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COMPARATIVE FLORAL ECOLOGY AND BREEDING SYSTEMS BETWEEN SYMPATRIC POPULATIONS OF *NOTHOSCORDUM BIVALVE* AND *ALLIUM STELLATUM* (AMARYLLIDACEAE)

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Abstract—We compared the floral biology of two sympatric populations of closely related species over two seasons. In 2018, *Nothoscordum bivalve* (L.) Britton bloomed from April 23 to May 7 and *Allium stellatum* Nutt. Ex Ker Gawl bloomed from August 28 to October 4. Erect, white flowers of *N. bivalve* were scented and had septal nectaries. Erect, pink-purple flowers of *A. stellatum* had septal nectaries, no discernible scent, and a style that lengthened over the floral lifespan. Both species were pollinated by bees with the most common geometric mean of body dimensions between 2-3 mm. Most bees carried pure loads of the host plant's pollen. Despite phenological isolation, the two herbs shared three bee species. *Allium stellatum* was also pollinated by the beetle *Chauliognathus pensylvanicus* DeGeer (Cantharidae). Tepal nyctinasty ensured mechanical self-pollination in *N. bivalv*e. Protandry occurred in *A. stellatum*. In *N. bivalve*, the proportion of pollen tubes penetrating ovules was highest in bagged, self-pollinating flowers. However, in *A. stellatum* it was highest in exposed flowers and hand cross-pollinated flowers. Fruit set in *N. bivalve* was highest in exposed and bagged, self-pollinating flowers. In *A. stellatum,* fruit set was highest in both exposed and hand cross-pollinated flowers. Seed set was the same among all treatments for both species. We interpret these results as evidence that *A. stellatum* is a self-incompatible, obligate outcrosser. *Nothoscodum bivalve* is a self-compatible, obligate inbreeder employing mechanical and insect-mediated self-pollination. Outbreeding depression appears to occur in *N. bivalve* with a partial trend towards intraspecific cross-incompatibility.

Keywords: bees, beetles, fruit/seed set, pollen tubes, protandry, obligate inbreeder

INTRODUCTION

Understanding how plant reproduction is altered by human activity is essential for the conservation of species and the practice of agriculture. The Order Asparagales contains commercially important species with ornamental, medicinal, and agricultural cultigens (Pires et al. 2006; Christenhusz & Byng 2016). This is particularly important in the genus *Allium* ss and its allies in the Amaryllidaceae (Angiosperm Phylogeny Group 2009), previously Alliaceae (The Angiosperm Phylogeny Group 2003) and/or Liliaceae (Cronquist & Takhtadzhi͡an 1981). *Allium* contains > 800 species (Li et al. 2010) with *A. sativum* L., *A. cepa* L., and their allies experiencing nearly global cultivation (Block 2010).

Information on the pollination and breeding systems of native populations of *Allium* species and their allies in North America lags behind nearly two centuries of research on cultigens. In particular, *A. stellatum* (autumn onion or prairie onion) is considered endangered and critically imperilled in Tennessee (Crabtree 2016). It is distributed from Texas to Canada in states along the Mississippi river and can be found in prairies or rocky hills growing in calcareous soils over limestone (Churchill 1986; McNeal Jr & Jacobsen 2002).

Nothoscordum bivalve (crow poison) is considered imperilled and rare in Indiana and threatened in Ohio (Endangered, threatened, rare and extirpated plants of Indiana 2016; Rare native Ohio plants 2018-19 status list 2018). It occurs from the south-eastern United States, west to Arizona and down through South America. In North America, it is found growing in open woods, barren tracts of land, disturbed sites, and grasslands (Baskin & Baskin 1979; McNeal Jr & Jacobsen 2002).

Allium stellatum and *N. bivalve* are two closely-related species. In fact, *A. siculum* Ucria and *A. tripedale* Trautv. were once placed within the genus *Nothoscordum,* and *N. bivalve* was originally placed within the genus *Allium.* Presumably, the main character that segregates these two genera is the absence of pungent sulphur compounds in *Nothoscordum* ss (Rahn 1998). Both species are bulbous and increase via a combination of seeds and clonal bulblets (Baskin & Baskin 1979; McNeal Jr & Jacobsen 2002). Both species are also regarded as prolific floral resources for pollinators and as foods for other animals (Everitt & Drawe 1974; Block 2010). However, based on their current conservation status in North America, their floral ecology and breeding systems require more attention.

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Populations of *N. bivalve* flower early in the springtime, from April to mid-May (Baskin & Baskin 1979). The inflorescence is an umbel with white-cream coloured flowers. Tepals usually have a purplish-red mid-vein but floral scent appears absent (McNeal Jr & Jacobsen 2002; Davis 2005). There are no references to the secretion of floral nectar in this genus although septal glands have been identified within the lineage (Rahn 1998; Farkas et al. 2012). The pollinators of *N. bivalve* have not been identified but the flowers are associated with one oligolectic bee species, *Andrena nothoscordi* Robertson. Bees in the family Apidae and butterflies in the family Pieridae are pollinators of the allied species *N. gracile* (Aiton) Stearn (Vent.) Kunth (Fernández et al. 2009). White flowers with concealed nectaries tend to be moth or bee-pollinated (see review in Wilmer 2011). Self-isolation mechanisms and fruit set rates have not been described in this species but *N. fragrans* (Vent.) Kunth was found to be self-compatible (Dyer 1967).

Populations of *A. stellatum* bloom from July to October (McNeal Jr & Jacobsen 2002). Umbellate inflorescences produce flowers with deep pink tepals but floral scent remains undescribed (McNeal Jr & Jacobsen 2002). One study found that *A. stellatum* produces three septal nectaries below the "crest-like apical processes" (Choi et al. 2011). Flowers in the genus *Allium* ss usually produce a nectar that is exceptionally concentrated (Kumar & Gupta 1993). Molano-Flores (1999) interpreted *A. stellatum* as a generalist entomophile because the author observed solitary bees and syrphid flies on flowers. However, the authors made no collections of insects for vouchers or pollen load analyses of foragers. Purple flowers with concealed nectaries are associated with bee pollination (Willmer 2011). Field observations by Molano-Flores (1999) suggest that the species is protandrous but the authors did not test for stigmatic receptivity. The author studied the effects of population size on reproductive success in *A. stellatum* and interpreted the species as an outcrosser but this was based on preliminary, unpublished data. Molano-Flores also concluded that self-compatibility existed in the species but it appears that this conclusion was based primarily on calculating pollen-ovule ratios in the population as described by Cruden (1977).

Large, sympatric populations of both *A. stellatum* and *N. bivalve* offer an unusual opportunity at the Shaw Nature Reserve in Saint Louis County, Missouri. The pollination and breeding systems of both species can be studied in the same year at opposite ends of the flowering season. Therefore, the purpose of this paper is to describe and compare the floral ecology, pollination, and breeding systems between two closely related and sympatric species. The following questions are investigated. What is the flowering phenology of these species' populations and how long do individual flowers live? How are floral organs spatially and temporally arranged? Where do the pollination systems of these two species fall within the generalist-specialist continuum and how do putative pollinators compare in diversity and size? Do these species employ mechanisms that promote self- or cross-pollination?

MATERIALS AND METHODS

Population and Site

Field research and observations were made from April 14 to May 7, 2018 and from April 10 to May 3, 2019 for *N. bivalve*. Fieldwork and observations for *A. stellatum* were from August 26 to October 6, 2017 and from August 19 to October 2, 2018. The field site at the Shaw Nature Reserve, Gray Summit, Saint Louis County, Missouri (38°27'56.9"N 90°49'23.7"W) was usually visited on alternate days during the flowering seasons of both species. Populations of both species grew in a limestone glade, among short grasses, bordering an open woodland in compact and often shallow soils over the exposed bedrock. We estimated a population of approximately 10,000 flowering stems of *N. bivalve* and approximately 2,000 flowering stems of *A. stellatum* at our site within the reserve.

Floral Phenology and Lifespan

To describe the flowering phenology of each population, a 1x1 m plot was used, including at least 100-200 flowering stems. The number of individuals with at least one opened flower was tallied during every visit to the reserve. To describe the lifespan of individual flowers, 15 umbels and 1 flower bud from each were randomly selected and labelled with dated jeweller's tags. Upon the opening of the perianth, we recorded the number of days until the tepals and styles withered, the number of anthers that were dehiscent, and the number of collapsed (empty) anthers in these 15 flowers. Once the tepals opened, we measured the respective lengths of styles in the tagged flowers 2-3 times over a 4-6-day period until the flower withered.

Floral Presentation

We noted flower colour but could not use scent jars to record odour as cutting the flowers of *A. stellatum* produced the typical alliaceous odour. We sniffed flowers between 10:00 and 16:00. For *N. bivalve*, we isolated flowers in Ziploc bags to sample odour but this was discontinued because the odour of the plastic appeared to alter the natural flower odour. Flowers of *N. bivalve* were sniffed from 12:00 to 16:00. In fact, all observations for flowers of *N. bivalve* had to be made in the afternoon as flowers remained closed in the morning. We noted the time, temperature, and cloud cover in which tepal expansion began. Nectar production in both species was observed by bagging whole umbels in organza bags and allowing secretions to accumulate. To locate nectar glands in both species, we picked flowers and cleared them in 3:1 ethanol (95%): glacial acetic acid for 2-6 hours before transferring to 70% ethanol (see below). Specimens were observed under a dissecting microscope to define gland geography and then glands were excised, mounted in distilled water and observed under a compound light microscope. We recorded the height of the scape from the soil to the umbel in each species and measured the length of a tepal on flowers selected at random.

