ASSESSING POLLINATOR ASSEMBLY AND POTENTIAL ACROSS SPECIES RANGES IN THE GENUS *TRIODANIS* (CAMPANULACEAE)

Kate Tillotson-Chavez*, Sarah Lukavsky, Jennifer Weber

School of Biological Sciences, College of Agriculture, Life, and Physical Sciences, Southern Illinois University- Carbondale, USA

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*Corresponding author: kate.tillotson@siu.edu

Abstract—Plant-insect interactions are a key topic in evolutionary ecology, especially in the face of anthropogenic changes that threaten to disrupt these mutualisms. An in-depth pollinator survey for four pladint species in the genus *Triodanis* is performed here for the first time, sampling a large portion of their geographic ranges (i.e., four U.S. states). All species in the genus exhibit dimorphic cleistogamy with variability in mean allocation to open (chasmogamous) flowers among populations and taxa. The genus *Triodanis*, therefore, provides an opportunity for understanding possible associations between pollinator assemblies and variation in species, breeding system, and geography. To assess these relationships, we sampled four species or subspecies of *Triodanis* at eight field sites in four states. Sampling encompassed a broad area across the Midwestern U.S. and Texas, and across a gradient of anthropogenically disturbed habitats. We found that for species of *Triodanis*: 1) small bee and fly generalist pollinators showed some floral constancy to *Triodanis* flowers during feeding bouts but did not differentiate between species, 2) pollinator community varied in diversity and abundance across different habitats, 3) while allocation to open flowers varied among species and populations, we found no association between breeding system (or species) on pollinator identity or abundance. This study represents the first in-depth survey of pollinator visitors of *Triodanis* and serves as foundational knowledge about the natural history of this group, provides resolution for members of Campanulaceae exhibiting floral traits of generalist hosts, and important documentation of plant-insect interactions in an era of ongoing anthropogenic changes.

Keywords—Self fertilization, dimorphic cleistogamy, native pollinators, syrphid flies

INTRODUCTION

The intricate and diverse relationships between plants and insects are the subject of many cornerstone topics in evolutionary ecology. Contemporary pressures such as climate change, habitat destruction, emergent pests and pathogens, and urbanization threaten to disrupt some of these important associations. In flowering plants that rely on insects as vectors for pollination, fitness consequences of disrupted mutualisms may come in the form of pollen limitation. With the increase in anthropogenic change across global landscapes and the continuing lack of resolution on floral hosts for small bee and other generalist species, explicit characterization of pollinator assembly through natural history studies is of continuing importance (Burkle & Alarcon 2011). Specifically, to better understand these pollination systems, many more studies are needed that explicitly characterize variation in pollinator communities, not only across geographic ranges, but in the context of variability among species ranges.

Elucidating pollinator relationships spatially also addresses the potential for local pollen limitation, because pollination service can vary in both quantity (e.g., the number of visits or total pollen received) and quality (e.g., proportion of conspecific and viable pollen transferred; Brown et al. 2002). Spatial and temporal variation in pollinator community composition can result in heterogeneity in visitation for pollinators and overall composition of generalists and specialists (Herrera, 1996). Under scenarios of high pollen limitation, selection may favor the evolution of increased selfing, providing reproductive assurance in changing environmental conditions (Goodwillie et al. 2010; Devaux et al. 2014; Leibman et al. 2018). Range expansions and subsequent shifts in pollinator communities are one means by which populations become pollen-limited; and this may create a continuum in breeding strategies across a species range (Koski et al., 2019).

One variable breeding strategy is dimorphic cleistogamy, a mixed mating system that includes both closed, obligately selfing (cleistogamous) flowers and open (chasmogamous) flowers that have the potential to outcross. The genus *Triodanis* ((L.) Niewl; McVaugh, 1948) consists of seven dimorphic cleistogamous species, has a distribution throughout North and South America, and is part of the subfamily Campanuloideae, Rapunculus clade (Roquet et al. 2008; Wendling et al. 2011; Crowl et al. 2016), contained within the cosmopolitan family Campanulaceae. Cleistogamous (closed) flowers are generally theorized to be resource-cheap in comparison to chasmogamy (open flowered) which typically produces floral attractants such as a corolla, pollen for outcrossing, and nectar (Knight et al., 2005; Goodwillie et al., 2010). Variability in allocation to chasmogamous (open) or to cleistogamous (closed) flowers at the species level, however, has not previously been considered as a factor influencing pollinator assemblies.

