliids. The lineage of *Penstemon tubaeflorus* has relatively narrow tubes suggestive of Lepidoptera pollination, and butterflies visit at a low rate, but most visits are still by bees (Clinebell & Bernhardt, 1998). There are two other species with characters that might suggest weak adaptation toward Lepidoptera (*Penstemon albidus* and *Penstemon marcusii*). On occasion, sphingid moths can be seen working a patch of ordinary penstemons, but the same patches are generally worked by legitimate pollinators also. On the whole, the lability in pollination syndrome is almost entirely between melittophily and ornithophily despite some visitation by many other types of insects (bombyliids, moths, pollen-foraging flies, beetles, and flies that lick anthers). Within melittophily, different penstemons have adapted to bees in different ways (Wilson *et al.*, 2004). Variation in overall size seems to predispose some to visits from large bees (*Xylocopa*), and others to visits from smaller bees (*Osmia*). In other cases, size variation may not influence the identity of visitors but does determine where pollen is placed. For example, *Bombus* workers pass readily from very small-flowered (*Penstemon procerus*) to large-flowered (*Penstemon strictus*) species; the former place pollen on a bee's face, while the latter deposit it on the thorax. Some penstemons have widely opening anthers that make pollen freely available; others dispense restricted amounts of pollen from anthers that open only slightly (Castellanos *et al.*, 2006), but none has reached the full development of the buzz-pollination syndrome, which includes nectarlessness.

Discussion

A long-standing question in evolutionary ecology is whether interactions between plants and animals are conservative or labile. On the one hand, traits that mediate interactions might be more conserved than other traits because of the complex and/or discontinuous nature of the relationship (Mant *et al.*, 2002); if so, they would leave strong phylogenetic signals (e.g. Ehrlich & Raven, 1964; Thompson, 2005). Authors who survey floral diversity can often make generalizations about the pollination system of whole genera and higher taxonomic groups, which implies conservatism (e.g. Endress, 1994). On the other hand, the traits that mediate interactions might be evolutionarily labile and subject to repeated convergence because the direct effects of animals on plant fitness generate strong selection and may flip-flop between mutualism and antagonism, and/or because the community of animals is subject to frequent changes in space and time (Armbruster, 1992, 1993; Johnson *et al.*, 1998). Our example provides the clearest evidence yet in support of the latter view – but with a caveat. Although the pollination system of penstemons is fantastically labile, the shifts between adaptive strategies do not take place in an open market; the many penstemon lineages do not seem to have had equal access to the many pollination syndromes (unlike in *Disa* orchids; Johnson *et al.*, 1998). Instead, we see numerous shifts from melittophily to hummingbird ornithophily but no clear shifts to other modes of pollination. Hummingbird pollination appears to be an attractor to which bee-adapted penstemon flowers are especially predisposed. Furthermore, that shifts occur from melittophily to ornithophily and not (or rarely) backwards constitutes an example of 'speciational drive' (*sensu* Gould, 2002), that is, the phenomenon in which transitions in one direction are evolutionarily easier than transitions in the reverse direction.

Gould's (2002) favorite reasons for constraint were generally 'internal' limitations imposed by idiosyncratic patterns of genetic variation or developmental processes. In the evolution of pollinator transitions, we suspect that 'external' factors are the reason for constrained lability. Consider an ordinary melittophilous penstemon (with a short, wide, blue corolla tube, a landing platform, and concentrated nectar). Changing to a hummingbird flower (with a long, narrow, red tube, no platform, and more dilute nectar) would seem to be no more complicated genetically than changing to a hawkmoth flower (long, narrow, white tube, no platform, and more dilute nectar). Nevertheless, transitions to birds have been much more frequent in penstemons.

We suspect that the most important external cause of the phenomenon of many shifts toward ornithophily lies in the capacity of hummingbirds to extract nectar easily from open melittophilous flowers while transferring pollen at a high ratio of grains delivered to grains removed (compared with bees), even when the flowers are a poor fit morphologically. Previously, we reported that hummingbirds had a 1.66% pollen