Insect Visitor Collections and Analyses

We observed insects foraging on the flowers of each species over two blooming periods. We collected 10-20 insect

specimens that we observed foraging for nectar and/or pollen on the study species each day at the site between 10:00 and 17:00. Insect vouchers were euthanized in killing jars with fumes of ethyl acetate. Specimens brought to the lab were stored in a freezer at -10°C for future measurements and pollen removal.

The protocol for both the removal and identification of pollen from insects, including how pollen grains were stained and recorded, followed Bernhardt et al. (1984). The protocol for pinning, labelling, measuring, and co-referencing insects with their pollen load slides followed Edens-Meier et al. (2011). Insects were identified by Michael Arduser and were deposited in the Billiken Bee Lab, in the Department of Biology at Saint Louis University, MO (D17,18 (2017-2019).

To identify grains in the pollen load carried by each insect specimen, we first collected plant specimens in bloom around the site. Pollen from these plants was removed and stained with Calberla's fluid to make a pollen library. Plant specimens were pressed as vouchers and identified by Dr James Trager (Shaw Nature Reserve) and then deposited in the herbarium of the Missouri Botanical Garden. *Nothoscordum bivalve* co-bloomed with *Anemonella thalictroides* (L.) A. J. Eames & B. Boivin, *Camassia scilloides* (Raf.) Cory., *Castilleja coccinea* (L.) Spreng., *Cercis canadensis* L., *Delphinium tricorne* Michx., *Glandularia canadensis* (L.) Small, *Lithospermum canescens* (Michx.) Lehm., *Minuartia patula* (Michx) Mattf., *Oxalis violacea* L., *Phlox divaricata* L., *Prunus americana* Marsh., and *Viola sororia* (Willd.). *Allium stellatum* co-bloomed with *Aureolaria grandiflora* (Benth.), *Echinacea purpurea* (L.) Moench, *Heliotropium tenellum* (Nutt.) Torrey, *Rudbeckia missouriensis* Engelm. ex C.L. Boynt. & Beadle, *Ruellia humilis* Pohl ex Nees, *Solidago ulmifolia* Muhl. ex Wiild., *Trichostema brachiatum* L., and *Vernonia arkansana* DC (DW1-13 and DW24-31).

Pre-pollination Modes of Self-Isolation

Floral structure was observed and style growth was measured (see above) using digital callipers (Fisher Scientific, 14-648-17) to determine whether there was significant spatial isolation between stigmas and dehiscent anthers. To detect the presence of dichogamy, we compared the timing of anther dehiscence and stigmatic receptivity. Anther dehiscence was recorded in the tagged flower buds (see above). The timing of stigmatic receptivity was recorded by testing for peroxidase activity (Armbruster et al. 2002). Pistils from 25 flowers of *A. stellatum* and 14 flowers of *N. bivalve* on separate umbels were selected by age and submerged in a 3% hydrogen peroxide solution. If bubbles formed on the stigma it was recorded as receptive.

Bagged Flower Experiments

Bagging experiments in situ were conducted for both species to determine whether flowers self-pollinated in the absence of insect foragers. We selected 50 umbels of *N. bivalve* and 114 umbels of *A. stellatum* at random while their umbels were in bud and counted the number of buds on each inflorescence. Selected umbels were divided into two groups. One group was exposed to insects throughout the lifespan of the inflorescence, while the second group was isolated from pollinators in an organza bag for the lifespan of the inflorescence. Each umbel was labelled with a jeweller's tag and tied to a bamboo skewer so that bagged umbels did not collapse onto the ground.

To determine compatibility, hand-pollination experiments were conducted on selected flowers in bagged umbels of *N. bivalve*. Once flowers began opening in bagged umbels and reached the age of known stigmatic receptivity (see above), bags were removed temporarily for hand cross-pollinations. We labelled and then emasculated one flower per bagged umbel before its anthers dehisced to avoid contamination from mechanical self-pollination (see below). The flower was cross-pollinated with pollen from whole anthers removed from inflorescences at least one meter away from the bagged umbel. Pollen was applied by holding dehiscent anthers with forceps and rubbing them against the stigmas until a pollen film was visible to the naked eye. Self-compatibility was tested utilizing the remaining bagged flowers as mechanical self-pollination occurred reliably.

Hand-pollination experiments for *A. stellatum* were as above with one important exception. In this species, each bagged umbel contained one hand-crossed flower and one hand-selfed flower. In the second case, the receptive stigma was rubbed with pollen from an anther on the same inflorescence (geitonogamous cross) as anthers and stigmas did not mature at the same time in the same flower. That is why it was unnecessary to emasculate anthers in cross-pollinated flowers of *A. stellatum.*

Pollen-Pistil Interactions

We tested the success of each pollination treatment by examining pollen tube growth in pistils in both species. A selected number of exposed flowers were collected at least 48 hours after their stigmas became receptive in *N. bivalve* (N = 27) and *A. stellatum* (N = 25). Additionally, in *N. bivalve,* a selected number of bagged, automatous, self-pollinated flowers (N = 31) and half of the hand-crossed flowers (N = 33) were collected 48 hours after pollen was applied to their stigmas. In *A. stellatum,* half of the bagged, hand self-pollinated flowers (N = 21) and hand crossed-pollinated flowers (N = 22) were collected. We collected pistils 48 hours after hand-pollination because the stigmas and styles begin to dehydrate after this time. Dehydration destroys pollen tubes impairing their visualization under epifluorescence. The fixation, storage, and softening of pistils, as well as squashing and staining for viewing under epifluorescence using a Zeiss Axio, Imager.M2, follows Bernhardt et al. (1980). We viewed each specimen and recorded the number of pollen grains on stigmas, the number of pollen tubes in styles, the relative length of the longest pollen tube in relation to the length of the entire pistil, and the number of pollen tubes penetrating the ovary and its ovules.

Comparative Fruit and Seed Set

All remaining, labelled flowers and umbels were allowed to fructify. We returned to the site to record and collect fruit of *N. bivalve* two weeks after flowers were cross-pollinated.

We collected the fruit of *A. stellatum* five weeks after flowers were hand-pollinated. For unmanipulated, exposed and bagged umbels, we counted the number of fruit per umbel disregarding flowers that were used in the hand-pollination experiments. For hand-pollinated flowers, we recorded whether a fruit had formed. Capsules produced under all treatments were dissected and seeds were examined and counted. To calculate the conversion rate of ovules into seeds, we estimated the mean number of ovules per ovary. Roughly 15 flowers of each species that had been preserved in 70% ethanol (see above) were dissected and the number of ovules in each ovary was counted under a stereo microscope.

Statistical Analyses

For style length, an ANOVA was used to verify that data were normal and to check the homogeneity of variance of the data. Then a paired t-test was used to make comparisons between dates.

The number of pollen grains on stigmas for all treatments of both species was square root transformed and then compared with a two-way factorial ANOVA. Pollen grain germination in *N. bivalve* was compared using a one-way ANOVA. However, for all treatments in *A. stellatum,* an insufficient quantity of germination rates was calculated (due to a low frequency of stigmas that contained pollen grains) so statistical analyses were inappropriate. Data was zero-inflated for the proportion of ovules penetrated by tubes and the proportion of tubes that penetrated ovules so statistical analyses were inappropriate.

Given the various inequalities associated with the treatments and sample sizes, and also because fruit and seed set are measures of fitness (of which interspecific comparisons are meaningless), two-tailed t-tests for fruit set and a Kruskal-Wallis rank sum test for seed set was only performed between exposed and bagged umbels and species.

RESULTS

Floral Phenology and Lifespan

Nothoscordum bivalve flowers bloomed from April 23 until May 7 in 2018. Population flowering peaked on April 30 with 176 flowering stems in a square meter plot. *Allium stellatum* bloomed from August 30 to September 22 in 2017 and from August 28 to October 4 in 2018. Peak flowering occurred on September 11 with 78 flowering stems in a square meter plot (Fig. 1). The flowering of *N. bivalve* was relatively short-lived but flowering stems were at a higher density compared to *A. stellatum*. In comparison, the flowering of *A. stellatum* was twice as long as *N. bivalve*. Anthesis of *N. bivalve* overlapped with 12 other flowering species at the site, whereas anthesis of *A. stellatum* overlapped with 8.

The floral lifespan of *N. bivalve* was 2-3 days. Mean style length was 5.03 mm ($N = 12$, SEM $\pm$ 0.15) on the first day of anthesis and did not change significantly throughout the floral lifespan ($P = 0.3460$). Most anthers dehisced immediately upon tepal expansion and exposed their pollen on the first observation (first or second day of anthesis). Transition to empty, dehisced anthers occurred by the second observation (third or fourth day of anthesis; Fig. 2FIGURE 2).

Individual flowers of *A. stellatum* lasted 4-6 days. The mean style length grew from 4.61 mm ($N = 37$, SEM $\pm$ 0.25) on the first day to 6.18 mm ($N = 37$, SEM $\pm$ 0.17) 2-3 days later ($P = 0.0001$, Fig. 3). About half of the anthers finished releasing pollen on the first observation (first or second day of anthesis). By the second observation (second or third day of anthesis), all anthers had completed dehiscence (Fig. 4).