Floral morphology (i.e., corolla symmetry) differs widely between subfamilies of Campanulaceae, while secondary pollen presentation is found throughout Campanuloideae (Yeo 2012). Pollination syndromes also differ widely between subfamilies and the diversity in floral forms found is due in part to pollination pressures (Roquet 2008). Pollinator functional groups have been a significant driver of diversity in Campanulaceae (Lagomarsino et al. 2016). Despite considerable work in the broader Campanulaceae, there have been no in-depth pollinator studies across multiple species of the genus *Triodanis*, (but for brief surveys see: Robertson 1928; Tooker et al. 2006; Roquet et al. 2008; Basteri & Benvenuti 2010; Olesen et al. 2012). The most comprehensive list of pollinators and floral visitors come from historical records taken over 100 years ago in the Midwest (e.g., Illinois) and only categorized one species of *Triodanis* (*T. perfoliata,* formerly *Specularia perfoliata*) (Robertson 1928; Tooker et al. 2006). Overall, resolution of the explicit identity of pollinators varies highly for many members of Campanulaceae with small, rotate, flowers, across N. America. For species within Campanulaceae that exhibit open, rotate, or "generalist" flowers such as those found on *Triodanis* and its sister taxon, the Mediterranean endemic, *Legousia*, brief surveys have characterized visitation as restricted to small solitary, or eusocial polylectic bees, and flower visiting flies such as families Syrphidae and Muscidae (Robertson 1928; Tooker et al. 2006; Roquet et al. 2008; Basteri & Benvenuti 2010). While *Triodanis* is likely highly selfing due to the presence of dimorphic cleistogamy, this character trait has not been described in *Legousia*. Forces such as dispersal events, climate change, and the resulting change in pollinator assemblage, may influence increased autogamy for some lineages, resulting in the highly diverse floral forms seen in the Rapunculus clade (Beattie 1974; Roquet et al. 2008; Mitchell et al. 2009).

This study focuses on four of the seven species in the genus *Triodanis*. While some floras still consider *T. biflora* a subspecies of *T. perfoliata* (e.g., (Diggs et al. 1999)), preliminary phylogenetic analysis indicates monophyly between these two taxonomic units (in prep, Simmonds *unpublished data*). For the purposes of clarity in narrative, we decided to consider *T. perfoliata* and *T. bilfora* as unique taxonomic units, but ultimately, we draw no broad inferences based on this delineation with few direct comparisons between *T. perfoliata* and *T. biflora* in this paper. All four species (*T. biflora*, *T. perfoliata*, *T. lamprosperma*, *T. leptocarpa*) are annual plants with largely sympatric ranges from the east coast to the Midwest and south into Texas, with ranges for two taxa (*T. perfoliata* and *T. biflora*) extending into Mexico and South America (Diggs et al. 1999; Weakley 2020). Both *T. perfoliata* and *T. biflora* are very common in the contiguous U.S., and these cosmopolitan species often occur in areas of high human disturbance such as urban parks and agricultural fields, as well as grassland habitats (*pers. obs*). Due to both the cosmopolitan and restricted range sizes of different species of *Triodanis* (i.e., a single county for *T. texana*), we hypothesize that the diversity and abundance of pollinator species may vary significantly over spatial scales and among species for this weedy native annual. Previous work demonstrated that the breeding system of *T. perfoliata* varies based on abiotic environments (Ansaldi et al. 2019), and our four study species of *Triodanis* occur in a range of habitats (Diggs et al. 1999; Weakley 2020). Therefore, these species may also exhibit variable breeding systems among populations. Possible variability in allocation to chasmogamous (open) or cleistogamous (closed) flowers among populations and species of *Triodanis* may influence pollinator assemblies. Specifically, allocation to chasmogamous (open) flowers in this genus may play an important role in the presence and diversity of pollinator functional groups visiting species of *Triodanis*, though this has never been examined. Here we examine the pollinator community across a large portion of the geographic ranges for four species or subspecies of *Triodanis*. Our objectives were to capture the potential variation of pollinators across broad geographic space and species for multiple populations per species and to 1) explore differences in pollinator behavior among species of *Triodanis*, 2) characterize differences in abundance and diversity of pollinator functional groups for *Triodanis* across sampling localities and habitat types (i.e., high versus low disturbance), and 3) discuss the potential influence of allocation to chasmogamous flowers among species in each sampling location on pollinator communities (or the impact of pollinator on the production of chasmogamous flowers).

MATERIALS AND METHODS

STUDY SPECIES AND RANGES

The genus *Triodanis* contains seven annual species native to North- and South- America (Nieuwl, McVaugh 1945, 1948; Fernald 1946; Bradley, 1975). Floral morphology of chasmogamous flowers in the genus varies little to the naked eye between species and consists of a pentamerous, dish-shaped, actinomorphic corolla. Open flowers for all species are generally 1-1.5 cm in diameter; petals are purple to purple blue. Pollen presentation is secondary, the style elongates as the corolla opens and pollen is presented on a stylar brush in keeping with other members of Campanulaceae (Yeo 2012). Overall flower production generally begins with the creation of cleistogamous flowers and then chasmogamous flowers and is followed again with cleistogamous flowers (Gara & Muenchow 2021). This production on the inflorescence begins with cleistogamous proximal and a mix of chasmogamous and cleistogamous produced medially and distally (Trent 1942). Chasmogamous flowers are both outcrossing and self-fertile; stigma lobes curl backward towards the stylar brush as the corolla senesces (Trent 1940; Goodwillie et al. 2018). For *T. perfoliata*, flowers will remain open up to three days if not pollinated (Ansaldi et al. 2018). Anthers dehisce before anthesis, and stigma lobes open one to two days later (Yeo 2012; Goodwillie & Stewart 2013). Cleistogamous flowers of all species lack corollas and are obligately selfing. All species in the genus *Triodanis* fall within this continuum of mixed mating. Capsules of chasmogamous flowers and cleistogamous flowers are distinguishable by size, stem placement, and difference in calyx number with chasmogamous flower capsules presenting with five (rarely four or six) and cleistogamous flower capsules presenting with three. Variability in chasmogamous flower production between species is observable during peak flowering. Open flowers for *T. biflora* are typically presented one at a time at the stem apex, whereas *T. leptocarpa* and *T. perfoliata* often exhibit multiple open flowers open along a stem at once. Variability and intermediate forms of flowering may be present in hybrids (Gara & Muenchow 1990).

