Breeding systems and pollination ecology of *Uvularia grandiflora* (Colchicaceae)[1]

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Abstract. Hand-pollination experiments followed by epifluorescence microscopy of pistils of *Uvularia grandiflora* Smith (Colchicaceae) indicated a trend toward late-acting self-incompatibility. Pollen tube growth in pistil tissue of bagged but unmanipulated flowers (mechanical self-pollination) was insignificant. As each pistil produces three stigmatic lobes, insects deposited pollen on an average of more than two in open-pollinated flowers. Medium-sized male and female bees were dominant pollen vectors representing four genera (*Andrena*, *Lasioglossum*, *Nomada*, and *Osmia*) within four families (Andrenidae, Halictidae, Apidae, and Megachilidae). Male bees comprised greater than half (59%) of the bees collected. Pollen load analysis indicates that the majority of female bees collected on *U. grandiflora* carried the pollen grains of the host flower's pollen mixed with grains of one or more coblooming species (polylectic foraging). The majority of nectar-drinking male bees also carried pollen loads that included grains of at least one coblooming species. We recorded pollen morphotypes of 12 coblooming vernal species on bees carrying mixed loads. Gynes of *Bombus* species were infrequent visitors. We report the rediscovery of females of *Andrena uvulariae* and the first collection of males of *A. uvulariae* from *U. grandiflora*.

Key words: Bees, pistils, pollen tubes, pollination, self-incompatibility

Utech and Kawano (2002) recognized five species in the genus *Uvularia* all confined to the eastern temperate USA and southeastern Canada. Known commonly as bellworts, straw lilies, or merrybells, all five are conservative signature species in the vernal perennial flora of open woodlands and deciduous forests. Some species form dense colonies based on subterranean stolons (Whigham 1974, Wijesinghe and Whigham 1997, 2001). In such species, ramet production dominates over genet production, especially when colonies are established under dense canopies. Seed production is selectively advantageous when *Uvularia perfoliata* L. is exposed to forest disturbance and plants grow in canopy gaps (Kudoh *et al.* 1999).

Wilbur (1963) revised the genus and discredited previous reports of interspecific hybridization. In addition, he reviewed the presence of nectar glands on tepals of all five *Uvularia* species. The same author described the presence of a shallow nectariferous depression at the base of each tepal.

References to insect-mediated sexual reproduction in *Uvularia* species dates to the 19th century, although it is fragmentary and anecdotal. Robertson (1896) referred to bee visits of *U. perfoliota* and *Uvularia grandiflora* Smith flowering in Madison, WI, citing observations in an unpublished manuscript by Trelease. Whigham (1974) observed wingless staphylinid beetles in the flowers of *U. perfoliata* and concluded that they were the pollinators. McCall and Primack (1987) reported visitations by unidentified beetles, halictid bees, and gynes of a *Bombus* species on *Uvularia sessilifolia*.

McCall and Primack (1987) compared the fecundity of *U. sessilifolia* L. with two other woodland perennial herbs. Their hand-pollination experiments on *U. sessilifolia* showed that the species was self-incompatible but often parthenocarpic (producing seeds in the absence of sperm) when allowed to self-pollinate mechanically (autogamy) or self-pollinated by hand. They found

"unfilled" seeds in the capsule but did not determine whether they were the production of embryonic lethals or merely collapsed, unfertilized ovules.

Motten (1986) had similar results for the breeding system of *U. sessilifolia*. In this study, flowers cross-pollinated by hand showed an 83% fruit set with fruit containing greater than four seeds/fruit compared with 8% in flowers self-pollinated by hand, with each capsule producing one seed/fruit. However, Motten (1986) also found that the primary insect visitors were bees in the family Andrenidae. Pollen load analyses on 15 of the 18 andrenids collected showed that 14 bees carried the pollen of *U. sessilifolia* mixed with coblooming species.

The yellow flowers of *U. grandiflora* bloom midspring and produce both nectar and pollen. The geographic distribution of *U. grandiflora* in North America ranges from the east coast of Canada and the USA as far west as the Dakotas and as far south as Alabama (Utech and Kawano 2002). Robertson (1928) reported a flowering period for this species from April 12 to May 11 in Carlinville, IL. Seeds are dispersed from mid-July to early August (Seibert and Savidge 1991) by four different species of ants (Whigham 2004).

Early collections of floral foragers of *U. grandiflora* include Robertson (1896) and Graenicher (1906). Working in the Carlinville, IL area, Robertson (1896) collected females of three *Bombus* species and a male and female of two *Andrena* species respectively. Graenicher (1906) suggested that this species was pollinated in southern Illinois primarily by *Bombus pensylvanicus* (De Geer) (as *americanorum* Fabricius) but believed that smaller bees in the genera *Osmia* (Megachilidae), *Andrena* (Andrenidae), and unidentified members in the Halictidae were also effective pollen vectors. These observations were refined and much improved by Seibert and Savidge (1991), also working in Illinois. They found that the dominant pollinators of this herb were gynes (*n* = 23) and two workers of *Bombus griseocollis* De Geer and were the first to conduct hand pollination *in situ*. They found that *U. grandiflora* was self-incompatible.

There are two reasons for extending fieldwork and breeding experiments on *U. grandiflora*. First, we need to determine whether self-incompatibility in this species is early or late acting. In fact, recent evidence shows that prezygotic self-incompatibility in insect-pollinated, petaloid monocots is no longer restricted to late-acting incompatibility (Ren *et al*. 2019 and see review in Vance *et al*. 2004) and may represent an important apomorphy to map onto future phylogenetic trees. Second, evidence suggests that visiting insect diversity for woodland species often changes with years and sites (see reviews in Bernhardt and Edens-Meier 2010; Edens-Meier *et al*. 2011a; Bernhardt *et al*. 2013, 2014). Therefore, we ask which insects carry the pollen of *U. grandiflora*?