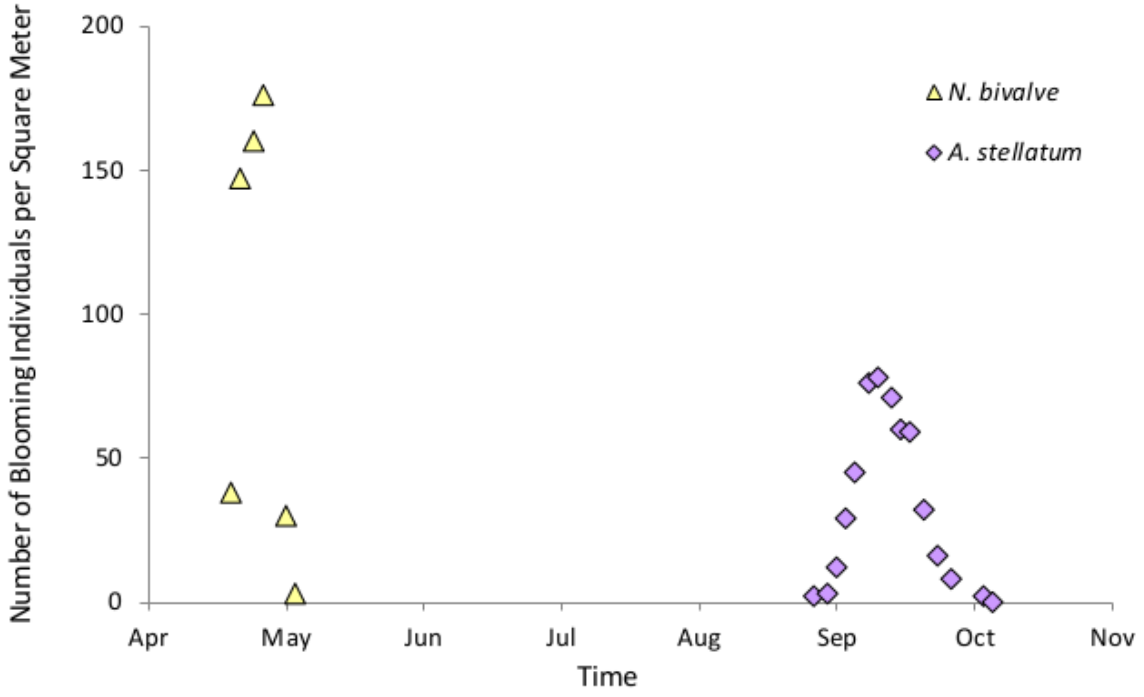

FIGURE 1. The number of umbels with at least one opened flower inside a square meter plot in 2018.

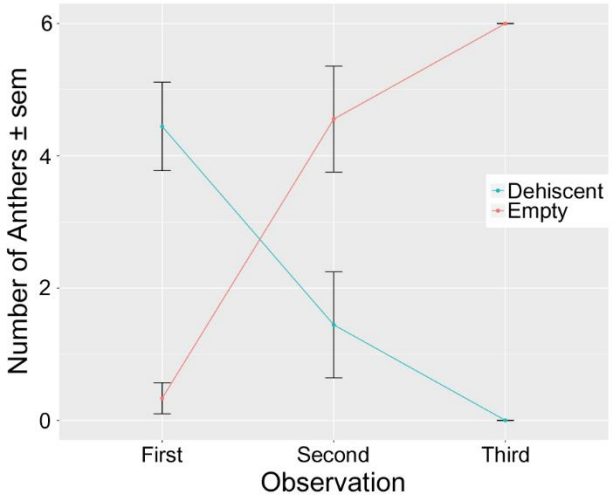

FIGURE 2. Mean number of dehiscent anthers offering exposed pollen vs. empty anthers in *N. bivalve* over 1, 3, and 6 days. Error bars are standard error of the mean ($N = 9$).

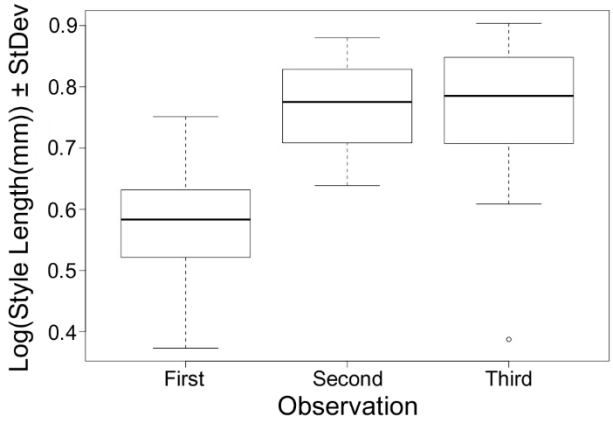

FIGURE 3. Log of style length in *A. stellatum* with observations made 2-3 days apart. Error bars are standard deviation ($N = 14$).

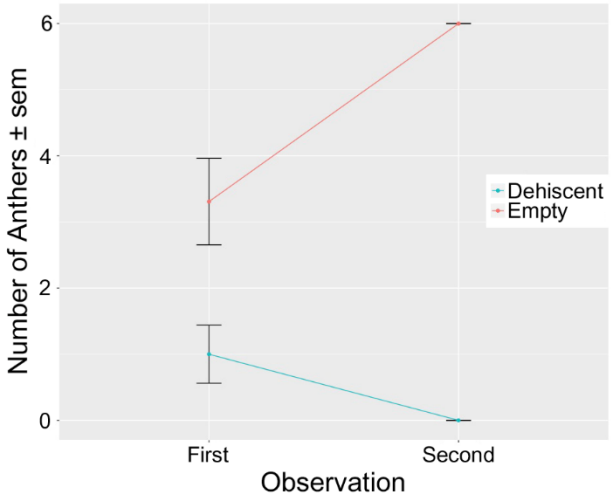

FIGURE 4. Mean number of dehiscent anthers with exposed pollen and emptied dehisced anthers in *A. stellatum* over 2-3 days. Error bars are standard error of the mean ($N = 14$).

Floral presentation

Floral attractants of *N. bivalve* included white tepals, yellow at their bases. Most flowers had a purple stripe running down the midvein on the abaxial sides of the three, outer tepals. Some flowers showed the same stripe on all six tepals. Flowers produced a faint–mild scent, reminiscent of commercial root beer and vanilla. During the floral lifespan, flowers usually opened between 12:30-15:00 and then closed sometime after 17:00. This process occurred each day for all flowers over their 2-3-day lifespans. At the end of the floral lifespan, tepals closed and didn't reopen. More reliably than the time of day or cloud cover, tepal expansion was most likely to occur when temperatures rose to 15°C or higher.

Nothoscordum bivalve had septal nectaries towards the base of the ovary (Fig. 5). In most cases, two nectaries were visible on each side of the septa with 6 glands for each ovary but numbers were variable (usually 3-9). Nectary positions also varied in this species. Some flowers produced glands midway along the ovary wall instead of basally and not all locules produced nectaries. Nectar collected between the base of the ovary and the three inner tepals.

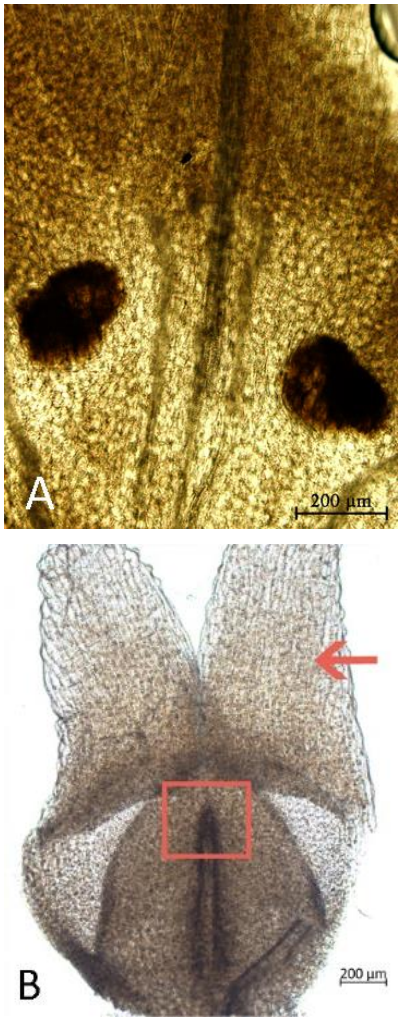

FIGURE 5. A) 2 Two darkened septal nectaries of *N. bivalve* near the bases of the two locules. B) an apical, septal nectary of *A. stellatum* (inside the red box) deeply recessed and covered by crest protrusions (see arrow).

TABLE 1. Abundance, sex, and mean body sizes of Hymenoptera collected on *N. bivalve* (2018-2019 collections pooled) and *A. stellatum* (2017 and 2018 collections pooled).