Triodanis perfoliata and *T. biflora* are widely occurring with ranges that encompass the eastern and midwestern United States and into parts of South America (Weakley 2020). The species *T. leptocarpa* and *T. lamprosperma* have more discrete ranges, both occurring through central Texas, north through Oklahoma, Arkansas, Kansas, and Missouri (GBIF). Species in the genus often occur in sympatry and have been found to readily hybridize, particularly *T. biflora* and *T. perfoliata* (e.g., Diggs et al. 1999; Goodwillie & Stewart 2013; Weakley 2020), though formal documentation of hybridization between these cosmopolitan species and those with smaller ranges is limited. Species can occur in cultivated or fallow fields, prairies, dry hills, wooded areas and along water edges, as well as highly human disturbed areas, such as degraded and mowed urban areas (Trent 1942; Weakley 2020; Gleason & Cronquist). Species bloom in spring and summer months dependent on local climate (April and May in central Texas, while more northern regions bloom in late May and June). Populations produce chasmogamous flowers for one to three weeks depending on both range and local conditions (Trent 1940).

SURVEY OF POLLINATOR AND ARTHROPOD ASSOCIATIONS OF *TRIODANIS*

We surveyed floral visitors of four species of *Triodanis* (*T. perfoliata, T. biflora, T. leptocarpa, T. lamprosperma*) in their native ranges across four U.S. states following general peak flowering phenology northward from Texas into Kansas, Missouri, and Illinois (sites *N* = 11; some heterogeneous sites were also sampled for microsite variation). Due to the large ranges of *T. perfoliata* and *T. biflora*, time restraints of peak flowering time, and necessity of understanding pollinator interactions when species occurred in sympatry, these cosmopolitan species were sampled within the narrower ranges of *T. lamprosperma* and *T. leptocarpa*. Standard sites surveyed had mixed species communities and consisted of more than one *Triodanis* species. Floral visitors were surveyed using standardized collection methods over a total of 22 days (about 3 weeks), from May 6th to June 15th of 2022, at *N* = 6 standard survey and *N* = 6 haphazard survey sites or 12 total (see Table 1). We used two approaches to classify our sites, a coarse but quantitative description was made using the Multi-Resolution Land Cover Consortium's National Land Cover (NLCD) database definitions (Table 1; Dewitz 2019). Though our microsite assessments were more useful for understanding local conditions, we include these land cover classifications here for a better understanding of the broader communities; these data may be useful for ongoing research efforts to understand pollinator communities. We also qualitatively assessed the level of anthropogenic disturbance at each site based on the microsite conditions. We subsequently considered sites that were in highly modified landscapes (e.g., cemetery, pasture) to be highly disturbed and sites that were dominated by grasses or managed (e.g., Konza Prairie Biological Station) to have relatively lower anthropogenic disturbance. Unfortunately, more elaborate site designations were not possible within the scope of our data. Standard sampling was completed at six of the twelve sites and consisted of intensive sampling for approximately three days during peak chasmogamous (open) flowering, in some cases inclement weather (e.g., rain) and early plant senescence reduced the sampling window. Sampling was divided into 30-minute blocks in the: mid-morning (MM), high-noon (HN), and mid-afternoon (MA), to capture a relatively wide spectrum of pollinators over most of a sampling day. Surveys were not formally conducted at dusk, dawn, or night, as relatively few pollinators were observed at these times (Tillotson-Chavez, pers. obs). Standard sites were sampled for up to 13.5 hours total, spread across samplers and sampled area. All possible insects visiting *Triodanis* were collected within these observation windows for this repeated sampling. Transect sampling was not feasible due to patchiness of plant populations and the small size and maneuverability of pollinators observed. Instead, the observation and sampling plots were rotated over the course of three days; no plot was sampled consecutively in a 24hr period. At these standard survey sites, areas were subdivided into (on average) six 1m x 1m observational plots per site by differences in microsite habitat and density (e.g., forest edge, mowed path, etc.). *Triodanis* individuals were generally clustered and patchy across a mixed landscape. Microsite sampling was determined using the highest density of flowering individuals in these habitat gradients. Overall, pollinator abundance for *Triodanis* was patchy and often few pollinators were collected in our standard sampling approach. To increase our sampling potential and better describe the pollinators of *Triodanis*, we performed additional haphazard sampling at six sites, consisting of brief and opportunistic sampling in patches with apparent insect activity. Observation and standard sampling plots could consist of one or more *Triodanis* spp. in flower at any time.