Materials and Methods. STUDY SITES. Two populations of *U. grandiflora* were studied in 2012 (April 1 to April 7) and 2013 (April 13 to May 7) at Cuivre River State Park, Lincoln County, MO. We had to repeat the open and bagged control groups in 2018 (April 17 to April 20) because our original number of collected specimens was inadequate for analyses. These two populations were used as a part of an ongoing field study on vernal pollinator networks at Cuivre River State Park (see Bernhardt *et al*. 2016, Edens-Meier *et al*. 2018, and Ren *et al*. 2019). To test our hypothesis that flowering displays are influenced by the environment, we compared plants growing on a WSW-facing slope in a clumped formation with one of a more random distribution growing on a NNW-facing slope.

We logged 62 hr at the field sites. In 2012, data were collected in early April from 10:00 am to 1:00 pm. In 2013, data were collected from mid-April to early May between 10:00 am and 12:00 pm.

Although *U. grandiflora* is not considered rare in Missouri, plants are usually found sparsely distributed throughout woodlands. The populations were 3.62 km apart and located in dry-mesic loess/glacial till woodlands. As members of this genus are known to be stoloniferous, we counted and measured flowering stems. The Turkey Hollow Trail (THT) population grew on a WSW-facing slope (39.0170N, 90.9252W) and produced approximately 62 flowering stems, some occurring in massive, presumably vegetative clumps (Fig. 1). The Big Sugar Creek Trail (BSC) population grew on a NNW-facing slope (39.0492N, 90.9317W) and produced approximately 25 flowering stems annually (Fig. 2). Most flowering stems at this site were individually dispersed along the slope. A pressed specimen of *U. grandiflora* (voucher # 6452255) and coblooming plants from these sites

FIG. 1.   Turkey Hollow Trail field site at Cuivre River State Park. Note *Uvularia grandiflora* (yellow flowers) clumped next to fallen log on steep hillside.

FIG. 2.   Big Sugar Creek field site at Cuivre River State Park. *Uvularia grandiflora* are dispersed on hillside.

were deposited in the herbarium of the Missouri Botanical Garden, St. Louis, MO.

MEASUREMENTS. Fifteen stems from each site were measured on April 3, 2012 (10:00 am to 2:30 pm) from the terminus to the level of the humus layer. The length and width of 15 flowers at each site were measured in 2012 (April 3) and 2013 (April 20 THT; April 21 BSC). Length was measured from the base of the floral receptacle to the terminus of the longest tepal. Width was measured at the widest distance from the terminus of one tepal directly opposite on the corolla. The number of flowers was counted on 15 stems in 2012 and 2013 at both sites.

FLORAL ATTRACTANTS AND REWARDS. The tepals of *U. grandiflora* are described as golden yellow (Utech and Kawano 2002), but no ultraviolet (UV) analysis has been described to date to determine the presence of UV reflectance. Ultraviolet photography procedures followed Verhoeven *et al.* (2019). To determine whether flowers were fragrant, we smelled open flowers and also placed individual flowers in clean, capped scent jars. The cap was removed and the contents were smelled as described by Bernhardt *et al.* (2016). We checked the base of tepals to see if they had nectar glands as described previously by Wilbur (1963).

As this is a flower of early spring, we wanted to know if the tepals and the interior floral chamber surrounding the anthers and stigmas were warmer than the ambient air. Tepal temperature protocol and equipment followed Bernhardt *et al.* (2016), whereas recording the temperature of the floral chamber followed Edens-Meier *et al.* (2018).

ANALYSES OF OPEN, INSECT-POLLINATED FLOWERS *VS*. BAGGED (UNMANIPULATED) FLOWERS. Flower buds ($n = 26$ buds on 26 stems) were labeled with jeweler's tags on April 17, 2018. A total of 13 buds was bagged under tulle, whereas 13 buds remained unbagged and open to the environment. Both bagged and open flowers were harvested 3 days later. Each group of flowers was placed in a labeled glass container and fixed in a 3:1 solution of 95% ethanol:glacial acetic acid that was made up *in situ*. Flowers were fixed for 3 hr before storing in 70% ethanol. To prepare each flower for epifluorescence photomicrography, the flower was softened in a 10% solution of sodium sulfite at 45 °C for 10 min. It was then taken through two baths of distilled water (10 min each) before the flower was dissected, and the gynoecium was excised. Each gynoecium was placed on a glass slide. The three styles were teased apart to avoid overlapping; the ovary was butterflied with a single-edged razor blade and bathed in three to four drops of decolorized aniline blue. The softened tissues were spread gently by placing a glass coverslip over the specimen and applying pressure to the coverslip with the tip of a probe. All subsequent protocols, procedures, and storage of specimens followed Edens-Meier *et al.* (2011b). Zeis Axioscope 40 and Zeiss Axioscope Imager M2 were used to examine the specimens. We recorded the number of stigmas bearing pollen grains and tubes, the number of pollen grains on each stigma, the number of pollen tubes in the gynoecium, and the length of the longest penetrating pollen tube in each gynoecium.