Insect Taxon	Number	% Females	Length (mm)	Width at Widest Part (mm)	Thoracic Depth (mm)	Body Size (mm^3)	Body size, Geometric Mean (mm)
Nothoscordum bivalve							
Andrena nasonii Robertson	1	0	7.78	2.06	2.35	37.66	3.35
Andrena nothoscordi	28	64	7.63	2.25	1.97	33.74	3.23
Andrena perplexa Smith	2	100	11.53	3.53	2.9	118.03	4.91
Andrena rubi Mitchell	2	0	8.88	2.66	2.32	54.68	3.8
Augochlorella aurata	3	100	6.7	2.22	1.65	24.54	2.91
Augochlorella persimilis	45	100	6.38	2	1.73	21.98	2.8
Augochloropsis fulgida Smith	1	100	7.65	2.99	2.3	52.61	3.75
Bombus bimaculatus Cresson	1	100	18.06	9.47	7.6	1299.8	10.91
Ceratina calcarata	2	0	6.36	2.14	1.49	20.28	2.73
Ceratina dupla Say	1	0	6.19	1.96	1.36	16.5	2.55
Ceratina mikmaqi Rehan and Sheffield	1	0	5.99	2.1	1.61	20.25	2.73
Ceratina strenua Smith	2	0	5.15	1.64	1.17	9.88	2.15
Hymenoptera (unidentified)	1	-	3.92	0.86	0.94	3.17	1.06
Lasioglossum versatum Robertson	4	100	6.64	2.2	1.93	28.19	3.04
Lasioglossum zephyrum Smith	1	100	5.84	1.69	1.23	12.14	2.3
Allium stellatum							
Apis mellifera Linnaeus	14	100	12.77	4.5	3.78	216.87	6.01
Augochlora pura Say	1	100	8.4	2.4	1.95	39.31	3.4
Augochlorella aurata	5	80	9.97	3.3	2.49	81.83	4.34
Augochlorella persimilis	11	91	5.81	1.84	1.53	16.32	2.54
Bombus fraternus Smith	1	0	20.84	8.34	7.59	1319.2	10.97
Bombus impatiens Cresson	3	100	15.59	6.34	5.38	531.76	8.1
Ceratina calcarata	4	75	6.97	1.92	1.42	19	2.67
Ceratina sp.	1	100	5.61	1.73	1.15	11.16	2.23
Coelioxys octodentata Say	1	100	9.81	3.41	3.02	101.03	4.66
Coelioxys sayi Robertson	1	100	12.02	3.47	3.59	149.74	5.31
Formicidae (unknown)	1	100	3.89	1.13	1.05	4.62	1.66
Heriades sp.	1	100	6.26	1.85	1.47	17.02	2.57
Lasioglossum callidum Sandhouse	1	100	6.92	2.16	1.65	24.66	2.91
Lasioglossum coreopsis Robertson	8	88	4.51	1.41	1.06	6.77	1.89
Lasioglossum hitchensi Gibbs	2	100	4.48	1.55	1.06	7.29	1.94
Lasioglossum tegulare Robertson	2	100	4.46	1.69	1.3	9.8	2.14
Megachile brevis Say	1	100	10.89	4	3.24	141.13	5.21
Megachile mendica Cresson	3	33	9.48	3.41	2.76	89.42	4.47
Xylocopa virginica Linnaeus	1	100	21.98	8.83	6.74	1308.1	10.94

In contrast, flowers of *A. stellatum* were pink to purple, produced no discernible scent, and did not close and reopen following the first day of tepal expansion. Flowers of *A. stellatum* contained 3 septal nectaries. Each nectary was found in a deep depression towards the apex of each of the three locules. A crest protruded from the top of the ovary and extended to the 3 inner tepals. Each crest formed a covering over a cavity where secreted nectar collected. Nectar was found between the ovary and tepals as described for *N. bivalve*.

Mean floral dimensions of *N. bivalve* include a style length of 5.03 mm (N = 12, SEM ± 0.15) as stated above, a tepal length of 10.38 mm (N = 15, SEM ± 0.35), and an umbel height of 9.84 cm (N = 15, SEM ± 0.47).

Mean floral dimensions of *A. stellatum* included a style length that grew from 4.61 mm (N = 37, SEM ± 0.25) to 6.18 mm (N = 37, SEM ± 0.17), a tepal length of 5.66 mm (N = 16, SEM ± 0.18), and an umbel height of 33.65 cm (N = 20, SEM ± 1.4).

Foraging Insect Diversity and Size

We collected 103 foraging insects on flowers of *N. bivalve*. The majority were female, solitary or facultatively social bees (e.g. *Augochlorella* and *Ceratina* species) representing three families including the Andrenidae (*Andrena*), Apidae (*Ceratina*), and Halictidae (*Augochlorella* and *Lasioglossum*). The exception was *Andrena nothoscordi* with males representing 36% of specimens. Species abundance and mean body size of Hymenoptera collected on both study species are given in Tab. 1. *Augochlorella persimilis* Viereck was the most commonly collected bee on *N. bivalve* with *Andrena nothoscordi* the second most frequently collected. Female bees were observed foraging for pollen and nectar and flew from inflorescence to inflorescence in a matter of seconds.

We collected 157 foraging insects on flowers of *A. stellatum*. The majority were female beetles and bees. Of the 67 specimens of *Chauliognathus pensylvanicus* (Cantharidae) collected and measured, 75% were female. These beetles foraged for nectar and copulated on flowers but remained stationary on individual umbels for hours. Their geometric mean of body dimensions was 2.2 mm. Bee collections included the families Apidae (*Bombus*, *Ceratina*, *Xylocopa*, and non-native *Apis*), Halictidae (*Augochlorella*, *Lasioglossum*), and Megachilidae (*Coelioxys, Heriades, Megachile*). These included solitary, facultatively social (*Augochlorella persimilis*, *Lasioglossum coreopsis*) and eusocial taxa (*Apis, Bombus* species). The majority of bees collected were females, including all worker specimens of *Apis mellifera* and *Bombus* species. The frequency of male specimens collected varied from 0% for 13 bee taxa to 9%-100% for *A. persimilis* (N = 11 specimens) and *Bombus fraternus* (N = 1), respectively. Collections indicated that *A. stellatum* was visited most frequently by *Apis mellifera*, *Augochlorella persimilis* and four *Lasioglossum* species. Bee behaviour differed from observations in *N. bivalve*. We noted that the smaller bees appeared to struggle as they forced their heads under the ovary crests.

Bee richness on *A. stellatum* (18 taxa) was slightly higher than on *N. bivalve* (15 taxa). *Augochlorella aurata* Smith, *A. persimilis*, and *Ceratina calcarata* Robertson were found foraging on the flowers of both study species. The geometric mean of body dimensions of Hymenoptera taxa collected on flowers of both species peaked between 2-3 mm (Fig. 6). Bee body size of foragers collected on *N. bivalve* were slightly smaller than those collected on *A. stellatum* due to the presence of the much larger members of the Apidae (e.g. *Apis, Bombus, Xylocopa*) foraging on the latter.

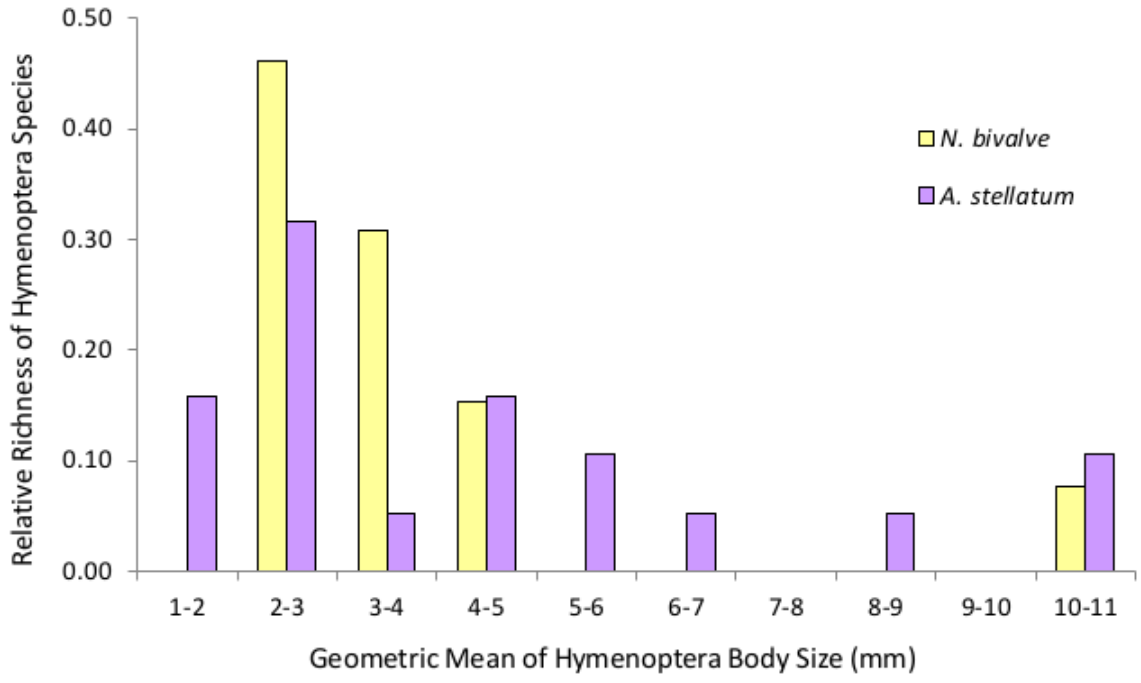

FIGURE 6. Relative richness of Hymenoptera species that carried host pollen according to geometric means of body dimensions.