Collection of insects was completed using an InsectaVac Aspirator (BioQuip) and insect nets. In each case, before floral visitors were collected their behavior was noted, such as: *Triodanis* species visited when co-occurring, habit when feeding (e.g., pollen foraging, basking or resting on petals), contact with chasmogamous reproductive organs,

Table 1. Site Descriptions. State, site name, site code, standard (6 sites) or haphazard (6 sites) sampling (see Methods), and *Triodanis* species present. Additionally, local habitat description, NLCD land cover designation (see Methods, Dewitz 2019), dates of sampling, and average temperature at time of sampling.

State	Site Name	Site Code	Sampling Method	*Triodanis* Species Present	Land Cover Description	NLCD Classification	Dates Sampled	Average Temperature (F)
TX	Stengl Field Station	SMTX	**Standard**	*T. biflora*, *T. perfoliata*	Developed, open space	Deciduous forest	5/8-5/10	89
TX	Stengl (Power Line)	SPTX	Haphazard	*T. lamprosperma*	Grassland, herbaceous	Deciduous forest	5/9	95
TX	DFW Lawn (Kim Sasan)	KSTX	Haphazard	*T. biflora*	Developed, high intensity	Developed, low intensity	5/15	90
TX	DFW Trophy Park	TPTX	Haphazard	*T. biflora*	Shrub/scrub	Developed, low intensity	5/15	88
MO	Fort Leonard Wood	FLWMO	**Standard**	*T. biflora*, *T. perfoliata*	Developed, open space	Deciduous forest	6/3-6/5	78
MO	Bohigian Conservation Area	BCMO	**Standard**	*T. perfoliata*	Grassland, herbaceous	Deciduous forest	6/3-6/5	77
KS	Konza Prairie	KPKS	**Standard**	*T. perfoliata*, *T. leptocarpa*	Grassland, herbaceous	Grassland, herbaceous	5/15, 6/13-6/15	89
KS	Cattle Field (Jeff Taylor)	JTKS	**Standard**	*T. perfoliata*	Pasture/hay	Grassland, herbaceous	5/15, 6/13-6/15	89
IL	Tower Grove Cemetery	TGCIL	**Standard**	*T. biflora*	Developed, open space	Deciduous forest	6/7-6/9	81
IL	Lake Murphysboro	LMIL	Haphazard	*T. biflora*	Developed, open space	Deciduous forest	6/7-6/9	90
IL	Southern Illinois University	SIUIL	Haphazard	*T. biflora*	Developed, medium intensity	Developed, medium intensity	6/7-6/9	82
IL	Fults Hill Nature Preserve	FHNPIL	Haphazard	*T. perfoliata*	Deciduous forest	Deciduous forest	6/10-6/11	84

and changes in feeding habits over the course of a day. Only pollinators and floral visitors that made contact with open flowers of *Triodanis* species were collected and treated as viable pollinators. Haphazard collections of pollinators were taken for a more robust sampling and are noted (see Table 1).

Collected floral visitors were pinned, sorted into functional groups (i.e., flies, bees; Fig. 1), and identified (Carril & Wilson 2021; Discover Life). Documented natural histories were used (Willmer 2011; Camilo et al. 2017; Carril & Wilson 2021; Discover Life) to assess possible generalism or specialism of species collected across functional groups; results were compared to field observations. Body measurements of bee species were taken from averages used in identification (Carril and Wilson 2021). Specialism to *Triodanis* was not assigned unless a pollinator species could be verified only to visit *Triodanis* flowers across the sampled range of that *Triodanis* species. Species range maps were also used to assess the likelihood of encountering specific bee and fly species across the ranges of the four *Triodanis* species sampled (GBIF; Carril & Wilson 2021). Due to very low differentiation in pollinator communities among *Triodanis* species, assessment of pollinator preference among species was performed qualitatively (see Results).

Finally, to evaluate potential as effective pollinators, pollen load scores were calculated on collected insects using methods adopted from

Figure 1. Pollinators in-situ and collected for identification. Top row: 1. Possible *Lasioglossum sp*. resting on *Triodanis* flower during feeding. 2. *Hemipenthes sinuosa* feeding on *Triodanis*. Bottom row: 3. *Ceratina dupla* collected from Missouri. 4. *Hoplitis pilosifrons* with distinctive pollen collection on the underside of the metasoma. Images 1 & 2 courtesy of T. Simmonds.

(Tepedino et al. 1999; Chisausky et al. 2020). Three regions of the head (dorsal, anterior, and ventral), and anterior and ventral regions of the thorax and abdomen were examined for pollen deposition. Pollen scores were compared using a weighted mean; downweighing each head score by ⅓ due to the number of subsections measured and averaging all eight scores. Scores of scopal pollen for bee species were excluded but noted, as scopal pollen is not readily available for pollination (Weinman et al. 2023). Pollen load scores for each region were counted using a score from 0 to 5; 0: no pollen grains present, 1: 1+ pollen grain on that region, 2: pollen grains separated by >1mm, 3: pollen grains separated by <1mm, 4: nearly complete pollen coverage of region, 5: multiple layers of pollen covering the area. Scores were compared between bee species, flower fly species, and bee flies.

