THE POLLINATION ECOLOGY OF FIVE SPECIES OF *PENSTEMON* (SCROPHULARIACEAE) IN THE TALLGRASS PRAIRIE¹

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Abstract

The floral ecology of Penstemon cobaea Nutt. var. cobaea, P. cobaea var. purpureus Pennell, P. digitalis Nutt. ex Sims, P. grandiflorus Nutt., P. pallidus Small, and P. tubaeflorus Nutt. was studied by sampling populations at nine prairie sites in Illinois, Kansas, and Missouri. All five species show protandry, but the receptive stigma lies only 2 mm away from the two pairs of fertile stamens. Bagging experiments on three Penstemon spp. show that only P. digitalis sets seed when insect visitors are excluded. Flowers of all five species exhibit a horizontal presentation of the corolla and emit a slightly discernible scent. However, the presumed pollination systems of Penstemon spp. correlate with corolla form and the size of the floral sinus. The tubular, white flowers of P. tubaeflorus appear to be pollinated by a combination of diurnal Lepidoptera and some native bees favoring a dorsal deposition of pollen on mouthparts and upper thoraces. The four remaining species have gullet- or bell-shaped corollas ornamented with violet-purple blotches or lines. These species appear to be pollinated primarily by polylectic/polyphagic bees (including six Bombus spp.), with P. digitalis and P. cobaea visited infrequently by the rare Penstemon wasp, Pseudomasaris occidentalis. Queens of Bombus pennsylvanicus subsp. pennsylvanicus forage primarily on the large, gullet flowers of P. grandiflorus and both varieties of P. cobaea. Queens of Bombus nevadensis subsp. auricomus prefer those P. digitalis and P. pallidus showing a reduced gullet or bell form. Bee pollination in four Penstemon spp. may operate within a two-tiered system. Large-bodied Bombus spp., Megachile brevis, and anthophorids (Synhalonia hamata and Anthophora terminalis) carry dorsal depositions of Penstemon pollen because they contact anthers and stigmas while they forage exclusively for nectar. In contrast, smallbodied members of the Anthophoridae (Ceratina), Colletidae (Hylaeus spp.), Halictidae (Augochlorella, Halictus, Lasioglossum), and Megachilidae (Hoplitis and Osmia) forage actively for Penstemon pollen encouraging repeated, ventral contact with the sexual organs of the flowers. Bombus queens were more prevalent at large Penstemon populations (especially P. cobaea). Bombus workers were collected primarily on restored sites. The importance of small bees as pollinators appeared to vary indirectly with *Penstemon* population size.

Penstemon (Scrophulariaceae: Cheloneae) is a North American genus of about 270 species (Wolfe et al., 1997) distributed from Alaska to Guatemala. Within the Great Plains, Freeman (1981) recognized 22 species of Penstemon in two subgenera and five sections. The Upper Mississippi Valley supports two additional species, Penstemon arkansanus Pennell and P. hirsutus (L.) Willd., suggesting that 24 Penstemon species are native to midwestern American prairies.

Despite the species richness of *Penstemon* in North America, analyses comparing life-histories within this genus lag far behind classical (Pennell, 1935; Keck, 1938) and molecular (Wolfe & Elisens, 1993) taxonomies. We lack significant literature on breeding systems in *Penstemon* compared to other scrophulariaceous genera (e.g., *Pedicularis*; Macior, 1982) distributed through the Northern Hemisphere (Kampny, 1995). References to the pollination biology of *Penstemon* by Pennell (1935) were derived primarily from predictions that were based on floral morphology. In contrast, what literature does exist on *Penstemon* pollination often shows a lack of consensus regarding the efficiency of different bee taxa as true pollen vectors.

Field studies of *Penstemon* pollination began with Robertson (1892, 1929), who noted protandry in "*P. laevigatus*" (= *P. digitalis*) and "*P. pubescens*" (= *P. pallidus*) in Illinois and collected a total of 20 different bee species in their flowers. Rob-

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ANN. MISSOURI BOT. GARD. 85: 126–136. 1998.

¹This work is part of the first author's doctoral dissertation being prepared in the Dept. of Biology, Saint Louis University, under the direction of P. Bernhardt. Financial support for fieldwork has been provided by the Conservation Federation of Missouri (Bell Conservation Scholarship), the Kansas City Garden Club, the Missouri Botanical Garden (Litzsinger Road Ecology Center), the Missouri Department of Conservation, the Missouri Prairie Foundation, and the St. Louis office of the Nature Conservancy. Donald Hardin provided assistance in the field and in the preparation of tables and graphics. Stanley Sawyer provided statistical advice. Charles Michener and colleagues identified the Hymenoptera and referred other insect specimens to appropriate authorities. We thank Paul Wilson and an anonymous reviewer for helpful comments and criticisms.