BREEDING SYSTEMS. We used only one flower/scape. While flowers were in full bud (tepals not
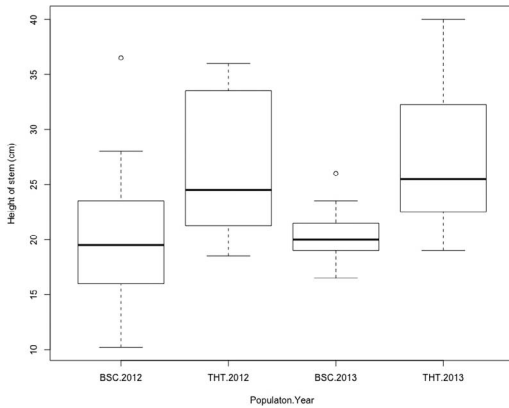
FIG. 3. Comparison of stem height of *Uvularia grandiflora* between two study locations in 2012 and 2013.

open but yellow color visible), we isolated them under tulle. When the tepals separated we divided them into two categories. Hand-mediated self-pollinated flowers ($n = 11$) received pollen from the dehiscent anthers within the same flower. This pollen was applied exclusively to the introrsive sides of the stigmatic lobes (the pollen-receptive sites) with a clean toothpick. Cross-pollinated flowers ($n = 10$) were emasculated and received only pollen from coblooming flowering stems located at least 1 m away. We did not attempt cross-pollinations between flowers belonging to stems within the same clump. Flowers were harvested 24 to 48 hr after each treatment and processed as above.

INSECT OBSERVATIONS AND COLLECTIONS. Insect activity on flowers was observed from 9:00 am to 12:00 pm and on separate occasions observations continued until 5:30 pm over the flowering seasons for 2 consecutive years. To witness foraging inside the flowers, tepals were removed and insect activity was videotaped using a Sony HDR-CX760V digital high-definition video camera recorder.

Insects visiting flowers were caught using individual ziplock bags. Vapors of 95% EtOH were used to euthanize captured specimens (see Edens-Meier *et al.* 2018). The body of each euthanized insect was then placed on a separate glass slide and bathed in one to two drops of either 95% EtOH or ethyl acetate, carefully scraping the body with a pin to dislodge grains in scopae or corbiculae. Once the solvent evaporated, the residue on the slide was stained with Calberla's

fluid, a coverslip was applied, and the slide and pinned insect received coreferenced labels. Pollen load analyses and micrography followed protocols in Bernhardt *et al.* (2013, 2014). Measurement of insects (length, width, and thoracic depth) followed Edens-Meier *et al.* (2011a). When insect specimens could not be identified to species, they were identified to genus. Specimens were stored in the fumigated insect cabinet in the Camilo Billikin Bee Laboratory at St. Louis University pending deposition in the entomological museum at The University of Kansas in Lawrence, KS.

EXPERIMENTAL DESIGN AND ANALYSES. The overall design is a contrast between two populations (THT *vs.* BSC) and 2 yr (2012 and 2013). Therefore, we used a two-way analysis of variance for comparing means for the various flower and plant traits measured. The data were log transformed to meet the assumptions of the test. Given that there are only single populations with distinct environmental parameters, this research design represents a pseudoreplication (Hulbert 1984). To account for this, we treated populations as a random effect (Underwood 1997).

To test the hypothesis that there might be a difference between the two populations, due to differential environmental conditions, in the height at which flowers are presented, we used an analysis of covariance (ANCOVA), with height of the tallest flower as the response variable, the height of the stem as the independent variable, and population as the covariate. For this analysis, we combined the data for both years. All analyses were performed in the R computational environment (v.3.4.1, R Core Team 2017).

Results. FLORAL PHENOLOGY AND MEASUREMENTS. During the hot, dry, early spring of 2012 (see Edens-Meier *et al.* 2017), plants were observed in bloom by March 3 (personal communication, Bruce Schuette). In 2013, plants were observed in bloom from April 11 through May 5. Flowering stems produced one to three flowers at both sites. There was a significant difference in stem height ($F = 23.12$, $P < 0.0001$), with the THT population having larger means both years (Fig. 3). Alternatively, there were no differences between years ($F = 0.35$, $P = 0.556$) or interactions between populations and years ($F = 0.215$, $P = 0.814$).

There were no significant differences between years ($F = 2.07$, $P = 0.156$) or populations ($F = 1.03$, $P = 0.903$) or interactions ($F = 2.36$, $P =$
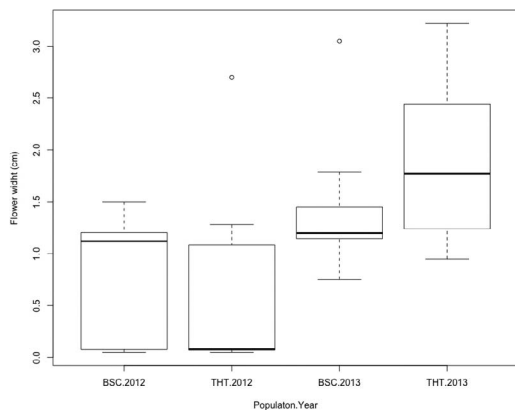
Fig. 4. Comparison of flower width of *Uvularia grandiflora* between two study locations in 2012 and 2013.

Fig. 6. Pistil of bagged *Uvularia grandiflora* showing a few pollen grains on stigmatic surface and a few germinating pollen tubes.

0.130) in flower length. Alternatively, there was a significant year effect ($F = 38.52$, $P < 0.0001$) for flower width, with 2013 flowers being significantly wider than in 2012 (Fig. 4). There was no significant effect for population ($F = 0.40$, $P = 0.812$) or interactions ($F = 2.99$, $P = 0.089$).