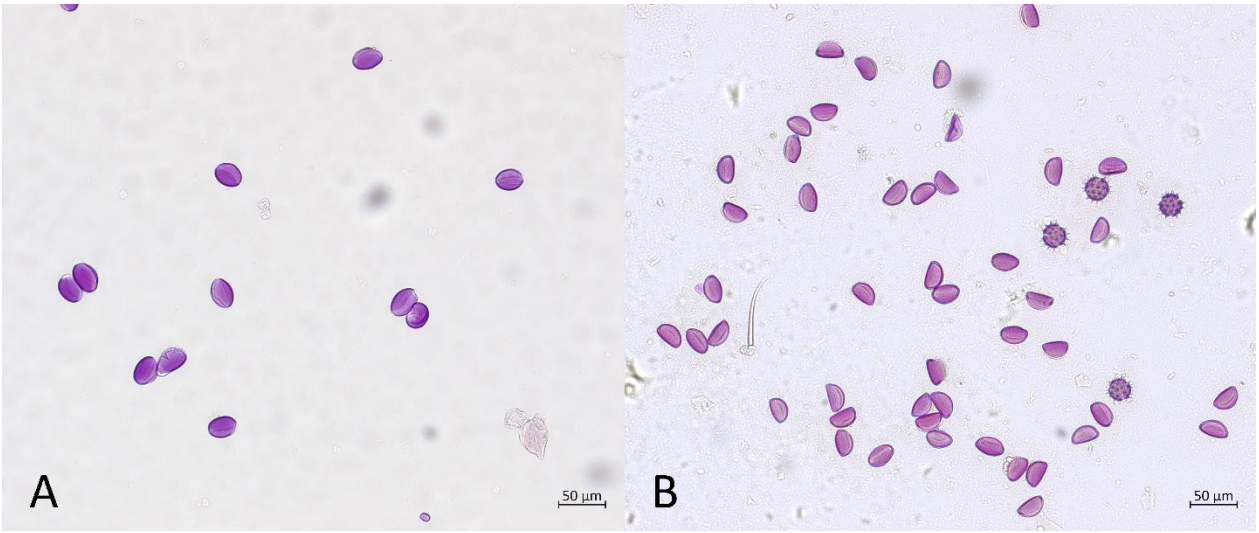

FIGURE 7. Representative sections of pollen loads. A) Pure pollen load from *Augochlorella persimilis* collected on *N. bivalve*. B) Mixed pollen load (note spiny grains of an unidentified member of the Asteraceae) from a *Chauliognathus pensylvanicus* collected on *A. stellatum*.

Pollen Load Analyses

Grains of both study species were psilate, monosulcate, and about 30 µm in length (Fig. 7). In *N. bivalve*, 80% of foraging insect taxa analysed carried *N. bivalve* pollen while 75% carried pure loads of *N. bivalve* pollen. Of the total (*N* = 103) insect specimens analysed, 88% carried only the pollen of *N. bivalve*.

In *A. stellatum*, 89% of all foraging insect taxa carried *A. stellatum* pollen while 67% carried pure loads of *A. stellatum* pollen. Of the total (N = 158) insect specimens analysed, 62% carried only the pollen of *A. stellatum* (Tab. 2TABLE 2).

Only 6% of specimens found on *N. bivalve* and 27% of specimens found on *A. stellatum* were carrying the pollen of co-blooming species mixed with the host-flower's pollen. Analyses of these mixed pollen loads (Tab. 3) shows foraging on different co-blooming species according to season. Bees of *N. bivalve* carried identifiable grains of *Oxalis violacea*, *Taraxacum officinale* (L.) Weber ex F.H. Wigg, and *Delphinium tricorne*. Almost all foraging species carried two pollen species if they carried mixed loads.

In *A. stellatum*, the majority of grains mixed with the pollen of the host flowers belonged to co-blooming members of the Asteraceae, followed by *Aureolaria grandiflora* and *Ruellia humilis*. As above, the majority of insects carrying mixed loads carried two, recognizable pollen morphotypes.

Pre-pollination Modes of Self-Isolation

Herkogamy was absent in *N. bivalve* since style length and anther height were the same and did not change over time. Dichogamy was also found to be absent in *N. bivalve*. The hydrogen peroxide test showed that only 3 out of 8 stigmas were active prior to anther dehiscence while 5 out of 5 stigmas showed activity in flowers in which anthers were dehiscent. Stigmas showing a positive response to the peroxidase test were characterized by a slightly swollen appearance (Fig. 8A). We observed a mechanism for mechanical self-pollination in *N. bivalve* during the opening and closing of tepals as described above. Field observations showed that during the period of perianth closure, the tepals pressed the dehiscent anthers against the receptive stigmas initiating self-pollination.

While style length and anther height were initially equal in *A. stellatum*, the style grew significantly as the flower aged (Fig. 3). Style length correlated positively with stigmatic receptivity. The mean length of styles that did not respond to the peroxidase test was 6.04 mm (SEM ± 0.215) while the mean length of receptive styles that did respond to the same test was 7.15 mm (SEM ± 0.375). Anther dehiscence occurred on the first and second day after the tepals opened (Fig. 4). Seven out of seven stigmas responded to the peroxidase test after the second day once all six anthers were empty and abscised. Open flowers in this species did not close their tepals at night.

Pollen-Pistil Interactions

Tab. 4 shows rates of pollen deposition and pollen tube growth in flowers exposed to insects vs. bagged, self-pollinating (mechanical autogamy) and bagged, hand cross-pollinated pistils of *N. bivalve*. Results indicate that pollen deposition on stigmas occurred regularly even in the absence of insect activity. In fact, when protected from insects, bagged flowers retained far more pollen grains on their stigmas (F = 5.456; df = 2, 151; P = 0.0052). The rate of pollen grain germination was consistent irrespective of bagged, open, and hand-pollination treatments (F = 1.795; df = 2, 83; P = 0.172). In all three treatments, we noticed that nearly all pollen tubes appeared to cease growing, becoming jagged and thickened as they approached the ovary. The few tubes that entered the ovary resumed straight and thin growth. Only a fraction of pollen tubes produced by cross-pollination entered the ovaries but none of these ever entered the ovules. The proportion of ovules penetrated by pollen tubes within 48 hours was highest in bagged flowers allowed to self-pollinate. The proportion of pollen tubes that penetrated ovules within 48 hours was also highest in bagged flowers (Fig. 8A, C, E; 9).

TABLE 2. Pollen load analysis for pollinators found on *N. bivalve* and *A. stellatum* (collections pooled as in Table 1). Values are total numbers of insect specimens collected with various pollen load compositions.

Insect taxon	*N. bivalve* only	*N. bivalve* + other	Other only	No pollen
Diptera				
Diptera (unidentified)	-	-	-	1
Bombyliidae (unidentified)	1	1	-	1
Hymenoptera				
Augochlorella persimilis	44	1	-	-
Andrena nasonii	1	-	-	-
Andrena nothoscordi	28	-	-	-
Andrena perplexa	1	1	-	-
Andrena rubi	1	1	-	-
Augochlorella aurata	3	-	-	-
Augochlorella fulgida	1	-	-	-
Bombus bimaculatus	-	1	-	-
Ceratina calcarata	2	-	-	-
Ceratina dupla	1	-	-	-
Ceratina mikmaqi	1	-	-	-
Ceratina strenua	2	-	-	-
Hymenoptera (unidentified)	-	-	-	1
Lasioglossum versatum	3	1	-	-
Lasioglossum zephyrum	1	-	-	-
Lepidoptera				
Hemaris diffinis	1	-	-	1
Protographium marcellus	-	-	-	1
Vanessa atalanta	-	-	-	1
Grand Total	91	6	0	6
	A.stellatum only	*A. stellatum* + other	Other only	No pollen
Coleoptera				
Chauliognathus pensylvanicus	48	18	-	-
Chrysomelidae (unidentified)	7	-	-	10
Diabrotica undecimpunctata	2	-	-	-
Diptera				
Syrphidae (unidentified)	2	-	-	-
Hemiptera				
Lygaeus sp.	1	-	-	-
Miridae (unidentified)	-	-	-	1
Hymenoptera				
Apis mellifera	11	3	-	-
Augochlorella aurata	5	-	-	-
Augochlorella persimilis	4	6	-	1
Augochlorella pura	1	-	-	-
Bombus fraternus	-	1	-	-
Bombus impatiens	-	3	-	-
Ceratina calcarata	1	3	-	-
Ceratina sp.	1	-	-	-
Coelioxys octodentata	1	-	-	-
Coelioxys sayi	1	-	-	-
Formicidae (unidentified)	-	-	-	1
Heriades sp.	-	1	-	-
Lasioglossum callidum	1	-	-	-
Lasioglossum coreopsis	5	1	1	1
Lasioglossum hitchensi	-	2	-	-
Lasioglossum tegulare	1	1	-	-
Megachile brevis	-	1	-	-
Megachile mendica	2	1	-	-
Xylocopa virginica	-	1	-	-
Lepidoptera				
Hesperiidae (unidentified)	4	-	-	-
Lepidoptera (unidentified)	-	-	-	2
Grand Total	98	42	1	16

TABLE 3. Mixed pollen load analysis for Hymenoptera found foraging on *N. bivalve* and *A. stellatum* (collections pooled as in Table 1). Values are total numbers of insect specimens collected with the specified pollen taxa in mixed loads.