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ertson was convinced that pollination was effected exclusively by long-tongued bees probing for nectar because small-bodied, short-tongued bees were excluded from the floral throat by the ornamented staminode. Clements and Long (1923) collected floral foragers on *P. gracilis*, in the Colorado foothills, concluding that Osmia and Hoplitis spp. (Megachilidae) were the most common floral visitors. Straw (1956) insisted that the pseudomasarid wasppollinated Penstemon spectabilis Thurb. was derived from a stabilized hybrid between bee-pollinated P. grinnellii Eastw. and hummingbird-pollinated P. centranthifolius (Benth.) Benth. This has since been discredited by Grant (1994; see also Wolfe & Elisens, 1993). Crosswhite and Crosswhite (1966) studied insect-pollination of P. gracilis and P. pallidus in southern Wisconsin and northern Illinois. Unlike Robertson, they emphasized the role of small, solitary bees, especially Osmia and Hoplitis spp. Crosswhite (1965) provided the only information on compatibility systems in the genus, concluding that P. pallidus was self-compatible. Lawson et al. (1989) analyzed the pollen loads of bees collected on Penstemon haydenii S. Wats. in Nebraska, noting an abundance of Hoplitis and Osmia, but did not comment on which bees regularly contacted the receptive stigmas.

Here we report the results of four years of field studies on six Penstemon taxa in five species: Penstemon cobaea var. cobaea, P. cobaea var. purpureus, P. digitalis, P. grandiflorus, P. pallidus, and P. tubaeflorus. Continued studies of pollination systems of this genus in the American Midwest are needed for two, overlapping reasons. First, further analyses and descriptions of pollination mechanisms in Penstemon will make it possible to map pollinator shifts onto a phylogenetic tree (see Armbruster, 1993; Goldblatt et al., 1995). Second, Penstemon spp. are native to prairies that are reduced and much endangered habitats through the American Midwest. Basic information on pollen dispersal in prairie Penstemon spp. should contribute ultimately to conservation policies and restoration projects.

MATERIALS AND METHODS

The taxonomy of *Penstemon* species follows Yatskievych and Turner (1990). Floral phenologies are based on censuses (as specified below) at each study site for one to four flowering seasons.

POPULATIONS

Penstemon cobaea Nutt. var. cobaea (pollinators collected 24 May-22 June 1994–1997, N = 116). Sixteen populations of this taxon on or near the

Konza Prairie (Kansas) have been studied, most of them small (< 30 blooming stems). The Konza Prairie Research Natural Area is an 8616-acre preserve managed by Kansas State University for the Nature Conservancy. Two large populations (> 500 blooming stems) were studied: (1) in 1995 in White Pasture near the northeastern corner of the Konza in an area considered more floristically rich in tallgrass prairie forbs than much of the rest of the site; and (2) in Wright Prairie, adjacent to the part of Konza known as the Texas Hog Pasture, for which access was provided by Valerie Wright.

Penstemon cobaea var. purpureus Pennell (28 May-4 June 1995-1996, N = 15). Three populations of approximately 100 flowering shoots each were monitored in roadside glades in Christian, Ozark, and Taney Cos., Missouri. All three sites are listed on the Missouri Department of Conservation Rare Plant Inventory.

Penstemon digitalis Nutt. ex Sims (30 May-5 July 1994–1996, N = 325). This species was studied on four sites, two tallgrass prairie restorations in eastern Missouri and two tallgrass prairie relicts in western Missouri: (1) Litzsinger Road Ecology Center of the Missouri Botanical Garden, St. Louis Co., Missouri. This wet mesic prairie restoration was seeded in 1989 and still contains a preponderance of weedy, early successional prairie species. (2) Shaw Arboretum Experimental Prairie of the Missouri Botanical Garden, Franklin Co., Missouri. This site is about 25 years old and is more diverse than Litzsinger, but not so diverse as the natural communities. (3) Paint Brush Prairie, Pettis Co., Missouri. The colony studied is in a relatively impoverished part of the site, but is adjacent to floristically rich prairie. There are notable differences, discussed below, between the floral foragers here as compared to the restorations. (4) Hi-Lonesome Prairie, Benton Co., Missouri. The study area here is a *Penstemon* monoculture embedded in common lenticular sedges (Carex spp.).

Penstemon grandiflorus Nutt. (21–30 May 1996– 1997, N = 44). This species was studied in a population of about 50 blooming plants on and near an ungrazed prairie haymeadow on the Poole Ranch in Geary Co., Kansas, about 2.8 miles south of Interstate 70 on Kansas Hwy. 177. This species is extremely rare on the Konza Prairie proper.