As plants in the THT population were taller than the ones in BSC, we addressed the question if this resulted in differential height of floral presentation. The ANCOVA was significant for the independent variable ($F = 314.9$, $P < 0.0001$; Fig. 5) as well as the interaction between site and stem height ($F =$
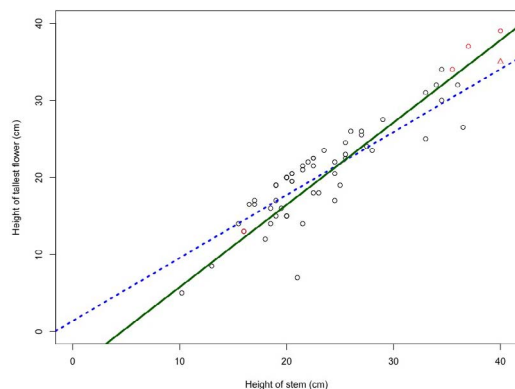

Fig. 5. Regression lines for the highest flower (cm) *vs*. stem height (cm) for two study locations (Turkey Hollow Trail [THT], solid green; Big Sugar Creek [BSC], dashed blue). Red marks represent plants with two flowers, and the triangle represents the single stem with three. Lines had significantly different slopes, with the THT location being steeper than the BSC.
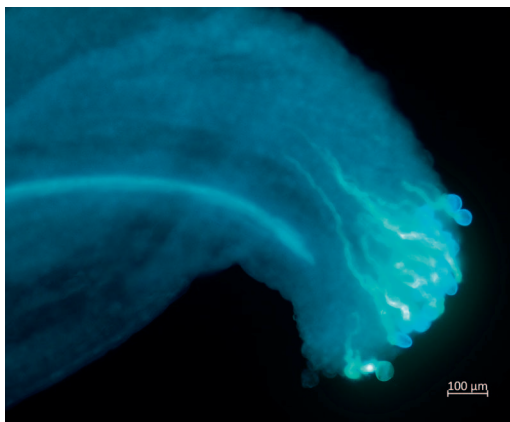
3.921, $F = 0.0358$). The two populations had a floral display at different height increments. Specifically, the slopes of the lines differed (BSC: $b = 0.8169$, $P < 0.0001$, $R = 0.6898$; THT: $b = 1.0681$, $P < 0.0001$, $R = 0.8585$).

FLORAL ATTRACTANTS AND REWARDS. Ultraviolet patterns were not detected. No discernible odor was detected in either fresh flowers or flowers in vials. Nectary pockets were found at the base of the six tepals as described by Wilbur (1963).

No significant difference was found between the interior floral chamber temperature *vs*. ambient air temperature. In addition, there was no significant difference between floral tissue (tepal) temperature and ambient air temperature.

ANALYSES OF BAGGED AND OPEN INSECT-POLLINATED FLOWERS. Only three of the 13 bagged flowers of *U. grandiflora* had pollen on their stigmas (Fig. 6) and the differences were very highly significant ($W = 11$, $P < 0.0001$). There were also significant differences in the total number of pollen grains, with one of three stigmas on the same gynoecium having pollen grains ($W = 11$, $P = 0.0002$). Finally, there were also significant differences in pollen tube numbers, with 5 to 15 pollen grains found on a stigma lobe and 5 to 15 pollen tubes were found penetrating style tissue, but the longest pollen tube only penetrated less than a quarter of the style length. In contrast, 12 of 13 pistils of flowers exposed to insects contained pollen grains on their stigmas ($W = 11$, $P = 0.0001$, Fig. 7). Of the 12 gynoecia bearing pollen, pollen grains were deposited on one to three stigma lobes. The
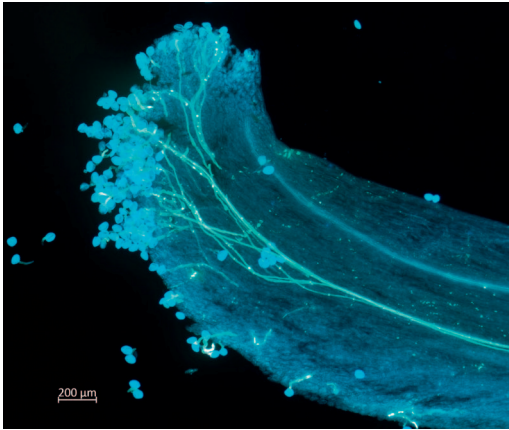
Fig. 7.   Pistil of open *Uvularia grandiflora* clearly demonstrating deposition of pollen grains on stigmatic surface and germination of many pollen tubes.

Fig. 8.   *Andrena carlini* foraging on open flower of *Uvularia grandiflora*.

number of pollen grains deposited on stigma lobes ranged from 4 to 328. The number of pollen tubes penetrating style tissue ranged from 1 to 234. Three gynoecia contained pollen tubes penetrating the ovaries.

Hand-Mediated Pollinations. Eight of the 10 hand-crossed pollinated pistils of *U. grandiflora* showed pollen germination on all three stigmatic lobes, tube penetration of the style, and entrance into the ovary. When 11 pistils were self-pollinated by hand, we also observed germination on all three lobes, with pollen tube penetration to at least half the length of the style. However, only one pistil in this treatment contained one pollen tube entering its ovary. One pistil showed aberrant swollen pollen tubes in the style tissue. There were no statistical differences in the number of pollen grains on the stigma between hand-crossed and hand self-pollinated treatments ($W = 4$, $P = 0.667$), nor in the index of pollen tubes in the style ($W = 5$, $P = 0.437$). Alternatively, there was a significant difference in the index of the number of pollen tubes that reached the ovary, with the hand-crossed-pollination treatment being significantly higher ($W = 9$, $P = 0.015$).