Insect taxon	Pollen taxon				
	N. bivalve	*Oxalis violacea*	*Taraxacum officinale*	*Delphinium triocorne*	Unidentified Eudicots
Andrena perplexa	1	1	-	-	-
Andrena rubi	1	-	-	1	-
Augochlorella persimilis	1	-	-	1	-
Bombus bimaculatus	1	1	1	-	-
Bombyliidae (unidentified)	1	-	-	-	1
Lasioglossum versatum	1	-	-	-	1
Grand Total	6	2	1	2	2
	A. stellatum	*Aureolaria grandiflora*	*Ruellia humilis*	Unidentified Asteraceae	Unidentified Eudicots
Apis mellifera	3	1	-	3	-
Augochlorella persimilis	6	1	2	2	4
Bombus fraternus	1	1	-	1	1
Bombus impatiens	3	3	-	2	1
Ceratina calcarata	3	-	-	3	1
Heriades sp.	1	-	-	1	-
Lasioglossum coreopsis	1	-	-	-	1
Lasioglossum hitchensi	2	1	-	1	-
Lasioglossum tegulare	1	-	-	-	1
Megachile brevis	1	-	-	1	-
Megachile mendica	1	-	-	-	1
Xylocopa virginica	1	-	1	-	-
Grand Total	24	7	3	10	14

shows rates of pollen deposition and pollen tube growth in flowers exposed to insects vs. bagged, hand self-pollinated and bagged, hand cross-pollinated pistils of *A. stellatum*. In contrast, exposed flowers retained 2 to 3 times more pollen grains on their stigmas than hand self- and cross-pollinated flowers, respectively ($P = 0.0052$). The rate of pollen grain germination over 48 hours was highest in hand cross-pollinations. Rates in which pollen tubes in pistils outnumber pollen grains on stigmas are the result of pollen grains being dislodged during the softening and squashing process as tube development renders grains empty of cytoplasm. As in *N. bivalve*, only a fraction of pollen tubes reached the ovaries within 48 hours to penetrate ovules. The proportion of ovules penetrated by pollen tubes within 48 hours was highest in exposed and hand cross-pollinated flowers. The proportion of pollen tubes that penetrated ovules within 48 hours was also highest in exposed and hand cross-pollinated flowers (Fig. 8B, D, F; Fig. 9).

Comparative Fruit and Seed Set

In *N. bivalve*, flowers exposed to insects (76%, $N = 138$ flowers on 22 umbels) and bagged flowers allowed to mechanically self-pollinate (65%, $N = 141$ flowers on 20 umbels) showed a much higher rate of fruit set compared to bagged, hand cross-pollinated flowers (9%, $N = 23$ flowers; Fig. 11). There was no significant difference between fruit set in the flowers exposed to insects and the bagged, mechanically self-pollinating flowers ($P = 0.1763$, $T = -1.376$). The mean number of ovules per ovary was 13.94 ($N = 17$). The mean number of seeds per fruit in *N. bivalve* was 11 in exposed flowers ($N = 24$, SEM ± 0.43; 0.80 seeds per ovule), 12 in bagged, mechanically self-pollinating flowers ($N = 24$, SEM ± 0.90; 0.82 seeds per ovule), and 7.5 in bagged, hand cross-pollinated flowers ($N = 4$, SEM ± 2.5; 0.54 seeds per ovule; Fig. 11FIGURE 11).

In *A. stellatum*, fruit set was high and similar in flowers exposed to insects (46%, $N = 1582$ flowers on 37 umbels) and in bagged, hand-crossed flowers (42%, $N = 19$ flowers). Fruit set was far lower in both flowers that were bagged and unmanipulated (4%, $N = 1590$ flowers on 36 umbels) and flowers that were bagged and hand self-pollinated (5%, $N = 19$ flowers). Exposed and bagged flowers had absolute and very highly significant differences in fruit set ($P < 0.0001$). The mean number of ovules per ovary was found to be 6.25 ($N = 12$). In fruits produced by each treatment, there was a mean of 3.0 seeds in flowers exposed to insects ($N = 22$ fruits, SEM ± 0.27; 0.47 seeds per ovule), 2.2 seeds for

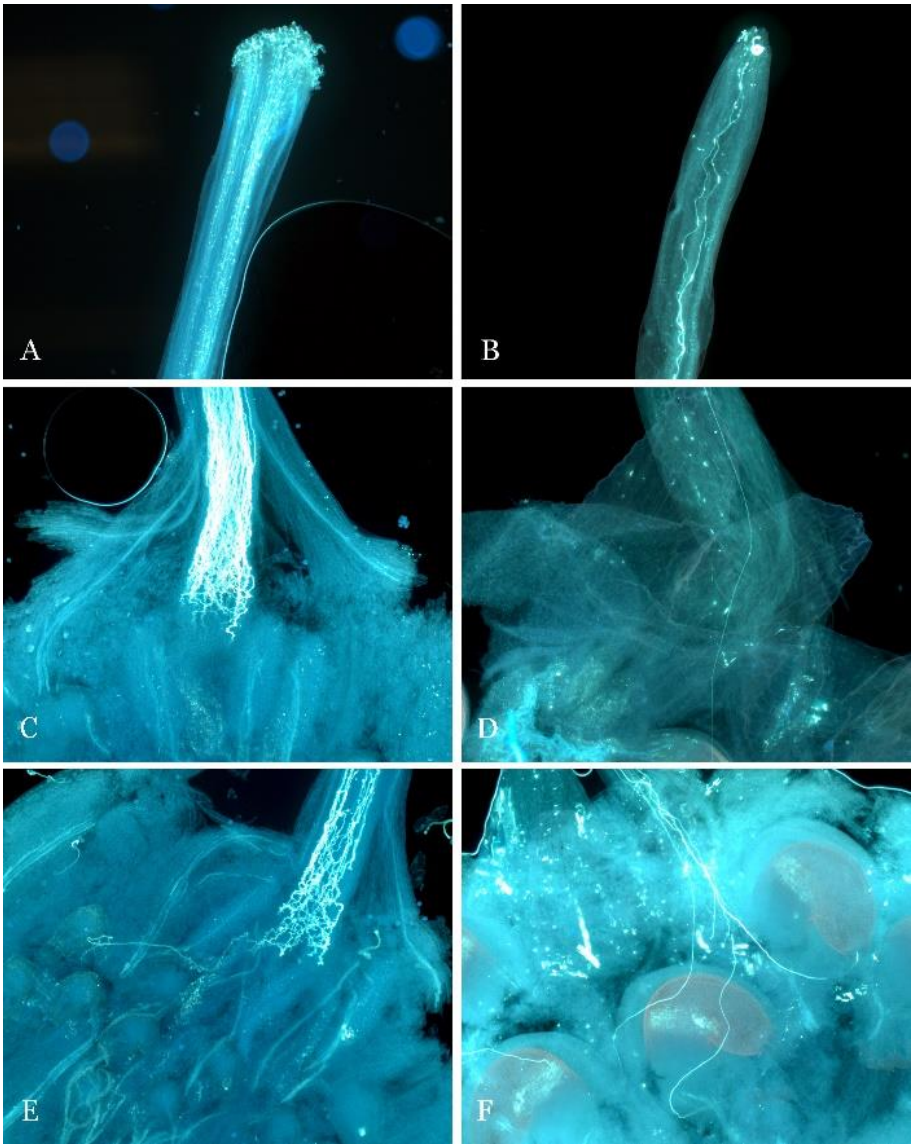

FIGURE 8. Epifluorescence of pollen tubes in exposed pistils *of N. bivalve* (A, C, E) and *A. stellatum* (B, D, F). (A, B) Deposition on stigma and initial penetration of the style. (C, D) Tubes at the bases of styles (note jagged, arrested growth in C). (E, F) Tube penetration of ovaries and ovules (note jagged growth discontinues upon entering the ovary in E).

bagged, unmanipulated flowers (N= 19 fruits, SEM ± 0.35; 0.35 seeds per ovule), 2.0 in bagged, hand self-pollinated flowers (N= 1; 0.32 seeds per ovule), and 2.6 in bagged, hand cross-pollinated flowers (N = 7 fruit, SEM ± 0.57; 0.41 seeds per ovule). According to a Kruskal-Wallace rank sum test, seed set was not significantly different between exposed and bagged fruit for both *A. stellatum* and *N. bivalve* (P = 0.6403).

DISCUSSION

Floral Phenology and Lifespan

The flowering period of *N. bivalve* occurred in April and May as predicted by Baskin & Baskin (1979). Anthesis was relatively short, lasting 2 weeks. This is typical of many vernal flowering species (Seymour 1969).

In contrast, *A. stellatum* bloomed from August through October, although it has been recorded in bloom as early as July in other regions (McNeal Jr & Jacobsen 2002). Anthesis in *A. stellatum* was twice the length of *N. bivalve*. This slower strategy suggests *A. stellatum* may have evolved to ensure insect-mediated modes of pollination where populations are less dense and possibly less clonal than those of *N. bivalve* based on our plots. Although our two populations of *N. bivalve* and *A. stellatum* are sympatric, share similar floral morphologies, share a few pollinator species, and belong to the same lineage, it is obvious that floral phenology is currently the main factor preventing interspecific hybridisation.