Penstemon pallidus Small (15–24 May 1996– 1997, N = 71). Two sites were used for this taxon in both 1996 and 1997: (1) Fults Hill Prairie, Monroe Co., Illinois. This area is perched atop the Mississippi River bluffs and is considered the best surviving undisturbed loess hill prairie along the Illinois bluffs. (2) Paint Brush Prairie, Pettis Co., Table 1. Pollen loads of foragers collected on Penstemon spp.

	Penstemon pollen only	Penstemon + other species	Other species only	No pollen	Total
	Large Gul	let-Corolla Sp	ecies		
Penstemon cobaea var. cobaea	U				
Bees					
Augochlorella striata (Provancher) F	2	0	0	0	2
Bombus nevadensis auricomus (Robt.) O	4	1	0	0	5
Bombus nevadensis auricomus W	2	0	0	0	2
Bombus pennsylvanicus (DeGeer) Q	19	8	2	1	30
Bombus pennsylvanicus W	1	0	0	0	1
Ceratina strenua (Smith) F	0	1	0	0	1
Halictus ligatus Say F	1	0	0	0	1
Hoplitis pilosifrons (Cresson) F	21	21	3	5	50
Hoplitis pilosifrons M	0	0	0	1	1
Lasioglossum (= Dialictus) spp. F	6	2	0	1	9
Synhalonia hamata (Bradley) F	1	0	0	0	1
Others					
Bombylius sp.	4	0	0	3	7
Euphoria sepulchralis (Fab.)	0	2	0	4	6
					$\Sigma = 116$
Penstemon cobaea					
var. purpureus					
Bees					
Bombus pennsylvanicus ()	0	0	0	1	1
Hoplitis pilosifrons F	ĩ	2	0	î	4
Lasioglossum imitatum (Smith) F	î	0	0	0	1
Osmia distincta (Cresson) F	1	1	0	0	2
Synhalonia rosae Robertson F	1	0	0	0	1
Xylocopa virginica L. F	0	1	1	1	3
Others					
Bombylius sp	0	0	0	1	1
Pseudomasaris sp. F	1	0	0	î	2
r seutomusurus sp. r		Ū	0		$\Sigma = 15$
Ponsteman grandiflarus					
Page					
	1	0	0	0	,
Augochlorella persimilis (Viereck) F	1	0	0	0	1
Augochiorella striata F	11	2	1	4	10
Bombus griseocouis (DeGeer) Q	5	1	0	0	1
Honlitis pilosifrons E	3	0	0	0	3
Lasioglossum (= Dialictus) spn F	6	7	0	1	14
Megachile brevis Say M	1	0	0	0	1
Xylocopa virginica M	1	0	0	0	î
Aytocopa buginad M		0	0	U	$\Sigma = 44$
	Small Cu	llet-Corolla Su	ecies		
Penstemon digitalis	Sman Ou	net-Corona Sp	ecles		
Bees					
Anthophora terminalis					
Cresson F	6	0	0	1	7
Augochlorella striata F	3	1	0	3	7
Bombus bimaculatus Cresson O	2	0	1	2	5
Bombus bimaculatus W	24	8	6	3	41
Bombus bimaculatus M	5	0	0	0	5
Bombus fraternus (Smith) W	8	1	1	0	10

Table 1. Continued.

	Penstemon pollen only	Penstemon + other species	Other species only	No pollen	Total
Bombus griseocollis Q	0	1	1	2	4
Bombus griseocollis W	3	5	0	1	9
Bombus impatiens Cresson W	9	1	1	0	11
Bombus nevadensis Q	28	2	6	9	45
Bombus nevadensis W	5	5	4	2	16
Bombus pennsylvanicus Q	3	3	1	2	9
Bombus pennsylvanicus W	7	6	1	2	16
Ceratina spp. F	7	5	0	5	17
Hoplitis pilosifrons F	6	2	0	1	9
Hylaeus spp. F	2	0	0	2	4
Lasioglossum (= Dialictus) spp. F	36	5	3	12	56
Osmia spp. F	11	0	0	1	12
Synhalonia hamata F	22	13	2	2	39
Synhalonia hamata M	1	0	ō	ō	1
Others					-
		0	0	0	,
Pseudomasaris sp. F	1	0	0	0	1
Pterourus troitus troitus L. F	1	0	0	0	$\Sigma = 325$
D					2 - 325
Penstemon patitaus					
Bees					
Apis mellifera L. W	1	0	0	0	1
Augochlorella striata F	0	0	1	1	2
Bombus bimaculatus Q	2	0	0	1	3
Bombus bimaculatus W	1	1	0	0	2
Bombus nevadensis auricomus Q	0	0	0	2	2
Bombus pennsylvanicus Q	0	0	0	2	2
Ceratina spp. F	2	0	0	5	7
Hoplitis pilosifrons F	13	1	0	6	20
Hoplitis producta (Cresson) F	1	0	0	0	1
Lasioglossum (= Dialictus) sp. F	1	1	0	0	2
Osmia spp. F	15	3	0	6	24
Synhalonia rosae M	3	0	0	2	5
					$\Sigma = 71$
	Tubular	r-Corolla speci	ies		
Penstemon tubaeflorus					
Bees					
Anthophora abrupta Say F	0	0	0	2	2
Anthophora abrupta M	1	0	0	2	3
Anthophora ursina Cresson F	1	0	0	0	1
Augochlorella striata F	0	0	0	1	1
Bombus pennsylvanicus O	3	0	0	0	3
Osmia distincta F	2	0	0	0	2
Others					
Pterourus troilus troilus F	1	0	0	2	2
Pterourus troilus troilus r		0	0	1	0 1
r terourus trottus trottus M	U	0	0	1	5 - 16
					2 - 10 55 - 507
					22 = 301