Insect Observations and Collections. Over two seasons, we collected a total of 71 insects (70 bees and one fly, Table 1). Bee foraging was greatest on warm, sunny days. Most bees were observed to land midway on the length of the tepals or toward the apices of the tepals before crawling upward and into the floral chamber and largely disappear-

ing from view (Fig. 8). A few entered the floral chamber by flying directly up the aperture made by the loosely funnel-form perianth. To see what was occurring once a bee entered the floral chamber, we intentionally removed three adjacent tepals to expose the androecium and gynoecium. We subsequently videotaped a male *Andrena* species as it entered the floral chamber and contacted the stigma lobes while it foraged for nectar at the bases of the tepals https://www.youtube.com/watch?v=qiCNsnkss_U.

Four families of bees were represented, of which 59% were males (Table 1). The majority of bees collected belonged to seven *Andrena* species ($n = 49$). However, all specimens of *Andrena uvulariae* were collected only from the Turkey Hollow site (Fig. 9). Nineteen *A. uvulariae* ($n = 11$ females, $n = 8$ males) were collected as these bees foraged or slept (one) in the flowers. The second most commonly collected genus, *Osmia*, was represented by four species ($n = 17$), of which 88% were males. In addition, we observed male bees attempting to deter a female bee (possibly *Osmia bucephala* Cresson) from visiting the flowers as seen in a video made in 2013, https://www.youtube.com/watch?v=LQ9g_wiuaag. This type of agonistic behavior was frequently observed.

Gynes of *Bombus* species were observed infrequently. One gyne was observed but not captured foraging on *U. grandiflora* when the ambient temperature was only 7.22 °C. Only one

Table 1.   Bee and fly species captured while foraging on *Uvularia grandiflora* (2012–13) including sex, number (*N*), length (mm), width (mm), thoracic depth (mm), and standard deviation values (SD) for each measurement. NA = not applicable.

Species	Sex	*N*	Length	SD	Width	SD	Thoracic depth	SD
Andrena carlini	Female	4	12.2	1.4	4.1	0.5	3.5	0.4
Andrena carlini	Male	6	9.9	0.6	3.0	0.3	2.5	0.3
Andrena cressonii	Female	1	9.2	NA	3.1	NA	2.8	NA
Andrena erigeniae	Male	1	7.9	NA	2.4	NA	1.5	NA
Andrena pruni	Female	1	12.0	NA	4.1	NA	2.9	NA
Andrena rugosa	Female	2	10.4	1.8	3.2	0.30	2.8	0.3
Andrena tridens	Female	5	9.9	0.7	3.1	0.2	2.5	0.3
Andrena tridens	Male	11	8.2	0.7	2.3	0.1	1.9	0.2
Andrena uvulariae	Female	11	7.4	0.6	2.3	0.2	1.7	0.3
Andrena uvulariae	Male	7	5.7	0.7	1.9	0.4	1.4	0.2
Bombus bimaculatus	Gyne	1	18.0	NA	8.6	NA	6.9	NA
Lasioglossum cattellae	Female	1	5.4	NA	1.8	NA	1.2	NA
Lasioglossum subviridatum	Female	1	6.2	NA	1.7	NA	1.1	NA
Nomada luteoloides	Male	1	8.3	NA	2.1	NA	1.5	NA
Osmia atriventris	Male	1	8.3	NA	2.6	NA	2.1	NA
Osmia bucephala	Male	5	11.8	0.8	4.4	0.2	3.4	0.4
Osmia lignaria	Male	1	8.9	NA	3.2	NA	2.4	NA
Osmia pumila	Female	2	6.8	1.1	2.7	0.0	2.1	0.2
Osmia pumila	Male	8	6.3	0.7	2.1	0.2	1.6	0.2
Bombylius sp. 1	Unknown	1	8.5	NA	3.8	NA	2.2	NA

Bombus bimaculatus Cresson was captured while foraging (Table 1). On one occasion, a gyne of *Bombus* (near) *impatiens* was captured in a plastic bag at the Turkey Hollow site while it foraged in a flower of *U. grandiflora*. Upon release, she immediately flew to a clump of *U. grandiflora* in flower and visited a total of five flowers before exiting the site.

INSECT MEASUREMENTS. The largest bee captured while foraging on *U. grandiflora* was *B. bimaculatus*, whereas male *A. uvulariae* were the smallest

FIG. 9.   *Andrena uvulariae* (female).

(Table 1). Bee size ranged from 5.22 to 17.97 mm in length (Table 1). Most of the bees (74%) were large (>10 mm), whereas 26% of the bees were of medium size (4–7 mm) as classified by Edens-Meier *et al.* (2018).

POLLEN LOAD ANALYSES. The majority of bees collected on *U. grandiflora* carried the pollen of this species. However, pollen loads of 57 bees showed that only two carried pure loads of the host flower's pollen (Table 2). *Andrena uvulariae* carried the greatest number of pure loads of pollen, but some males and females carried mixed pollen loads (Table 2). All the remaining bees and one fly carried a minimum of at least one other coblooming species mixed with grains of *U. grandiflora*. The bee carrying the greatest number of pollen morphotypes was a male of *O. bucephala*, with seven additional pollen morphotypes in which the pollen of *U. grandiflora* was mixed with *Claytonia virginica* L., *Cercis canadensis* L*., Phlox divaricata* L., *Anemonella thalictroides* (L.) Spach, *Corydalis* sp., an unidentified member of the Asteraceae, and an unidentified tricolpate eudicot grain. In all, bees with mixed pollen loads visited a total of 12 plant species. Ten of the 12 vernal species were known to secrete nectar. We could not determine whether *A. thalictroides* and *Hydrastis canadensis* L. secreted nectar. The most commonly identified

Table 2.   Pollen load analyses of insects collected on *Uvularia grandiflora* in 2012 and 2013 (pooled). f = female; m = male.