The shorter floral lifespan of *N. bivalve* compared to *A. stellatum* was expected for a species with a shorter flowering period. Also, the self-compatible flowers of *N. bivalve* probably live just long enough to self-pollinate via tepal closure on the first and second night. Trends towards facultative-obligate self-pollination tend to shorten floral lifespan (Aizen 1993). Conversely, Gao et al. (2015) concluded that dichogamous species with perfect flowers, such as *A. stellatum,* are more likely to have longer floral lifespans as more time is needed to keep male and female reproductive

organs separated. In *A. stellatum,* styles increased in length over the longer floral lifespan until the stigmas surpassed the height of dehiscent, aging anthers. This may be interpreted as a weak mode of herkogamy coupled with protandry. While increases in style length were small, they may still help prevent insect-mediated autogamy and contribute to self-isolation in this species due to the relatively small body sizes of the dominant pollinators (see above).

Floral Presentation

In *N. bivalve*, tepal displays were consistent with McNeal Jr & Jacobsen (2002) but our descriptions of both floral scent and septal nectaries are novel. The presence of septal nectaries in this species is not particularly surprising as the genus *Nothoscordum* represents a comparatively recent segregation from *Allium* ss in which septal nectaries are common (Rahn 1998). However, nectaries of *N. bivalve* were highly variable in size, location, and number. Therefore, it is possible that there is only a weak selective pressure on nectary development in this population since insects are not required to effect pollination (see above). While tepal length was in agreement with McNeal Jr. & Jacobson (2002), we note that umbel heights in our population were 25-50% shorter than described previously. Perhaps our plants invested less photosynthate in the construction of scapes as they grew in sites with little topsoil on porous limestone. A second explanation is that this population grew in an area with little seed predation so tall scapes were not selectively advantageous (Cariveau et al., 2004).

Our inability to detect a scent in flowers of *A. stellatum* appears consistent with the lack of description in the literature. The presence of septal nectaries with crests was reviewed by Choi et al. (2011) for *A. stellatum*. What is the function of these crests? We suspect they restrict the foraging of certain nectar-robbing insects that regularly visit small, radially symmetrical flowers. Specifically, insects that are not strong enough or lack proboscoides long enough to push under the crests will be denied access to the reward. As these plants bloom in late summer, they may be exposed to more nectar thieves including tiny flies, thrips, and parasitic wasps. Furthermore, pollinators struggling to place their heads under ovary crests may be more likely to make passive contact with reproductive organs. Concealed nectaries are associated with pollination by bees, butterflies, and moths (Willmer 2011). In *A. stellatum*, our measurements of tepal length and umbel height agreed with McNeal Jr & Jacobson (2002).

Foraging Insect Diversity, Size, and Pollen Load Analyses

Our population of *Nothoscordum bivalve* was bee-pollinated but the foraging habits of 14 taxa and bee genders varied. Females of *Andrena nothoscordi* (Andrenidae) typically specialize on the collection of pollen from the host species (i.e. oligolectic) but a few have been collected on *Physaria filiformis* (Rollins) O'Kane & Al-Shebaz (Brassicaceae; Edens-Meier et al. 2011). We also note that 36% of our foragers were males. This bee species made up 30% of the specimens caught and analyzed. In contrast, *Augochlorella persimilis* (Halictidae) made up 49% of bee specimens but no males were collected. In the literature, females of North American, *Augochlorella* species collect pollen from a wide variety of angiosperms

TABLE 4. Analysis of pollen tube development in pistil squashes of hand pollinated flowers in *N. bivalve* and *A. stellatum.*

	N. bivalve			*A. stellatum*		
	Exposed	Auto-Self	Hand-Cross	Exposed	Hand-Self	Hand-Cross
Number of pistils sampled (N)	27	31	33	25	21	22
Number of pollen grains per stigma	78	114	58	2.8	0.67	0.32
Number of pollen tubes per style	28	17	14	2.8	1.2	1.8
Proportion of grains that germinated	0.40	0.23	0.35	1.2	2.5	5.0
Proportion of pistils with pollen tubes	0.96	0.94	0.91	0.72	0.52	0.45
Proportion of longest tube length to style length	0.86	0.74	0.52	0.94	0.72	0.82
Proportion of ovaries penetrated by tubes in pistils with tubes	0.41	0.35	0.33	0.88	0.64	0.50
Proportion of ovaries with penetrated ovules to styles with tubes	0.074	0.14	0	0.81	0.18	0.50
Proportion of ovaries with penetrated ovules to ovaries with tubes	0.18	0.40	0	0.93	0.29	1.00
Proportion of ovules penetrated by tubes	0.017	0.049	0	0.18	0.067	0.091
Proportion of tubes that penetrated ovules	0.0083	0.048	0	0.54	0.14	0.48
Proportion of ovules penetrated by tubes to number of grains	0.0020	0.0063	0	0.59	0.83	1.70

(Ordway 1966; Clinebell & Bernhardt 1998; Boyd et al. 2011). It is interesting that all but one specimen of *A. persimilis* carried pure pollen loads. This suggests that, in early spring, generalist foraging bees have fewer options and, in fact, only 6% of all bee specimens (N = 95 bees) collected on *N. bivalve* carried pollen of other co-blooming species. The temporary "faithfulness" of these bees to *N. bivalve* may also have been due to the sheer size of the flowering population. The small sizes of bees visiting *N. bivalve* proved relatively uniform. This may be due to the relative scarcity of native, large-bodied bee taxa flying at this time of year.

One species of beetle, *Chauliognathus pensylvanicus*, was commonly collected on *A. stellatum*. While this insect was observed foraging and collected only in the second year of this study, specimens composed half of all pollen loads analysed from 27 insect taxa. Bee foragers on *A. stellatum* represented 18 bee taxa of which six included males. None of the bee taxa collected and identified on *A. stellatum* were associated with oligolectic foraging on *Allium* species. The frequency of bees collected on *A. stellatum* carrying pollen of at least one other co-blooming species was 41% (N = 61 bees) suggesting that generalist bees foraging from late summer through autumn have a far broader choice of flowering species. Bee body size on *A. stellatum* was more variable as flowers attracted some of the larger members of the Apidae ss including naturalized *A. mellifera*. By this time of year, *Apis* hives and *Bombus* colonies should be at their peak in the production of neuter workers.

While *A. stellatum* received visits from three more bee taxa than *N. bivalve*, it received fewer individuals of each taxon compared to the intense visitation of *A. northoscordi* and *A.*

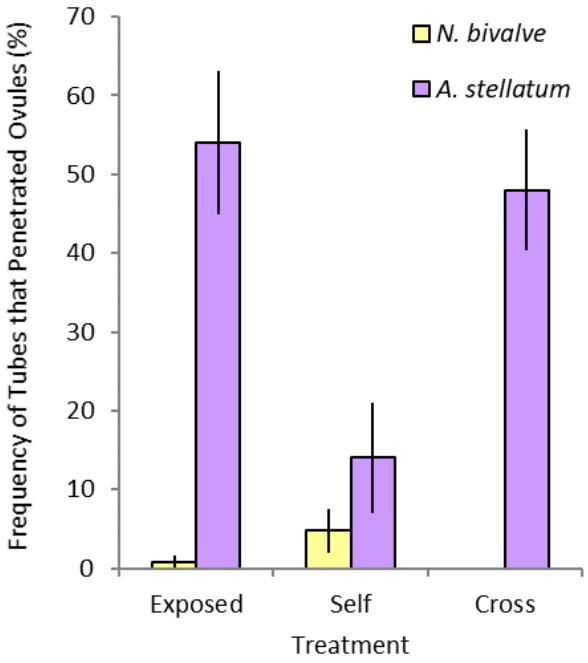

FIGURE 9. Percent of ovules penetrated by tubes compared to the number of pollen tubes per style. In *N. bivalve*, no tubes in cross-pollinated flowers penetrated ovules. Error bars are standard error of the mean.

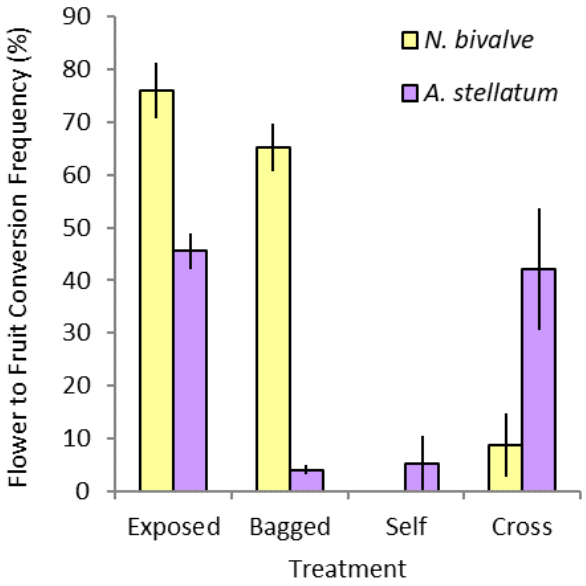

FIGURE 10. Mean percentage of fruit per flower per umbel of *N. bivalve* (*N* = 20-23) and *A. stellatum* (*N* = 19-37). Error bars are standard error of the mean.