F = Female, M = male, Q = queen, W = worker.

Missouri. This Osage Plains prairie is particularly rich in late spring forbs which co-flower with penstemons.

Penstemon tubaeflorus Nutt. (4–5 June 1996, N = 17). This species was studied at Long Bald, a dolomite glade in Caney Mountain Conservation Area, Ozark Co., Missouri. This is a large and spectacular glade surrounded by dry oak woods, and it contains a wealth of wildflowers, including the Ozark endemic Echinacea paradoxa (Norton) Britton.

Taken together, the study areas of this project represent a diversity of *Penstemon* habitats across the tallgrass prairie biome. Although we have pooled the floral forager lists in this paper (Table 1) by *Penstemon* taxa to conserve space, we emphasize the value of repeated samples of the same study areas over several years in evaluating the fidelity of floral foragers to specific penstemons.

The sites themselves are, for the most part, wellknown nature preserves in Illinois, Kansas, and Missouri, for which there is a large literature available (e.g., Evers, 1955; Freeman & Hulbert, 1985; Ochs, 1993; Toney, undated; Solecki et al., 1986). These references contain much information on site history, location, ecology, and floristic composition.

Floral fragrance. Whole flowers of each species were placed in clean, glass, stoppered vials for periods up to two hours, following Buchmann et al. (1978). At the end of the two-hour period, the vials were uncorked and smelled.

Bagging experiments. To determine the potential role of self-compatibility and mechanical selfpollination (autogamy) in the absence of insect visitation, inflorescences were bagged during the flowering season. Nylon stockings or wood and mesh exclusion cages were placed over ten flowering shoots, in bud, of *P. cobaea* var. *cobaea*, twelve of *P. cobaea* var. *purpureus*, six of *P. digitalis* and three of *P. grandiflorus* for the length of individual flowering periods. Infructescences were examined for the production of fruit and seed within two months following the withering of the last flower on the shoot.

Pollinator analyses. Field observations recorded foraging behavior of insects on, and within, *Penstemon* flowers. This included noting when foragers collected nectar and/or pollen and whether insects contacted anthers and stigmas while foraging.

The collection and analyses of floral foragers on *Penstemon* flowers represent the harvest of 35–50 insects/site each year. As these *Penstemon* populations represent protected species on nature pre-

serves, overcollection of prospective pollinators may lower seedset. Otherwise, the protocol for netting, killing, and processing specimens followed Bernhardt (1990a, b) and Bernhardt and Weston (1996). Floral foragers were collected on penstemon blossoms and killed in jars poisoned with ethyl acetate.

To verify the presence of *Penstemon* pollen, each euthanized insect was placed on a separate, clean glass slide and bathed in a few drops of absolute ethanol. At this time additional masses of pollen packed onto scopae and corbiculae were teased or scraped off with a dissecting needle. When the ethanol had evaporated, the pollen film left on the glass slide was stained with two to three drops of Calberla's fluid (Ogden et al., 1974). Because insects were sacrificed in a communal killing jar, there is the danger of pollen contamination as bodies of different insects contact each other (see Bernhardt & Weston, 1996). Therefore, we excluded pollen counts of less than 50 grains on insects since the vast majority of floral foragers were extremely hairy and liable to pick up loose grains due to static electricity. Pollen loads were counted on each slide as follows: a total of 200 pollen grains were counted for each insect in increments of five. If there were more than 150 grains of Penstemon pollen in the count (> 75%), the load was classified as a "Penstemon Pollen Only Load." If more than 150 grains of non-penstemon pollen was present the load was called an "Other Species Only Load." If more than 50 grains of both Penstemon and at least one other non-Penstemon species were present, this was called a "Penstemon + Other Species Load." If less than 200 grains were present on the slide, the load was scored as a "No Pollen Load."