Variation in Pollinator Communities Across Sampling Locations

We compared pollinator community structure between survey sites to better understand trends of pollinator habitat and composition within and between sites. Pollinators of sites were characterized by the presence and abundance of pollinator species, scoring the latter as highly abundant (5+ individuals), abundant (3+ individuals), or present (1 individual). Observations and samples were taken from a range of both highly developed anthropogenically disturbed areas (e.g., Dallas Fort Worth: DFW and a suburban backyard: KSTX) and preserved natural grassland or forested areas (e.g., Konza Prairie Biological Station: KPKS) across all states sampled. Co-flowering species at each site sampled were also identified (see Supp. Table 1). Genus level diversity (e.g., pollinator abundance, genera richness, Menhinick's index, Simpson and Shannon-Wiener (S-W) diversity indices, and Pielou's evenness were calculated in the R package vegan (Oksanen et al. 2022) and was used to understand variability in pollinator assembly for *Triodanis* species across sites. Samples collected were pooled across microsite plots and sampling period for standard sampled sites and metrics were calculated using the pollinator assemblages of each of these sites. Richness was calculated in relation to genera present across sites; sample-based rarefaction could not be used due to lowest percent coverage of 0. With the S-W index sensitive to the presence of rare genera and Simpson weighted for common, Pielou's evenness was also used to understand variability in abundance across sites. For the sites that had this standardized sampling we performed t-tests to examine any differences in metrics of diversity between sites with relatively high disturbance (*N* = 4 sites) compared to sites with lower levels of disturbance (*N* = 2 sites; R Core Team, 2018).

BREEDING SYSTEM ALLOCATION

Across sample sites, whole individual vouchers of mature plant specimens were assessed for the total number of chasmogamous (open) and cleistogamous (closed) flowers produced. Floral type (chasmogamous or cleistogamous) can be accurately distinguished by calyx number in all species (e.g., Ansaldi et al. 2018) and percent of chasmogamous flowers (pCH) out of all flowers produced can be quantified on individual stems at maturity. At least 10 mature individuals per population (unless the population numbered less than 10) and each microsite within populations were surveyed in a standard procedure. The percentage of chasmogamous flowers (pCH) was calculated as the average pCH across individuals and across populations for each species with sufficient sampling. Due to very low differentiation in pollinator communities among *Triodanis*, assessment of pollinator preference for populations or species with relatively higher allocation to open flowers (pCH) was performed qualitatively (see Results). A brief qualitative assessment of the standing crop of nectar was performed in the field using microcapillary tubes, sampling in mid-morning to minimize dew accumulation from overnight in flowers previously open. We acknowledge that our nectar sampling could have been influenced if foragers depleted nectar early in the morning, before we sampled.

RESULTS

POLLINATOR ASSOCIATIONS OF *TRIODANIS*

Overall, we sampled a total of 126 individual pollinators across two pollinator functional groups (i.e., flies, bees) at 10 field sites in four states during peak flowering of *Triodanis* species (May-June; Table 2). For all *Triodanis* taxa sampled, we documented floral visitors and potential pollinators. Over our sampling localities, we observed no variability among species of *Triodanis* for pollinator functional groups (see Table 2), with small bee species and flies (Syrphidae and Bombyllidae) predominating in pollinator assemblies of *Triodanis* (for standard sampled sites, small bees (39.6%), flies (60.4%); Table 2). Polylectic, small (~6mm) solitary or eusocial bees composed all bee species collected and observed (see Table 2); no pollinators classified as oligolectic or large bee species (e.g., *Bombus*) were found to visit *Triodanis*. There was no indication in the wide variety of pollinators sampled and observed that *Triodanis* has specialist pollinators. While generalist pollinators and floral visitors were dominant, honeybees (*Apis mellifera*) a common generalist, was absent even in highly developed urban areas consistent with the absence of medium sized bees across our spatial sampling. Bees lacking scopal hairs, such as males of *Ceratina strenua*, were intermittently present. No corbiculate bees were collected or observed. While butterflies and moths were present throughout the sites sampled, they were incredibly rare floral visitors on *Triodanis*, (*pers. obs.*; including night visiting moths) and their visitation was only observed casually, outside of standard survey sampling. When viewed, visitation was brief, and we were unsuccessful in collecting moth species from flowers in haphazard sampling. In general, of pollinators collected, 61 individuals were small solitary or eusocial bees, across six families, with 11 genera, and 19 distinct species represented. For flies and other floral visitors, we collected 62 specimens, across three families and three genera, representing four distinct species (Table 2).

In observations of pollinator foraging, most pollinators that visited *Triodanis* appeared to show floral constancy (i.e., restriction of visitation to one or more similar floral species in a foraging bout) and did *not* appear to differentiate among co-occurring *Triodanis* species. On foraging bouts, we observed pollinators exhibiting some constancy between open flowers of all *Triodanis* species present. Not all floral visitors were as consistent, with syrphid fly species *Toxomerus marginatus* and *T. geminatus* (Syrphidae), both highly general

Table 2. Results of pollinator surveys across field sites and microsites within field sites. Floral host *Triodanis* species abbreviations: Tb= *T. biflora*, Tp= *T. perfoliata*, Tla= *T. lamprosperma*, Tle= *T. leptocarpa*. Sex of pollinators (M=Male, F=Female), and polylecticism (generalist pollinator) is noted. This is not noted for species in Bombyliidae as there is less resolution for generalist species in that family. Collection time of day is noted by Mid-Morning (MM), High-Noon (HN), and Mid-Afternoon (MA). Sites marked with * signify abundance. *=Abundant, **=Highly abundant (see Methods) in our sampling.

