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Author(s): J. D. Gardner and M. Spivak

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A Survey and Historical Comparison of the Megachilidae (Hymenoptera: Apoidea) of Itasca State Park, Minnesota

J. D. GARDNER¹ AND M. SPIVAK

Department of Entomology, University of Minnesota, 1980 Folwell Avenue, 219 Hodson Hall, St. Paul, MN 55108

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ABSTRACT The University of Minnesota Insect Collection holds a rich collection of bees from Itasca State Park, MN, from 1937 and 1938. This collection formed the historical baseline data for comparison with a new survey conducted from 2011 to 2013, to measure changes in bee species over the last 75 yr. Bees were collected with timed net surveys and trap nests at eight different sites within the park. Megachilidae were the focal family for the current study, due to their importance as commercial pollinators and their unique nesting habits. Species richness and diversity of Megachilidae in the new survey were both significantly lower than that of the historical collection but were not significantly different when species accumulation curves were extrapolated to estimate the true species richness. Eleven species in the historical collection were not rediscovered, while three species not previously collected in Itasca State Park were found in 2011–2013.

KEY WORDS bee, diversity, long-term change

Bees in decline have been a major topic of concern in recent years. The Forgotten Pollinators (Buchmann and Nabhan 1996) brought the issue to public attention, and it became a media sensation when the honey bee Colony Collapse Disorder was discovered in 2006. Although the term “CCD” only applies to honey bees, wild bees are also suffering losses when compared with historical data. This has been well-studied in bumble bees, with particular species of concern identified (Williams 1982, Berenbaum et al. 2007, Kosior et al. 2007, Colla and Packer 2008, Goulson et al. 2008, Grixti et al. 2009, Cameron et al. 2011). Trends are not so consistent for the remaining wild bees (Bartomeus et al. 2013). Some studies documented clear species declines (Frankie et al. 2009, Burkle et al. 2013), some found little change or a blend of declines and increases (Marlin and LaBerge 2001, Banaszak et al. 2003, Tanács et al. 2009), and at least one documented an increase in bee species richness and diversity (Grixti and Packer 2006).

More research is needed to document wild bee declines, and determine what the primary factors precipitating those declines are and which species or higher taxa should be considered of particular concern. A substantial historical collection of bees from Itasca State Park, MN, a protected pine forest and wetland area since 1891, provided an opportunity to resurvey the site and compare bee fauna across time.

The family Megachilidae was the focus of this study, although other bees were collected and will be analyzed in a future publication. Megachilidae are found

worldwide wherever there are flowers to support them. There are slightly >4,000 described species worldwide and 600 in the United States, making it the third largest of seven recognized bee families (Ascher and Pickering 2012). The number of species in Minnesota is unknown, but if the bees of Wisconsin are a reliable guide, it is probably close to 60 or 70 (Wolf and Ascher 2009). Most nest aboveground in pithy stems or wood tunnels, such as those left by wood-boring beetles (Cane and Neff 2011). Except for the small basal subfamilies Lithurginae, Fideiinae, and Pararhophitinae, Megachilidae share the unique behavior of using foreign material from the environment to line their nests, as opposed to the glandular secretions used by other bees (Litman et al. 2011).

Megachilidae were selected for this study because of their importance as managed pollinators (Bosch and Kemp 2002, Pitts-Singer and Cane 2011) and their possibly greater sensitivity to habitat changes. Aboveground megachilid nests are a more changeable resource than the soil used by ground-nesting bees, either positively through ecological succession (Grixti and Packer 2006) or negatively through urbanization (Frankie et al. 2009).

Itasca State Park

The University of Minnesota has a long history of collecting at Itasca State Park, making this an ideal location at which to study changes in bee populations. Itasca State Park covers ≈132 square kilometers in northern Minnesota, primarily within southern Clearwater County but touching northern Becker and Hub-

¹ Corresponding author, e-mail: gard0228@umn.edu.

bard Counties. It is important to distinguish Itasca State Park from Itasca County, which is actually located over 60 kilometers to the east. The park is heavily forested with pine and aspen, and also contains numerous lakes and wetlands. Bee collecting for the current study focused on roadsides, paths, and clearings, where flowers were most abundant.