Insect length was recorded by measuring six pinned specimens of each species, gender, and/or caste. The body length of each specimen was measured from the labrum to the apex of the abdomen (Bernhardt & Weston, 1996).

Insect specimens were identified by C. D. Michener, R. Brooks, and colleagues at Snow Entomological Museum, University of Kansas, and by M. Arduser of the Missouri Department of Conservation. Vouchers are deposited at the Snow Museum. Bee genera follow Michener et al. (1994).

RESULTS

Floral phenology. Midwestern Penstemon spp. are found in bloom from early May until early July (Fig. 1). Flowering seasons overlap broadly between species. Penstemon pallidus is the first species to bloom in spring, while the two varieties of *P. cobaea* Volume 85, Number 1 1998 Clinebell & Bernhardt Pollination Ecology of *Penstemon*



Figure 1. Floral phenology of Penstemon spp. (1994-1997).

are usually the last to begin flowering. *Penstemon digitalis* has the longest flowering period.

Bagging experiments. In both varieties of *P. co-baea*, no fruits were produced in the absence of visiting insects. In *P. grandiflorus* approximately 50% of the flowers produced full-sized capsules to-tally devoid of seed (parthenocarpy). In *P. digitalis*, the vast majority of flowers produced seed-containing capsules.

Floral presentation. All Penstemon spp. are protandrous with the stigma located within 2 mm of the two pairs of fertile anthers. All Penstemon spp. present their flowers horizontally with respect to the peduncle.

Flowers of all *Penstemon* spp. produce a barely discernible fragrance reminiscent of ripe canteloupe. *Penstemon tubaeflorus* is the only species studied with flowers that are pure, translucentshiny white to the human eye. Flowers of *P. grandiflorus* appear pink-mauve with faintly discernible nectar guides. *Penstemon digitalis* and *P. pallidus* have white corollas with the floral throat streaked with narrow, light purple lines. *Penstemon cobaea* var. *cobaea* has pink or white corollas, while corollas of variety purpureus are deep purple (although we have records of red or white morphs in Taney Co., Missouri). Corollas of both varieties of *P. cobaea* have deep purple blotches and/or broad lines on the throat.

The corollas of *P. tubaeflorus* (sinus 4–6 mm wide) have the most narrow floral tubes. The corollas of both varieties of *P. cobaea* (sinus 18–25 mm wide) and *P. grandiflorus* (15–18 mm wide) form large, bilabiate gullets. In contrast, the corol-

las of *P. digitalis* (sinus 8–12 mm) and *P. pallidus* (4–7 mm) form small gullets.

Floral foragers. Approximately 600 insects were caught foraging on *Penstemon* flowers. *Penstemon tubaeflorus* was the only species that was visited consistently by the butterfly *Pterourus troilus*, but captures do not reflect the density of field observations (Table 1). The head and proboscis of this butterfly contacts stigmas and dehisced anthers while it probes for nectar, with pollen loads deposited on the proximal portion of the proboscis (Table 2). Bees greater than 10 mm in length cling to the petal lobes while probing for nectar, acquiring dorsal depositions of pollen on their heads (Tables 1, 2).

In all remaining species pollination appeared to be dominated by Hymenoptera, with both varieties of *P. cobaea* also visited by beeflies (*Bombylius* spp.) that carried *Penstemon* pollen while contacting the stigmas (Table 2). Bumblebee flower-beetles (*Euphoria sepulchralis*, Scarabaeideae) collected on *P. cobaea* var. *cobaea* carried loads of *Penstemon* pollen, but they consumed basal floral organs and we were unable to determine whether they contacted stigmas (Table 2).

Penstemon digitalis and P. cobaea var. purpureus were visited by females of Pseudomasaris occidentalis (Vespidae). These wasps contacted the stigmas while swallowing pollen and/or foraging for nectar at the base of the corolla tubes, so pollen deposition was both ventral and dorsal.

The foraging behavior of bees was determined by physical size. Bees greater than 10 mm in length (Anthophora, Bombus, Megachile, and Synhalonia