Family	Species	Site ID	Host	Polylectic	Time of Day	Sex
Hymenoptera						
Andrenidae	*Andrena nasonii*	FLWMO	Tp	Yes	Hn	F
Apidae	*Ceratina arizonensis*	SPTX	Tla	Yes	HN	F
	Ceratina dupla	BCMO*	Tp	Yes	HN	F
	Ceratina strenua	FLWMO*, KSTX**	Tb, Tp	Yes	MM, HN	M, F
Colletidae	*Colletes inaequalis*	FLWMO*	Tp	Yes	HN	F
Halictidae	*Augochlora pura*	FHNPIL*	Tp	Yes	HN	F
	Augochlorella aurata	FHNPIL*, KPKS**, BCMO*	Tp	Yes	MM, HN	F
	Augochloropsis sumptuosa	KPKS	Tla	Yes	HN	F
	Halictus confusus	KPKS, SMTX*	Tb, Tp, Tla	Yes	MM, HH	F
	Halictus ligatus	FLWMO	Tb	Yes	MM	F
	Halictus tripartitus	KSTX*	Tb	Yes	HN	F
	Lasioglossum coeruleum	LMIL	Tb	Yes	HN	F
	Lasioglossum tegulare	SMTX	Tp	Yes	HN	F
Megachilidae	*Heriades carinata*	FHNPIL*, KPKS*	Tp	Yes	MA, HN	F
	Hoplitis pilosilofrons	SMTX	Tb	Yes	MA	F
Diptera						
Bibionidae	*Plecia* --	SMTX				
Bombyliidae	*Hemipenthes sinuosa*	SMTX**, SFTX**, JTKS	Tb, Tp, Tla		MM, HN	
Syrphidae	*Toxomerus marginatus*	FLWMO**, KPKS*, TGCIL**, SIUIL**	Tb, Tp, Tle	Yes	MM, HN, MA	
	Toxomerus sinuosa	FLWMO**, TGCIL**, SIUIL**	Tb, Tp	Yes	MM, HN	

species, visiting co-occurring fly-pollinated species (e.g., *Heracleum mantegazzianum*, *Lepidium virginicum*) and some bee species visiting showier flower species and *Triodanis* (e.g., *Lysimachia arvensis*; see Supp. Table 1). The bee fly species *Hemipenthes sinuosa* (Bombyliidae), was common only at the Stengl Field Site in central Texas. While this species showed no observable preference between the three *Triodanis* species found there (*T. perfoliata, T. biflora, T. lamprosperma*), it did show floral constancy between *Triodanis* individuals within microsite observation areas on foraging bouts (*pers. obs.*). Visitation habits included hovering followed by landing on the corolla and probing for nectar. Bee fly species have long tongues in comparison to other flower visiting flies and are generally covered in dense hairs, whose effectiveness at harboring and carrying pollen has typically been underestimated (Kastinger & Weber 2001). Feeding habits were dependent on weather conditions in that flies seemed most active on days exhibiting full sun, no wind, and a UV Index of 10 or higher, like findings summarized by Kastinger & Weber (2001).

Pollen scores allowed overall pollen presence to be quantified and compared between the three pollinator functional groups: bees, bee flies, and syrphid flies. Differences in mean pollen load scores were variable between functional groups (mean pollen score, sample size): bees (1.095, N = 57), bee flies (0.630, N = 12), and syrphid flies (0.081, N = 27). Any deviation from the totals listed above for pollinators collected and those in which pollen load was quantified was caused by general degradation to the specimen. We note that the presence of pollen on a pollinator does not necessarily confirm the delivery of pollen between conspecific flowers.

Table 3. Metrics of diversity across sites with standard sampling surveys. Sites indicated with * were determined to exhibit high anthropogenic disturbance (see Methods and Table 1 for site descriptions). Diversity indices were calculated for genera of pollinators to *Triodanis*. This includes abundance of individuals across genera and genera richness. Menhinick's index (D) to control for sampling effort. Pielou's Evenness (J) to understand both evenness and dominance. Shannon-Wiener Diversity (H') for testing in relation to rarer species. Simpson's Diversity (λ) for measuring dominance of more common species.