Species (gender)	*n*	*Uvularia* only	*Uvularia* + other species	Other species only	No pollen
Diptera					
Bombylius sp. 1 (f)	1	0	1	0	0
Hymenoptera					
Andrena carlini (f)	4	0	1	2	1
Andrena carlini (m)	6	0	4	1	1
Andrena cressonii (f)	1	0	0	0	1
Andrena erigeniae (m)	1	1	0	0	0
Andrena pruni (f)	1	0	1	0	0
Andrena rugosa (f)	1	0	0	1	0
Andrena tridens (f)	5	1	2	1	1
Andrena tridens (m)	7	4	2	0	1
Andrena uvulariae (f)	11	7	4	0	0
Andrena uvulariae (m)	6	3	2	0	1
Bombus bimaculatus (f)	1	0	1	0	0
Lasioglossum catellae (f)	2	0	2	0	0
L. subviridatum (f)	1	0	0	1	0
Nomada luteoloides (m)	1	0	0	1	0
Osmia atriventris (m)	1	1	0	0	0
Osmia bucephala (m)	5	0	3	0	2
Osmia lignaria (m)	1	0	1	0	0
Osmia pumila (f)	1	0	2	0	0
Osmia pumila (m)	1	1	3	0	4

pollen mixed with the pollen of *U. grandiflora* was a combination of *Cercis canadensis* and a *Prunus* species.

Discussion. Floral Phenology and Measurements. Despite differences in floral phenology in 2012 and 2013, plant height remained constant within the two populations. Plants at the THT site were consistently taller both years than at the BSC site. Several possible and potentially overlapping explanations exist. This includes environmental differences, diverging genetic origins, and different temporal demographies. The simplest explanation is that the taller plants at the THT site grew on a WSW-facing slope and received more sunlight than plants growing on the NNW-facing slope at the BSC site, resulting in the production of more photosynthate directed into the production of taller stems. In contrast, floral width appears to parallel phenological changes between 2012 and 2013. Adaptive plasticity in floral display is a common strategy of many animal-pollinated flowers (Harder and Johnson 2005). This is probably driven by the fact that many of the pollinators are insects whose metabolic rate is environmentally determined (Vicens and Bosch 2000). In turn, site-specific phenology and flowering display influence the quality and quantity of seed production (Kudo

2006). What specific mechanism(s) is (are) driving the differential floral display between the two populations in our research remains to be investigated.

Floral Attractants and Rewards. The yellow flowers of *U. grandiflora* offer nectar and pollen as rewards. Yellow is a common visual attractant in vernal insect-pollinated petaloid monocots in North America. We find yellow flowers in *Trillium*, *Erythronium*, *Cypripedium*, *Calochortus*, *Hypoxis*, *etc.* (see Vol. 26 of *Flora of North America*). The lack of temperature differences within and outside the floral chamber is not surprising since the yellow color pattern is uniform. *Cypripedium parviflorum* has an inflated chamber and there is no significant change in temperature during the cool spring weather (Edens-Meier *et al.* 2018). It is more likely that floral temperatures will change when pigmentation patterns are dark, such as those found in *Viola pedata* (Bernhardt *et al.* 2016), which also grows at Cuivre River.

Analyses of Open Insect-Pollinated Flowers and Breeding Systems. Seibert and Savidge (1991) conducted hand-pollination experiments on a population of *U. grandiflora* in Trelease Woods (Urbana, IL). They concluded that this species was "mostly self-incompatible." Our bagging experi-

ments indicate that mechanical self-pollination is minimal but this does not answer the question of what happens if a bee visits more than one flower on the same scape or ramet. Our fluorescence studies suggest that there is a late-acting self-incompatibility system following experimental hand-pollinations. Specifically, pollen tubes produced by self-pollination do enter the style but appear less likely to enter the ovary compared with tubes produced by cross-pollination over the same length of time. This is common in other petaloid monocots (Gibbs 2014).

INSECT OBSERVATIONS AND COLLECTIONS. The majority of bee species collected exiting the flowers of *U. grandiflora* belonged to polylectic lineages associated with the pollination of other vernal herbs in the Midwest (Edens-Meier *et al.* 2011a, b, 2018; Bernhardt *et al.* 2016; Ren *et al.* 2019). The only exception is *Andrena uvulariae*, but some females of this species also foraged on other plant species. Claims that this bee species is an obligate oligolege require more tests of pollen load analyses as mixed loads are found on other specialist species regarded previously as foraging specialists (Bernhardt and Walker 1996).

Mitchell (1960) first described the *A. uvulariae* female. Since then, members of this species have remained elusive. The male of this species has not yet been described. Within the past few years, however, *A. uvulariae* has been found, usually collected by bowl traps, in several locations and habitats in the USA, primarily in and east of the Appalachians (Ascher and Pickering 2017). Specimens have also been discovered in some older collections from the eastern USA, often misdetermined as the very similar *Andrena ziziaeformis* Cockerell (Ascher and Pickering 2017). Notably, the only known locations west of the Appalachians in addition to our Cuivre River sites are several widely separated locations in the Missouri Ozarks (Arduser and A. Harmon-Threatt, unpublished), in central Indiana (R. Jean, personal communication), and in SE Wisconsin (L. Anchor, unpublished), this despite extensive and ongoing collecting efforts in Illinois, Indiana, and Missouri, where *Uvularia* species, particularly *U. grandiflora*, are relatively common. Our current research adds new information on the foraging behavior of the female *A. uvulariae*, and suggests one simple reason why this species has remained so elusive while populations of *Uvularia* species are so broadly distributed throughout northeastern USA. It is

possible that small, foraging bees are concealed within the corolla and remain undetected. Its relative scarcity—if real—west of the Appalachians suggests that *A. uvulariae* may have restrictive or unique microhabitat requirements, particularly since our entire collection of this species originated exclusively from only one, the drier and less mesic, of our two sites.