The University of Minnesota Insect Collection contains at least 1,398 bees from the park, including 293 Megachilidae, from 1911 to 1986. Most of this collecting took place in the early years and steadily dropped off after ≈1940, even though the University continues to maintain the Itasca Biological Station and Laboratories and holds summer session classes there to this day. This early collecting is almost certainly due to the influence of Alexander Hodson and Clarence Mickel, who co-taught Field Zoology at Itasca from 1936 to 1941, and one of Mickel's graduate students, H.E. Milliron, who worked on bumble bees (Milliron 1939).

The years 1937 and 1938 represent a peak in bee collecting, coinciding with a time when three of Clarence Mickel's graduate students (H. R. Dodge, A. E. Pritchard, and H. E. Milliron) were with him while he was teaching a summer course in Field Zoology. In all, 702 bees, including 174 Megachilidae, were collected during those 2 yr. A majority of these bees (64%) were collected by Mickel and his graduate students; Milliron was especially prolific, accounting for just >50% of the 1937–1938 bees. The other bees were collected by Mickel's Field Zoology students, comprising 59 known individuals over both years.

Although there are no class syllabi available for this Field Zoology course (only a brief mention in the 1937–1938 University of Minnesota Bulletin, available on the UMN Digital Conservancy), it seems likely that Mickel assigned an insect collection for his class, and the bees (along with other insects) were collected to fulfill that requirement. Milliron was at the park while studying methods of rearing a local caddisfly and its ichneumonid parasitoid, and indicated that he did some collecting via sweeping vegetation while searching for the parasitoid (Mickel and Milliron 1939). It is unlikely that all the bees were incidentally collected during this search, but it does give a possible clue to the methods Milliron used. Other clues suggesting a net collection include most of the bees having fluffy, un-matted hair, and some of them still carrying pollen.

Project Objectives

The purpose of this project was twofold. First, to conduct a survey of Itasca State Park, Minnesota, and assess current species richness of the megachilid bees, and second, to compare results with bees in the University of Minnesota Insect Collection, collected in the same location from 1937 to 1938. It is through historical comparisons such as this that changes in species composition can be detected, and if necessary, conservation practices developed.

Materials and Methods

Study Sites. In 2010, an initial trap nest trial was conducted on the southern edge of the University of Minnesota Biological Station and Laboratories (UMBSL) grounds. A cleared strip of land, ≈600 m in length, was chosen for study because it receives ample direct sunlight, contains abundant floral resources, and has several large patches of bare, sandy soil, all conditions favorable to bees. *Trifolium* spp. (Fabaceae), *Erigeron* spp. (Asteraceae), *Berteroa incana* L. (Asteraceae), and *Melilotus officinalis* (L.) Lamarck (Fabaceae) were the most common flowering plants in this area. The site suffers major disturbance when it is mowed in late June to control plant growth, destroying many of the flowers, but it is mostly recovered by mid-July.

In preparation for the 2011 field season, I (J.D.G.) examined Google Earth satellite images of Itasca State Park to locate clearings where flowers and bees would likely be found. Seven study sites throughout the park were found and deemed suitable for trap nesting, net collecting, or both, in addition to the 2010 UMBSL site, which is henceforth designated as site 1 (Fig. 1). Sites suitable for net collections were larger and contained more flowers than trap nest-only sites. The study sites, eight in total, represented a wide range of the habitat conditions found in Itasca State Park.

Site 2, the most densely forested location, was on a service road directly adjacent to a small lake, a short distance from the eastern park boundary. It was chosen for the early spring-blooming willows next to the lake; later in the spring, *Trillium grandiflorum* (Michaux) Salisbury (Liliaceae) and other wildflowers bloom along the overgrown road.