Standard Site	Abundance across Genera	Genera Richness	Species Richness	Menhinick's index	Pielou's Evenness	Shannon-Wiener Diversity	Simpson's Diversity
				D > 0	J (0-1)	H' (1-5)	λ (0-1)
SMTX*	19	5	5	1.15	0.31	1.02	0.50
FLWMO*	25	5	5	1.00	0.28	0.95	0.46
JTKS*	1	1	1	1.00	N/A	0	0
TGCIL*	11	1	2	0.30	N/A	0	0
BCMO	31	2	2	0.36	0.09	0.14	0.06
KPKS	14	5	5	1.34	0.38	1.22	0.61

POLLINATOR COMMUNITY VARIATION ACROSS SAMPLING LOCATIONS

Several bee species were common between sites and species of *Triodanis* (e.g., *Ceratina strenua*, *Augochlorella aurata*: see Table 2), while a few of these wide-ranging generalists were found at only one site (see: *Lasioglossum coeruleum*, *Colletes inaequalis*; see Table 2). We found that the highly generalist syrphid fly (*Toxomerus spp.*) predominated accounting for >50% of sampled pollinators in developed (low to medium intensity) areas containing both *T. perfoliata* and *T. biflora* in more northern states (MO, IL; see Table 2). The species *T. lamprosperma* and *T. leptocarpa*, were not present for observation as floral hosts when sampling in these more disturbed localities. We found no statistically significant differences in any metrics of diversity between sites of relatively higher or lower disturbance (Tables 1, 3). However, we note that our sample size of sites that classified as low disturbance and had standardized sampling was quite low (N = 2). Nonetheless we observed some general trends worth noting. Overall, pollinators were the most abundant at BCMO (31), but richness in pollinator genera was greatest in preserved grassland and herbaceous habitat (Menhinick's index; SMTX D = 1.15, KPKS D = 1.34). Diversity indices (Shannon-Wiener, Simpson) suggest that grassland and herbaceous areas, designated as areas to conserve and promote plant diversity (Natural Areas 1986 Jan 24; Mission | KPBS | Kansas State University), may host relatively high pollinator genera diversity for *Triodanis* (SMTX H′ = 1.02 λ = 0.499, KPKS H′ = 1.22, λ = 0.612) in comparison to developed, anthropogenically disturbed sites (e.g., TGCIL H′ = 0, λ = 0). In general, both *Triodanis* species and pollinator genera diversity trended towards lower in developed areas (see Tables 2, 3).

BREEDING SYSTEM ALLOCATION

Similar to previous work, allocation to open, chasmogamous flowers for *T. perfoliata* was variable across populations (Ansaldi et al. 2018). For *T. biflora* the mean proportion of open flowers across individuals in a population (pCH) was much lower (SMTX 3.2%, FLWMO, 4.5%, TGCIL 6.6%) than *T. perfoliata* (SMTX 10.9%, FLWMO 14.2%, BCMO 21.2%; see Supp. Table 2), as was expected due to its flowering habit of producing relatively few chasmogamous flowers at a time (Trent 1942). We were unable to reliably assess breeding system variability for *T. leptocarpa* and *T. lamprosperma* due to a limited sample size of populations, or individuals within a population; however, pCH for *T. leptocarpa* was assessed for one site (KSTX 33.2%). This is the first study to quantify breeding system allocation across a wide geographic range for *T. biflora* (for smaller surveys see: Bradley 1975; Gara & Muenchow 1990). Data taken from previous field seasons for *T. perfoliata* (Beth H Ansaldi et al. 2018; Berg et al. 2019) reported a higher average pCH of 30.9% across six sites and three states, emphasizing the high extent of variability in this species. Aggregated data taken from areas with more anthropogenic caused disturbance (FLWMO, TGCIL) tended to have higher pCH than those with more intact "natural" areas across *T. biflora* and *T. perfoliata*, though sample sizes were insufficient to test this statistically. We found no indication for variation in pollinator preference, functional group, or species based on the relative production of chasmogamous flowers within or among populations and species of *Triodanis* (Table 2). Detailed description of nectar presence, quality, and volume was complicated by evaporation or dilution from condensation, but nectar presence was detected in several *T. biflora* individuals.

DISCUSSION

Here we documented that across a relatively large spatial scale (i.e., four states spanning the midwestern U.S. and TX) generalist solitary or eusocial small bee and fly species seem to be the primary pollinators of *Triodanis*. Though this study encompassed only four species of seven reported species of *Triodanis* ((L.) Niewl, McVaugh 1945, 1948; Fernald 1946; Bradley, 1975), it includes the two species with the largest ranges (*T. perfoliata* and *T. biflora*) and included multiple field sites with more than one species of *Triodanis* present. Sampling also included sites in Texas, which is where all species of *Triodanis* exhibit range overlap, including those not sampled here (e.g., *T. texana* and *T. coloradoensis* only occur in TX, but were not in this survey). We cannot discount that variability among years or across geographic ranges could reveal pollinator species not documented in this present survey. However, given the consistency in functional groups across this survey, it seems very likely that these pollinators would functionally be generalist small bees and flies.