transfer efficiency compared with a 1.63% efficiency for bumblebees when visiting the melittophilous *P. strictus*, and when visiting the ornithophilous *Penstemon barbatus* the bird's efficiency rose to 2.48% (Castellanos *et al.*, 2003). We suspect that sphingid moths, for example, are less effective pollinators of penstemons. Penstemons have only four anthers, and when one watches sphingids visit melittophilous penstemons, they do not appear to remove pollen. Another factor is that hummingbirds are inquisitive enough to investigate many flower designs, and they have sufficient spatial memory to return to plants that have been rewarding (Healy & Hurley, 2001). Furthermore, in temperate western North America, the energetically costly migrations of hummingbirds take them into habitats with disparate flowers where the birds must take what is available. These considerations make it likely that hummingbirds will frequently become visitors to bee-adapted flowers, as long as those flowers have enough nectar flow to make visits profitable. Melittophilous penstemons may be under particularly strong selection to obtain many bee visits, resulting in more generous nectar secretion schedules than in many other bee-pollinated plants (Williams & Thomson, 1998; Wilson *et al.*, 2006; also Cruden *et al.*, 1983). For penstemons, therefore, the pathway from bees to birds is *permitted* by constitutional similarities of the foraging modes of the two pollinator types; it is *made available* by ecological circumstances that impel birds to poach from flowers belonging to the 'illegitimate' syndrome; and it can be *driven* by the birds' less wasteful delivery of pollen (Castellanos *et al.*, 2003). Another reason for thinking that this constellation of external forces is more important than internal morphogenetic constraint is that similar repeated shifts to bird-pollination syndromes have occurred in many distantly related plants (Stebbins, 1989; Grant, 1994), where the developmental genetics are presumably different.

The signature of constrained lability also appears in some other pollination systems. Bruneau's (1997) study of *Erythrina* illustrates it well. There are about 112 *Erythrina* species, and all appear to be bird pollinated. None has evolved to be bee or moth pollinated. However, there have been repeated shifts from passerine pollination to hummingbird pollination. This involves many phenetic characters, including the tubularity of the corolla and the architecture of the inflorescence. Bruneau suggests that changes in nectar chemistry are central to the shifts between pollinators. Then there is *Pedicularis*, comprising some 500 species, almost all of which are to a greater or lesser extent bumblebee pollinated (although *Pedicularis densiflora* attracts hummingbirds too: Macior, 1986). In *Pedicularis*, there have been many convergent changes in the floral characters that affect the mechanism of bumblebee pollination and the reward system (Ree, 2005). *Pedicularis* has kept to one kind of pollinator but has adapted to use that type in different ways: characters such as the length and orientation of the beak that places pollen on bees seem to have undergone considerable homoplastic evolution.

Are hummingbirds a one-way attractor? Our data are consistent with unidirectional lability, which would constitute a case of what Gould (2002) called 'speciational drive.' All of the inferred transitions could be explained parsimoniously on the hypothesis that flowers evolve from being bee pollinated toward being bird pollinated and never in the other direction. Unfortunately, in a poorly resolved phylogeny such as ours, with numerous species having the derived condition, it is difficult to use parsimony to distinguish between separate origins and reversal. Although there is no case where it is more parsimonious to postulate a reversal than multiple origins of ornithophily, there are many equivocal cases. However, in these cases, the several ornithophiles appear more heterogeneous in their ornithophily than the related melittophiles are in their melittophily (Supplementary Material). In trying to count convergences, we should not dismiss phenetic dissimilarity (as we might when inferring phylogenies). Convergence tends to make species similar only superficially. Close comparison of the species suggests that many similarities are homoplastic rather than homologous. Furthermore, if the two convergent species are far enough apart on the phylogeny, then the homoplasy does not mislead one in the task of counting convergences. Because there are so many species of penstemons, the homoplasy is striking in our study group. In a group with fewer species it might go unnoted, but the phenomenon could apply to a broad swath of angiosperm diversity. Not very far outward of the clade we call penstemons, there are additional origins of ornithophily in *Russellia* and *Tetranema*. In *Costus*, a monocot genus with large zygomorphic flowers, Kay *et al.* (2005) counted seven shifts from melittophily to ornithophily and no other shifts. In *Mimulus* section *Erythranthe*, Beardsley *et al.* (2003) reported two shifts from bees to birds. In North American *Aquilegia*, Whittall & Hodges (2007) have inferred two shifts from bees to birds, followed by several shifts to moth pollination, but no direct shifts from bees to moths, and no reversals. (An obvious difference between *Aquilegia* and penstemon is that *Aquilegia* has numerous stamens, which we hypothesize makes moths viable pollinators.) In the Loasoideae, Weigend *et al.* (2004) report two shifts from melittophily to ornithophily. In the Melastomataceae, Penneys & Judd (2005) report two other such shifts resulting in the genus *Charianthus* and separately the species *Tetrazygia fadyenii*. By contrast, Perret *et al.* (2003), studying Sinningieae (Gesneriaceae), found evidence for shifts from hummingbird to bee pollination as well as equivocal shifts in the usual direction. Considering all this work and more broadly Grant's (1994) list of western North American ornithophiles, it seems very likely that transitions from melittophily to ornithophily are far more frequent than transitions in the reverse direction.