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Floral forager	N = Number caught	$\overline{X} = \text{Length of} \\ \text{body* (mm)}$	Observed foraging for pollen	Observed foraging for nectar	Contacted anthers and stigma while foraging
Coleoptera					
Euphoria sepulchralis	6	12.5	_	+	_
Dintera					
Bombylius opp	0	12.0			
Bomoyitus spp.	0	12.0	_	+	+
Hymenoptera Bees					
Anthophora spp. F	9	12.1	_	+	+
Anthophora spp. M	3	13.3	_	+	+
Apis mellifera W	1	13.0	_	+	+
Augochlorella spp. F	31	7.2	+	_	+
Bombus gueons	119				1
B Line and the	110	10.5		Ŧ	Ŧ
B. bimaculatus B. mineses Ilis	8	18.5			
B. griseocouis B. payadansis	59	22.8			
B. pennsylvanicus	53	27.0			
Bombus work on	09	21.0			
Domous workers	90	15.0	_	+	+
B. bimaculatus	43	15.3			
B. fraternus B. minere Ilia	10	10.5			
B. griseocollis	9	19.5			
B. impatiens B. povadancia	0	12.0			
B. nevadensis B. pennsylvanicus	16	22 5			
Bambua malar	10	22.0			
Domous males	-				
B. bimaculatus	5		_	+	+
Ceratina spp. F	25	6.1	+	_	+
Hanlitia pilosifrone F	1	8.5	+	—	+
Hoplitis pilosifrons M	00	1.1	+	-	+
Hoplitis producta E	1	0.0	5	2	:
Hylaeus spo. F	1	6.5 5.0	+	_	+
Lasioglossum	4	3.0	Ŧ		Ŧ
(= Dialictus) spp. F	82	6.0	+	_	+
Megachile brevis M	1	11.0	_	+	+
Osmia spp. F	40	8.4	+	_	+
Synhalonia hamata F	40	15.3	_	+	+
Synhalonia hamata M	1	12.5	_	+	+
Synhalonia rosae F	1	14.2	-	+	+
Synhalonia rosae M	5	13.7	-	+	+
Xylocopa virginica F	4	21.4	?	?	?
Xylocopa virginica M	1	22.5	?	?	?
Wasps					
Pseudomasaris sp. F	3	19.0	+	+	+
Lepidoptera					
Pterourus troilus troilus F	4	22.5	_	+	+12
Pterourus troilus troilus M	1	22.0	-	+	+/?

Table 2. Size and behavior of floral foragers on Penstemon spp.

* Measurement refers to body length measured from the clypeus (excluding the length of the proboscis) to the tip of the abdomen.

F = female, M = male, Q = queen, W = worker.

spp.) entered the floral tube and probed for nectar, receiving dorsal depositions of pollen from dehisced anthers while contacting the stigmas (Table 2). These bees did not collect *Penstemon* pollen by scraping anthers with their legs. Although Xylocopa virginica was over 10 mm in length, bees of this species mostly obtained nectar from Penstemon flowers by piercing the base of the corolla tube without entering the floral throat. Observations of queen and worker Bombus spp. showed that these large bees typically foraged first on the lowest open flower on an inflorescence, and then climbed up the inflorescence following the spiral of open corollas and visiting flowers in both phases of protandry. All the large bee species were observed visiting the flowering shoots of several genets in succession.

In contrast, bees less than 10 mm in length (Augochlorella, Ceratina, Hoplitis, Hylaeus, Lasioglossum, and Osmia spp.) were not observed to probe for nectar. These bees were observed clinging upside-down and collecting pollen from dehiscent anthers. Penstemon pollen was deposited ventrally on these insects, and bees contacted stigmas while foraging for pollen. These small bees did not appear to discriminate between flowers in the young (dehiscent) versus old (empty) anther phases of protandry in Penstemon flowers based on corolla features. These bees were observed to enter the floral tube, cling to the old, empty anthers and contact the stigmas for a few seconds, but then they exited the flowers without attempting to scrape old anthers. These small bees were observed visiting several flowering shoots in succession before leaving the site.

Approximately 57% of all bees captured on Penstemon spp. carried pure loads of Penstemon pollen in their corbiculae or scopae (Table 1). An additional 20% carried Penstemon pollen mixed with the pollen of other co-flowering species. Mixed pollen loads included both nectar-producing (Baptisia spp., Delphinium spp., Onosmodium sp., Pycnanthemum sp., Rubus sp., and Teucrium sp.) and nectarless (Rosa spp., Schrankia nuttallii) species. Pollinaria of several Asclepias spp. were found attached exclusively to the first pair of legs.

Some queen bumblebees appeared to restrict their foraging to selected *Penstemon* spp. *Bombus pennsylvanicus* was most abundant on the large-gullet species: *P. cobaea* and *P. grandiflorus. Bombus nevadensis* was found primarily on the small, gulletbell-shaped corollas: *P. digitalis* and *P. pallidus.* No morphometric difference could be found between *B. pennsylvanicus* and *B. nevadensis* queens. Bumblebees were seldom observed or collected in flowers of populations of *P. cobaea* var. *cobaea* that consisted of < 50 flowering shoots (Konza Prairie sites 1995 and 1997).