Though we found no statistical difference in pollinator diversity between sites of relatively high or low anthropogenic disturbance, we did note some interesting trends. For example, there was more general variation in the diversity of pollinator assembly genera in human-maintained natural habitat areas, though diversity does not reflect the general abundance of bee pollinators at sites such as BCMO or the low abundance of pollinators generally at KPKS. High variability in pollinator abundance spatially and temporally within the same year in similar habitat is in keeping with previous research (Reverté et al. 2019). We detected no association of specialized pollinators or pollinator functional groups with *Triodanis* species or population level breeding system variation. Our study adds to our understanding of pollinator assemblies for Campanulaceae species exhibiting generalist traits. Rotate corollas, such as those exhibited by *Triodanis*, have also evolved several times in the genus *Campanula* (Blionis & Vokou 2001). Strong selection pressure from pollinators has led to convergent traits and tangled taxonomic relationships based on these morphological features in Campanulaceae. Switching to generalist strategies (i.e., open corolla, auto compatibility) in response to low efficiency pollinators such as flower visiting fly or small be species, similar to those found in this study, and subsequent lower male reproductive success (Lau & Galloway 2004) has been found for *Campanula (*Kobayashi et al. 1999). While efficiency comparisons between Lepidopterans and Hymenopterans as pollinators have been well documented, comparisons of efficiency of fly pollinators and bee species remain an area of low resolution. Spatial variability of abundance in potentially higher quality pollinators due to land use factors may cause pollen limitation (Gómez et al. 2010) but pollen limitation has only been assessed at a single site for one species in this genus (*T. perfoliata*, Ansaldi et al. 2018). Based on the trends in our data, future work should examine variation in pollen limitation both among species and across populations of *Triodanis* occurring in habitats exhibiting different levels of anthropogenic disturbance.

Understanding pollinator assemblies for species can add unique insights about the potential for gene flow among populations and species. For example, for solitary bees the best predictor of foraging range is that of body size, with smaller bees traveling shorter distances than larger bees (Gathmann & Tscharntke 2002). Overall, we found bees with a mean body length of 6 mm to visit species of *Triodanis*, all of which are solitary or eusocial bees (Carril & Wilson 2021). With pollinators showing floral constancy in localized areas and flight length limited in small bee and fly species, this may contribute to high population genetic structuring in *Triodanis*, in addition to that already present due to cleistogamy (Tackett et al. 2022). This hypothesis is consistent with high population genetic structuring described across 18 populations of *T. perfoliata* (Tackett et al. 2022). In addition, pollinators represent a key step in either facilitating or limiting cross-species hybridization. Hybridization has been documented across multiple species of *Triodanis*, leading to complicated evolutionary relationships (Crowl et al. 2016). While previous work has demonstrated that sympatric species of *Triodanis* can flower at the same time (Berg et al., in press) this is the first study to demonstrate that pollinators are indeed shared across species in sympatry.

With historical records, comparison of past and present pollinator communities is possible and is increasingly important as we try to characterize changes in pollinator assembly due to anthropogenic effects (Kharouba et al. 2019). Pollinators collected in this study generally affirmed narrow historical data for pollinators of *Triodanis* (reviewed in Roquet et al. 2008). These records were based on those of Charles Robertson for *T. perfoliata* from a 90-year-old survey in Illinois collected and observed in one field season in 1928 (Robertson 1928); of the 21 pollinators represented in this historical survey, six pollinators matched my survey. Missing from our survey compared to those of Robertson are bumblebee species (*Bombus griseocollis* and *Bombus pensylvanicus*), which if present may have been significant pollinators of *Triodanis* as has been shown for other members of North American Campanulaceae (Willmer et al. 2017; Koski et al. 2018). Overall, the general composition of the pollinators we collected aligned with the composition of functional groups in Robertson's survey apart from the two bumblebee species.

Notably, these historic records also indicate that the broadly distributed short horned plasterer bee *(Colletes brevicornis*) is a specialist of *T. perfoliata* (Robertson 1928; Discover Life -- AMNH_BEE00208252), yet it was not collected in my much broader spatial sampling. The absence of this species could simply indicate temporal variation in pollinator assemblies, or other stochastic factors impacting pollinator activity and presences (e.g., weather). However, our survey strongly indicates that generalist pollinators compose many of the pollinator assemblies for *Triodanis*. This line of inquiry lends anecdotal weight to the importance of vigorous pollinator surveys. Additional work is needed to clarify the potential relationship between the short-horned plasterer bee and *Triodanis.*

CONCLUSIONS

Descriptive life history studies continue to be increasingly important for documenting species of both pollinators and plants to better understand variation in floral morphology, factors influencing pollen limitation and gene flow, and to assess these relationships in an era of rapid anthropogenic changes. By characterizing pollinators across large spatial scales for four of seven annual species (*Triodanis*), we contribute meaningful data for how pollinator assemblies vary across ranges and habitat types (i.e., high and low disturbance). This study is one of the few to examine how variation in dimorphic cleistogamy could contribute to variation in pollinator abundance or assembly. In addition, this study confirms active pollinator visitation across species, adding key information for understanding patterns of hybridization in the genus *Triodanis*. Finally, this study is the first in-depth survey of pollinators for the genus *Triodanis*, and the first specific survey of this group in almost a century. Here, we document the key pollinators of these species; this work provides a foundation for future studies in *Triodanis* and contributes to the broader interest in documenting explicit variation in pollinator assemblies across ranges and between related species.

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AUTHOR CONTRIBUTION

KT-C and JW conceived this work. KT-C and SL carried out data collection. Statistical analysis was performed by KT-C. The manuscript was prepared by KT-C and JW.

DISCLOSURE STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data used to write this article are available as supplementary data in the online version of this article, see below. Please contact the corresponding author for further data requests.

APPENDICES

Additional supporting information may be found in the online version of this article:

Table S1: Co-Flowering Species by Site

Table S2: Floral Ratio Data

Table S3: Abundance Scores by Site

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