Male bees, of course, do not actively collect pollen. However, our pollen load analyses showed that the majority of male bees collected must have contacted the anthers passively as they foraged, presumably, for nectar. Most of these males probably take nectar from a range of coblooming species because our pollen load analysis showed that 19 of 30 male bees also carried grains from flowers other than the host species.

Why were 59% of bees collected on *U. grandiflora* males? The most likely explanation is that *U. grandiflora* at our sites blooms at a time of year in which male bees outnumber or are equal to the number of females. However, it is not unusual to find an aggregation of male bees on flowers that attract female bees that collect pollen from a narrow lineage of flowering plants (oligolectic foraging). This is well documented for bee taxa in which the females collect pollen from a narrow and closely related clade of angiosperms. The best-known examples of this include bees foraging on the genus *Cucurbita* (Hurd and Gorton 1971) and in some species in the genus *Conospermum* (Proteaceae; Houston 1989). High concentrations of male bees, though, are usually associated with flowers that are sexual mimics (e.g. *Ophrys*; Vereecken and Francisco 2014) and in some Neotropical orchids offering perfume rewards to *Euglossine* species (Roubik 2014). Our results were quite different because *U. grandiflora* flowers attracted male bees from three families (Andrenidae, Apidae, Megachilidae) representing three unrelated genera. The majority of males foraged on these flowers and some males appeared to be patrolling the flowers and were attacking or harassing bees of other species.

When records of bee collections on *U. grandiflora* are compared over a century, a complex picture of its pollinator guild emerges. In fact, this guild may be very labile over relatively short distances. Collections in Illinois favored large-bodied, eusocial *Bombus* species (Robertson 1896, 1928; Seibert and Savidge 1991) over smaller-bodied solitary species. Graenicher (1906) be-

lieved that in addition to being pollinated by *Bombus* species, smaller bees may also serve as effective pollen vectors. Our collections over two seasons indicate that Graenicher's predictions are correct at certain sites. Specifically, *Andrena* and *Osmia* species, medium- to large-bodied bees, were more common as agents of pollen dispersal than *Bombus* species in our research. Bee diversity, body size, and sex ratios differ from earlier studies. We encourage further studies of this plant species and on other vernal, woodland plants with broad, North American distributions to understand variation in the pollination ecology of endemic species.

Literature Cited

Ascher, J. S. and J. Pickering. 2017. Discover life bee species guide and world checklist (Hymenoptera: Apoidea: Anthophila). Draft 47. http://www.discoverlife.org/mp/20q?guide=Apoideaspecies. Retrieved December 29, 2017

Bernhardt, P. and R. Edens-Meier. 2010. What we think we know vs. what we need to know about orchid pollination and conservation: *Cypripedium* as a model lineage. Botanical Review 76: 204–219.

Bernhardt, P. and K. Walker. 1996. Observations on the foraging preferences of *Leioproctus* (*Filiglossa*) Rayment (Hymenoptera: Colletidae) in eastern Australia. Pan-Pacific Entomologist 72: 130–137.

Bernhardt, P., R. Meier, and N. Vance. 2013. Pollination ecology and floral function of Brown's peony (*Paeonia brownii*) in the Blue Mountains of northeastern Oregon. Journal of Pollination Ecology 1: 9–20.

Bernhardt, P., R. Edens-Meier, E. Westhus, and N. Vance. 2014. Bee-mediated pollen transfer in two populations of *Cypripedium montanum* Douglas ex Lindley. Journal of Pollination Ecology 13: 188–202.

Bernhardt, P., R. Edens-Meier, D. Jocson, J. Zweck, Z.-X. Ren, G. Camilo, and M. Arduser. 2016. Comparative floral ecology of bicolour and concolour morphs of *Viola pedata* L. (Violaceae) following controlled burns. Journal of Pollination Ecology 19: 57–70.

Edens-Meier, R., M. Arduser, G. Camilo, and P. Bernhardt. 2018. Comparative pollination ecology between two populations and two varieties of *Cypripedium parviflorum* (Orchidaceae) in Missouri, United States of America—Does size matter? Botanical Journal of the Linnean Society 186: 544–559.

Edens-Meier, R., M. Arduser, E. Westhus, and P. Bernhardt. 2011a. Pollination ecology of *Cypripedium reginae* Walter (Orchidaceae): Size matters. Telopea 13: 327–340.

Edens-Meier, R., M. Joseph, M. Arduser, E. Westhus, and P. Bernhardt, P. 2011b. The pollination biology of an annual endemic herb, *Physaria filiformis* (Brassicaceae) in the Missouri Ozarks following controlled burns. Journal of the Torrey Botanical Society 138: 287–297.

Edens-Meier, R.M., N. Vance, Y. B. Luo, L. Peng, E. Westhus, and P. Bernhardt. 2010. Pollen–pistil interactions in North American and Chinese *Cypripedium* L. (Orchidaceae). International Journal of Plant Sciences 17: 370–381.

Edens-Meier, R.., G. Brown, J. Zweck, M. Arduser, J. Edens, T. Dickson, H. Nonnenmacher, E. Westhus, and P. Bernhardt. 2017. Reproductive ecology of *Asclepias meadii* Torr. (Apocynaceae), a federally threatened species. Journal of the Torrey Botanical Society 144: 218–229.