The density of bumblebees belonging to the neuter, worker caste was highly skewed in the data set. Ninety percent of all workers of six *Bombus* spp. (Table 1) were restricted to the flowers of *P. digitalis* at the Litzsinger Road Ecology Center, a restored site. The remaining 10% were distributed among all remaining study sites for all *Penstemon* spp. Although the flowering period of *P. digitalis* at the Litzsinger Road site overlapped broadly with *P. digitalis* at the two remaining sites, the presence of worker-caste bumblebees on virgin prairies and on old restored prairies was only a fraction of the total catch of workers of *Bombus* species at a five-year restoration site.

The density and diversity of bees less than 10 mm long differed among the bee-pollinated Penstemon spp. (Table 1). Over 50% of all bees collected on P. cobaea var. cobaea were Hoplitis pilosifrons (Megachilidae). In contrast, approximately 45% of bees on P. grandiflorus were Augochlorella striata (Halictidae). Most of the small-bodied bees collected on P. digitalis were Lasioglossum spp. (Halictidae). Osmia spp. (Megachilidae) and Hoplitis pilosifrons were equally represented on P. pallidus. Note that most of these bee taxa were collected in smaller proportions on Penstemon flowers other than the modal Penstemon species (listed above) at each site (Table 1). These smaller bees were observed to visit more than one open flower on an inflorescence, and to visit several flowering shoots in succession. Small bees were rarely observed and collected on flowers of P. cobaea var. cobaea in populations of > 500 flowering shoots (Konza Prairie sites 1995 and 1997). These results on the foraging preferences of bumblebees versus small, solitary bees on P. cobaea populations were highly significant by chi square (T = 37.33, $P \ll 0.001$). Statistical procedures follow Conover (1980). Remembering that the square root of the chi square test statistic is approximately the absolute value of the test statistic of the normal distribution, we can estimate that there is an extremely low probability, on the order of one occurrence in a million, that these results are due to chance alone (Stanley Sawyer, pers. comm.). We also emphasize that we do not have a high enough level of sample replication in our data set to use parametric statistics, which would distinguish species effects from site effects, and acknowledge that we thus cannot make definitive comments here on this issue.

DISCUSSION

Pollen dispersal varies among prairie *Penstemon* species in the American Midwest. The putative trend toward butterfly pollination in *Penstemon tubaeflorus* is reflected by the constriction of the floral tube. Otherwise, there appeared to be few floral characters indicative of classic psychophily in this species (Barth, 1985; Proctor et al., 1996). For example, flowers were not held erect and lacked the characteristic pigmentation associated with "butterfly flowers." This suggests that this pollination system is recent and that this species may be derived from a bee-pollinated ancestor.

In the four remaining *Penstemon* spp., bee visitation was dominant, involving a broad diversity of potential pollinators representing four families of Apoideae. Furthermore, bee pollination in midwestern Penstemon spp. appeared to be a two-tiered system. Pollination by bees in most angiosperm systems is based on either passive or active contact (sensu Bernhardt, 1996) with the anthers. Pollination by passive contact implies that the bee first contacts dehiscent anthers and receptive stigmas while foraging for nectar, or other rewards, toward the base of the flower. Pollination by active contact suggests that the bee contacted the receptive stigma while foraging specifically for pollen. Both modes of pollination occurred in four Penstemon spp., but each pollination mode was performed by two different sets of bees. Large-bodied, long-tongued bees effected passive pollination, while small-bodied bees, with tongues of varying lengths, effected active pollination. Since Penstemon flowers were protandrous, this implies that active pollination required automimicry (sensu Dafni, 1993) to encourage cross-pollination. Small bees foraging for pollen were unable to discriminate initially between fresh, dehisced anthers and old, empty anthers, suggesting that pollen collection in *Penstemon* flowers represents a form of partial reinforcement for smaller bees.

We have found no previous citations of pollination of *Penstemon* spp. by bumblebee flower-beetles (*Euphoria sepulchralis*). These greenish scarabs are not hairy, but became dusted with *Penstemon* pollen in the process of feeding on nectar and floral organs while residing inside the base of the corolla tube for long periods of time. It is reasonable to suggest that they played a minor role in the pollination of *Penstemon* spp. on the Konza Prairie Research Natural Area. Likewise, uncommon pseudomasarid wasps probably contributed to the pollination of *P. digitalis* and *P. cobaea* var. *purpureus*. The literature suggests that pseudomasarids are important pollinators of some *Penstemon* spp. native to the west coast of North America (Straw, 1956).