Janson (1992) sketched out a macroevolutionary process caused by such inequalities in the rates of transitions between syndromes. He illustrated the process with seed-dispersal syndromes, but the same possibility presents itself with pollination syndromes. Thus, for certain types of flowers, lineages

may typically move from syndrome X to Y, and from Y to Z, but rarely from X to Z and rarely backwards (Whittall & Hodges, 2007). Janson's inequalities among transition probabilities is the same as Gould's speciational drive. (Because the process need not have anything to do with 'speciation' in the sense of the origin of reproductive isolation, we might have called it 'transitional drive'.) Such drive is caused by individual lineages adapting along the paths of least resistance as local ecological situations present themselves. Drive partially affects the proportions of penstemons of one syndrome or another. One might next ask, 'Why there are still so many melittophiles?' (Wilson *et al.*, 2006). Accepting that there are differences in the probabilities of transition (a high rate of change from melittophily to ornithophily and very low rates of all other changes), the clade should become more ornithophilous over time. One possible explanation for why there are still so many melittophiles is that this process has not had enough time to go to the limit. It takes special circumstances over many generations to allow for a shift, and despite the high number we have found, shifts are still rare occurrences. Alternatively and with allure for those interested in macroevolutionary dynamics, there could be countervailing clade selection, perhaps in the form of ornithophily disfavoring subsequent cladogenesis. Our lack of phylogenetic resolution hampers testing between the alternative possibilities, but the data do seem sufficient to say that there is speciational drive (without or with other macroevolutionary processes).

In our study, many of the convergences suggested by the molecular data were anticipated by traditional taxonomy. Ornithophiles and melittophiles have long been treated as close relatives within sections or subgenera of penstemons (Lodewick & Lodewick, 1999). In this sense, finding convergences toward hummingbird pollination would not surprise a penstemon specialist. But from the viewpoint of understanding speciational drives, the number of origins of ornithophily is astonishing and indicates to us that the hummingbird pollination niche is just waiting to be claimed by plants with flowers like penstemons in a way that other pollination niches are not. In addition to the factors considered above, such as rapid nectar replenishment, large sympetalous corollas may be a preaptation for bird pollination (Endress, 1996). This is supported by the observation that there are no ornithophiles nested within those clades of penstemons that have small flowers that fit around the tongues and faces of bees. Another predisposing factor may be having an anthocyanin pathway that can easily mutate from producing blue-purple pigments to hot-pink ones to scarlet and crimson ones (Zufall & Rausher, 2004; Rausher, in press).

Our documentation of numerous shifts toward ornithophily substantiates other contributions toward understanding floral evolution among penstemons. For example, Baker & Baker (1983) showed that the ornithophilous penstemons had higher sucrose:hexose ratios than the melittophiles (also Freeman *et al.*, 1984). Our analysis shows that this is through

convergence, not conservatism. Similarly, penstemons that are hummingbird pollinated have anthers that open more widely and present pollen more generously than relatives that are hymenopteran pollinated (Castellanos *et al.*, 2006). The analysis presented here shows that open anthers originated multiple times, which further supports the adaptive explanation of this feature.

Acknowledgements

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Supplementary Material

The following supplementary material is available for this article online:

Text S1 Enumerating the shifts in pollination syndrome.

Fig. S1 One among thousands of parsimonious trees, based on internal transcribed spacer (ITS) sequence data. Species of low relevance to tracing character evolution were pruned. Black lineages are inferred to be bee pollinated, white lineages to be hummingbird pollinated; and dashed lineages are equivocal.

Fig. S2 One among thousands of parsimonious trees, based on *trnCD/TL*. Species of low relevance to tracing character evolution were pruned. Black lineages are inferred to be bee pollinated, and white lineages to be hummingbird pollinated.

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