Gibbs, P. E. 2014. Late-acting self-incompatibility: The pariah breeding system in flowering plants. New Phytologist 203: 717–734.

Graenicher, S. 1906. Some notes on the pollination of flowers. Bulletin of the Wisconsin Natural History Society 4–6: 12–21.

Harder, L. D. and S. D. Johnson. 2005. Adaptive plasticity of floral display size in animal-pollinated plants. Proceedings of the Royal Society B: Biological Sciences 272: 2651–2657.

Houston, T. F. 1989. *Leioproctus* bees associated with Western Australian smoke bushes (*Conospermum* spp.) and their adaptations for foraging and concealment. Records of the Western Australian Museum 14: 275–292.

Hulbert S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54: 187–211.

Hurd, P. D. and L. Gorton. 1971. Squash and gourd bees (*Peponapis, Xenoglossa*) and the origin of the cultivated cucurbita. Evolution 25: 218–324.

Kudo, G. 2006. Flowering phenologies of animal-pollinated plants: Reproductive strategies and agents of selection; pp 139–158. *In* L. D. Harder and S. C. Barrett, eds. Ecology and Evolution of Flowers. Oxford University Press on Demand, London, UK.

Kudoh, H., S. Shibaike, J. Takasu, D. Whigham, and S. Kawano. 1999. Genet structure and determinants of floral structure in a temperate deciduous woodland herb, *Uvularia perfoliata*. Journal of Ecology 87: 244–257.

McCall, C. and R. B. Primack. 1987. Resources limit the fecundity of three woodland herbs. Oecologia 71: 431–435.

Mitchell, T. B. 1960. Bees of the eastern United States, Vol. 1. North Carolina Agricultural Experiment Station Technical Bulletin 141: 1–538.

Motten, A. F. 1986. Pollination ecology of the spring wildflower community of a temperate deciduous forest. Ecological Monographs 56: 21–42.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ren, Z. X., P. Bernhardt, R. Edens-Meier, J. Zweck, M. Arduser, H. D. Li, and H. Wang. 2019. Comparative pollen–pistil interactions and insect pollination in two *Hypoxis* species (Hypoxidaceae) in China and North America. Plant Systematics and Evolution 305:115–126. https://doi.org/10.1007/s00606-018-1556-6.

Robertson, C. 1896. Flowers and insects. XVI. *In* J. M. Coulter, C. R. Barnes, and J. C. Arthur, eds. The Botanical Gazette 21(XXI). University of Chicago Press, Chicago, IL.

ROBERTSON, C. 1928. Flowers and Insects: Lists of Visitors of Four Hundred and Fifty-Three Flowers (26). Carlinville, Illinois. The Science Press Printing Company, Lancaster, PA. pp. 141–142. https://doi.org/10.5962/bhl.title.11538.

ROUBIK, D. 2014. Orchids and Neotropical pollinators since Darwin's time, pp. 229–264. *In* R. Edens-Meier and P. Bernhardt, eds. Darwin's Orchids Then & Now. University of Chicago Press, Chicago, IL.

SEIBERT, T. F. AND J. A. SAVIDGE. 1991. Pollination and breeding biology of large-flowered bellworts. Canadian Field-Naturalist. 1053: 392–394.

UNDERWOOD, A. J. 1997. Experiments in ecology: Their logical design and interpretation using analysis of variance. Cambridge University Press, UK.

UTECH, F. H. AND S. KAWANO. 2002. *Uvularia*, pp. 147–150. *In* Flora of North America Editorial Committee, eds. Flora of North America: North of Mexico. Magnoliophyta: Liliidae: Liliales and Orcidales. (Vol. 26). Oxford University Press, New York, NY.

VANCE, N., P. BERNHARDT, AND R. EDENS. 2004. Pollination and seed production in *Xerophyllum tenax* (Melanthiaceae) in the Cascade Range of central Oregon. American Journal of Botany 91: 2060–2068.

VEREECKEN, N. J. AND A. FRANCISCO. 2014. *Ophrys* pollination: From Darwin to the present day, pp. 229–264. *In* R. Edens-Meier and P. Bernhardt, eds. Darwin's Orchids Then & Now. University of Chicago Press, Chicago, IL.

VERHOEVEN, C., Z. X. REN, AND K. LUNAU. 2019. False colour photograph: A novel digital approach to visualize the bee view of flowers. Journal of Pollination Ecology 23: 102–118.

VICENS, N. AND J. BOSCH. 2000. Weather-dependent pollinator activity in an apple orchard with special reference to *Osmia cornuta* and *Apis mellifera* (Hymenoptera: megachilidae and Apidae), Environmental Entomology 29: 413–420.

WHIGHAM, D. F. 1974. An ecological life history study of *Uvularia perfoliata* L. American Midland Naturalist 91: 343–359.

WHIGHAM, D. F. 2004. Ecology of woodland herbs in temperate deciduous forests. Annual Review of Ecology, Evolution, and Systematics 35: 583–621. doi:10.1146/ecolsys.35.021103.105708.

WIJESINGHE, S. K. AND D. F. WHIGHAM. 1997. Costs of producing clonal offspring and the effects of plant size on population dynamics of the woodland herb *Uvularia perfoliata* (Liliaceae). Journal of Ecology 85: 907–919.

WIJESINGHE, S. K. AND D. F. WHIGHAM. 2001. Nutrient foraging in woodland herbs: A comparison of three species of *Uvularia* (Liliaceae) with contrasting belowground morphologies. American Journal of Botany 88: 1071–1079.

WILBUR, R. L. 1963. A revision of the North American genus *Uvularia* (Liliaceae). Rhodora 65: 158–188.