Three patterns of interactions between *Penste*mon flowers and bees were documented. First, the

foraging specificity of queens of Bombus nevadensis to P. digitalis and P. pallidus versus that of B. pennsylvanicus to P. cobaea and P. grandiflorus provides an interesting dichotomy. The morphometric differences between the two Bombus spp. with respect to glossa length and body parameters are minor, according to taxonomic authorities (Medler, 1962). In both species, the combined length of the glossa and prementum is about 12.5 mm. Based on our collections, it would appear that B. nevadensis preferred *Penstemon* corollas with small gullets, while B. pennsylvanicus preferred corollas with large gullets and much expanded sinuses. (In contrast, both B. griseocollis queens, which rarely visit penstemons, and B. fraternus queens, which did not visit penstemons at all in this study, have a combined glossa plus prementum length of about 10 mm.) However, statistical analyses of the foraging preferences of queens of B. nevadensis and B. pennsylvanicus would require additional data sets from a far greater number of sites. In fact, we acknowledge that the above-mentioned relationship between certain Bombus queens and Penstemon spp. may actually reflect a combination of overlapping phenologies and biogeographies.

Second, bumblebee queens far outnumbered neuter workers on Penstemon flowers. This was to be anticipated considering the well known, annual life-cycle of Bombus spp. in the Northern Hemisphere, since Bombus queens always forage for pollen in spring until they raise a significant retinue of workers (Heinrich, 1979). What was not expected was the relative abundance of workers on restoration sites (N = 99) versus the relative absence of workers (N = 7) on both original, tallgrass prairies and true glade sites. We were unable to determine whether Bombus workers simply avoided Penstemon populations in "virgin" prairies and glades or whether *Bombus* colonies matured more rapidly within, or adjacent to, restored sites. Whatever the case, this pattern, if continuous, may have direct implications for future conservationists who emphasize the restoration of original pollination systems.

Third, collections of small-bodied, solitary bees indicated selective foraging preferences that correlated negatively with the size of *Penstemon* populations. This occurred exclusively in *P. cobaea* var. *cobaea* within sites on the Konza Prairie Research Natural Area. Over four seasons, 59 solitary bees were collected on populations with less than 50 flowering stems, but only four specimens were ever collected on populations with more than 500 flowering stems. These massively flowering populations of *P. cobaea* appeared to be pollinated primarily by *Bombus* queens. Why did solitary bees avoid the

greater resources offered by a much higher density of *Penstemon* flowers? Our only explanation is that Bombus queens outcompeted solitary bees for access to Penstemon corollas in larger populations, even though they did not compete for the same resource. Bernhardt (1993) mentioned older reports that female xylocopines (Anthophoridae) recognized and avoided flowers of Passiflora spp. that had been visited and marked with a glandular secretion by another female of the same species. Perhaps solitary bees recognized and avoided Penstemon flowers that had been visited and marked by Bombus spp. The comparative disinterest that Bombus queens showed to smaller populations of P. cobaea may have reflected both economic and energetic considerations. As bumblebees are strong fliers and trapline foragers (Heinrich, 1979; Bernhardt & Montalvo, 1979) they may have avoided smaller populations of flowering plants because such populations offered weaker visual/olfactory displays during peak flowering periods. While a traplining forager would seem most appropriate for the pollination of smaller, isolated populations, their visitation contradicts bumblebee economics (Heinrich, 1979). Small flowering populations provide inadequate rewards for both the bees and the larvae provisioned by the foraging queens (Bernhardt, 1990b).

Waser et al. (1996) argued that generalized pollination is the rule, not the exception, in most angiosperm species. Floral generalization becomes selectively advantageous when pollinator populations fluctuate, so that pollinator shifts can occur in angiosperm populations.

We do not accept that bee pollination in all midwestern *Penstemon* spp. must also reflect fluctuations in generalized trends favoring either large or small bees. We interpret bee pollination in four *Penstemon* spp. as two specialized syndromes concurrent within the *same* flowers. A coexisting, twotiered syndrome has been selected for pollination by both large-bodied, long-tongued, nectar-foraging bees and small-bodied, pollen foragers. Note that all *Bombus* spp. and most of the solitary bees (excluding *Osmia* s. str.) collected in this study are polylectic taxa in grasslands (Bernhardt, 1990a, b). Note also that the same bee species may adopt either active or passive pollen collection on different, coblooming flowers (Bernhardt, 1996).

With two bee-pollination syndromes operating in the same *Penstemon* flower, either syndrome can encourage outcrossing, regardless of demographics, in any *Penstemon* population. It seems naive to presume that dichotomous modes of pollination in the same flower usually represent shifts in generalized

patterns. For example, the older literature has long suggested that a two-tiered system has existed in some species pollinated by both birds and bees. Both birds and bees contribute to pollination in one species because two different modes of attractants and rewards overlap within the same flower (Grant & Grant, 1968; Breedlove, 1969; Macior, 1975; Grant, 1976; Schemske, 1978). If bees and homeothermic vertebrates can pollinate the same flower, we should also be able to postulate two different groups of bees pollinating the same flower for different rewards. Frankly, a generalized mode of pollination must always be implied when field studies fail to incorporate basic analyses of pollen loads and observations of foragers contacting receptive stigmas.

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