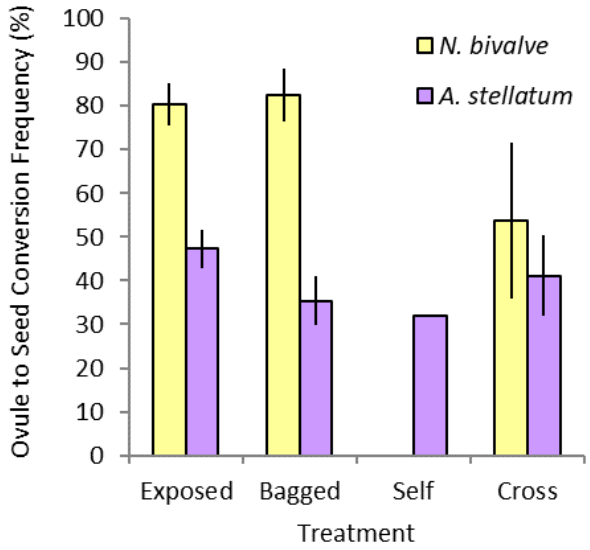

FIGURE 11. Mean percentages of seed per ovule per flower of *N. bivalve* (N = 4-24) and *A. stellatum* (N = 1-22). Error bars are standard error of the mean

persimilis on *N. bivalve*. *Augochlorella aurata*, *A. persmilis*, and *Ceratina calcarata* were shared by *N. bivalve* and *A. stellatum*. Since individual bees do not have lifespans that persist from April–September, this supports the literature reporting that these three species are either multivoltine, facultatively social, or eusocial (Ordway 1966; Rehan et al. 2016; Dibble et al. 2018).

Pre-pollination Modes of Self-Isolation

Dichogamy was absent in *N. bivalve*. This was unexpected since most species of *Allium* ss are protandrous (Rahn 1998). The function of flower closure at night (nyctinasty) has received several interpretations. Darwin & Darwin (1880) suggested it was an adaptation that reduced heat loss. Stahl

(1897) thought that closed flowers were less likely to accumulate water deposited by dew or rain. Grubb & Jackson (2007) concluded that closed perianths shielded ovaries from nocturnal herbivores. Minorsky (2019) even argued that closing petals makes it easier for predators to detect and hunt florivores by reducing visual, auditory, and physical clutter. However, in our study, tepal closure in *N. bivalve* forced dehiscent anthers to contact receptive stigmas functioning to ensure mechanical autogamy.

In contrast, *A. stellatum* is protandrous, supporting the simple observations of Molano-Flores et al. (1999). Our description of the elongation of the style over the floral lifespan is novel and suggests a weak form of herkogamy. *Allium stellatum* shows a trend towards outcrossing.

Pollen-Pistil Interactions

While pollen tube growth in *N. bivalve* became arrested at the bases of styles, this does not necessarily suggest that tubes showed late-acting rejection. This effect has been documented in many plant families (Sogo & Tobe 2006). In some *Quercus* species, pollen tube growth halts for over a year before entering the ovary (Cecich 1997). Arrested growth of pollen tubes may indicate that megasporogenesis is incomplete (Sogo et al. 2004; Sogo & Tobe 2006). It is particularly common in orchids (Arditti 1992) and in other lineages including some wind-pollinated trees blooming in cold weather (Arditti 1992; Sogo et al. 2004; Sogo & Tobe 2006). Sogo & Tobe (2006) suggested it is selectively advantageous when pollen tubes pause at the bases of styles because it gives late-arriving grains a chance to "catch-up". Consequently, all tubes are provided with an equal start when they enter the ovary which allows female selection of the fittest sperm (Sogo & Tobe 2006).

When pollen tube growth slows, tubes naturally become jagged and erratic. This is associated with late-acting incompatibility in other species (Edens-Meier et al. 2010; Lu et al. 2014). However, in *N. bivalve* jagged growth occurred in all pistils regardless of treatment. This included our bagged self-pollinations which showed high fruit and seed set. Therefore, jagged and erratic tubes in *N. bivalve* are probably little more than the developmental consequence of impeding tube growth temporarily at the bases of the style.

Arrested and jagged pollen tube production was not seen in *A. stellatum*. Far fewer pollen grains were deposited on stigmas and far fewer grains penetrated pistil tissue compared to *N. bivalve* regardless of treatment. Interestingly, the proportion of tubes that penetrated ovules in *A. stellatum* was much higher than in *N. bivalve*. However, if we could have allowed for > 48 hours to pass before harvesting flowers, this frequency would have probably increased in *N. bivalve* given its higher rate of fruit set.

In both species, the proportion of ovules penetrated by pollen tubes was lower than the conversion rate of ovules into seeds in all treatments. The most likely explanation for this is that our epifluorescence microscopy underestimated the actual number of penetrated ovules because we harvested flowers only 48 hours after hand-pollinations. High rates of fruit set suggest that pollen tubes may simply have not had sufficient time to reach ovules.

Comparative Fruit and Seed Set

Fruit set and seed set in populations of *N. bivalve* were 30% and 33% higher, respectively, than in *A. stellatum*. We presume that some fruits were lost in both populations due to factors including predation, disease, and weather. Patterns of reproductive success in the two species appeared to diverge along predictable lines associated with high yields in obligate inbreeders vs. lower yields in taxa that are primarily outcrossers (Whitehead et al. 2018). We note there are 96 *Allium* species found in North America and different species flower from spring through autumn (McNeal Jr & Jacobsen 2002). Future research can focus on whether the success of fruiting and seed set shows a correlation between flowering period and breeding systems.

Compatibility Systems

Based on our treatments, *N. bivalve* is obviously self-compatible as well as mechanically and obligately autogamous (sensu Ornduff 1969) at our site. Variations in self-compatibility, coupled with mechanical self-pollination as a "failsafe mechanisms" (sensu Schemske et al. 1978) occurs in a number of other spring ephemeral herbs as unpredictable weather lowers ambient temperatures and increases cloud cover depressing the activity of emergent, anthophilous but poikilothermic insects. This was best documented in *Sanguinaria canadensis* L. (Papaveraceae), which often flowers before *N. bivalve*, and may also attract pollen vectors in the genus, *Andrena*. Lyon (1992) noted that flowers of *S. canadensis* were dichogamous but mechanical self-pollination occurred regularly on the third day of the floral lifespan if ambient temperatures and light levels were too low to stimulate bee activity.

Our extremely extensive population of *N. bivalve* may be founded on both seed set following self-pollination and vegetative propagation via bulblets (Baskin & Baskin 1979). The most notable result of our two experimental series on fruit set and pollen tube penetration was that *N. bivalve* appears to express a novel mode of outbreeding depression (see review in Waser & Price 1994). While we observed penetration of ovules, fruit set, and seed set in hand-mediated crosses, these occurred at lower rates compared to bagged, autogamous flowers and flowers exposed to insects. We hand cross-pollinated flowers while stigmas were receptive and found large amount of pollen grains covering pistils and similar rates of grain germination between hand-pollinated and autogamous flowers. This verifies that our method of hand-pollination did not confound our results. Furthermore, we did not observe any damage to the pistil that could have been caused by emasculation, such as early wilting or infection. Therefore, our results suggest that *N. bivalve* expresses outbreeding depression as a product of intraspecific cross-incompatibility, a rare and understudied breeding system. This is not to be confused with interspecific cross-incompatibility (Maune et al. 2017) or even obligate autogamy (sensu Ornduff 1969).

The form of compatibility in *N. bivalve* appears similar to other known cases of intraspecific cross-incompatibility. However, those are cases of inbreeding depression caused by crossing self-incompatible but genetically similar cultivars

within the same domesticated species (Egea & Burgos 1996; Gómez et al. 2019). Intraspecific cross-incompatibility has never been observed in self-compatible populations, wild populations, or populations that have experienced outbreeding depression to our knowledge.

The fact remains that this species continues to offer attractants and rewards indicative of insect-pollination even though insects are not needed to effect self-pollinations. We note that this species has a broad and southerly distribution from the southern half of North America down into South America (McNeal Jr & Jacobsen 2002). In a list of species reviewed by Whitehead et al. (2018), breeding systems varied throughout natural distributions at the intraspecific level, from inbreeding to outcrossing. The next phase of field research should be to compare breeding systems in our population to more southerly populations. We suspect that obligate inbreeding and cross-incompatibility in our population of *N. bivalve* evolved more recently when the species spread northward into colder regions with unpredictable weather patterns depressing activities of vernal pollinators. Natural selection may have favoured changes in breeding systems while some traits that encouraged insect visitation remained. Perhaps this has also helped to expand the distribution of *Andrena nothoscordi* further north.

Based on the results from our hand-pollinations, *A. stellatum* showed a trend towards late-acting self-incompatibility. Pollen tubes produced following self-pollination entered ovaries but often failed to penetrate ovules within 48 hours while tubes in cross-pollinated flowers succeeded. This is inconsistent with the preliminary result of Molano-Flores et al. (1999). However, the previous authors assessed compatibility indirectly using pollen-ovule ratios as described by Cruden (1977). The only other test for breeding systems in the genus *Allium* is in the domesticated *A. cepa*, which is self-compatible (Currah & Ockendon 1978). There seems to be variation in the breeding systems of members of the genus, *Allium* and much more testing remains to be done.

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