Site 4 was in a large (>5,000 m²), dry forest clearing alongside another overgrown service road north and west of Lake Itasca. *Trifolium* spp., *Fragaria* spp. (Rosaceae), and *Achillea millefolium* L. dominated along the road. The east end of the clearing was mostly tall ferns, but the west end had only low vegetation, including *Vaccinium* spp. (Ericaceae), *Rubus* (*Rubus*) spp. (Rosaceae), and *Cirsium discolor* (Muhlenberg ex Willdenow) Sprengel (Asteraceae) among many other flowers. Sites 3 and 5 were to the east and west of site 4, respectively, in smaller clearings along the same path, with similar vegetation.

Site 6 was a roughly 0.36 km² expanse of rolling hills covered in grass and young pines, interspersed with small ponds, near the southwestern corner of the park. According to old maps at UMBSL, the site used to be a farm, and was probably abandoned sometime in the 1950s. It is now in the process of being restored to pine forest, but at the moment remains a mostly open meadow dominated by *Solidago* spp. (Asteraceae), *Fragaria* spp., *Campanula rotundifolia* L. (Campanulaceae), and *Leucanthemum vulgare* Lamarck (Asteraceae).

Site 7 was just west of Lake Frazier, near the middle of the southern edge of the park, along a forested trail used for snowmobiles in the winter and along the side of Highway 113. *Rubus* and *Fragaria* were extremely abundant in the forested area, while *Melilotus officinalis*



Fig. 2. Observation block trap nest with bamboo bundle.

bility to bees. The shelters were attached to 5-foot (1.5 m) metal garden stakes pounded into the ground, holding the shelters \approx 4 feet (1.2 m) above the surface. The stakes were regularly smeared with a thin layer of petroleum jelly and the immediate area cleared of tall vegetation to discourage ants and spiders from climbing up. When possible, the shelters were placed in dry ground with sparse vegetation, oriented to face east or southeast, with trees to the west to provide shade in the afternoon.

In 2011 and 2012, bundles of bamboo were also attached to the top of each shelter box (Fig. 2). Bamboo of 0.5" (1.27 cm) average outer diameter was cut into 12- to 22-cm lengths and held in 6" (15.24 cm) diameter plastic pipe segments, wide enough to fit 50–60 bamboo sticks inside. The pipe served as a shelter and was secured to the wooden shelter box with steel wire.

Nests were set up on-site in April and retrieved in September or October, when the bees had finished nesting. Over the winter, cocoons and prepupae were removed from the nests, put in individual gelatin capsules, labeled (Fig. 3), and stored at 4°C until spring. Occupied, but not visibly capped, bamboo nests were identified with X-ray photographs (3.00 mA, 5.00 mAs, 40 kVp) in 2011 and with a 2.7 mm OD rigid borescope

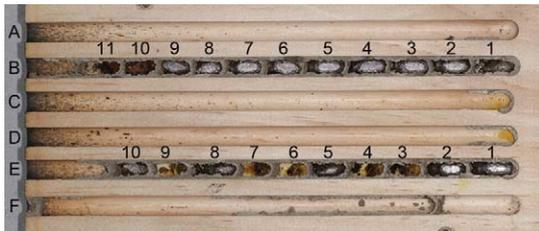


Fig. 3. Observation block showing labeling system for trap nest bees. Each block or reed is assigned a number. Each tunnel in a block is assigned a letter A-H (bamboo is assigned a lowercase "b"), and each cell is numbered in order of construction. Labeled bees can be matched with nest photos to assist in identification. Letters ran in reverse order in 2012.

in 2012; the remaining unoccupied tubes were reused in following years. Used observation block nests were cleared of debris and rinsed in a 50% bleach solution before reuse.

In spring, the capsules were kept at outdoor temperatures until emergence, at which point bees were collected. Photographs taken of the opened, undisturbed nests, combined with the individually labeled capsules, allowed accurate tracking of a specimen's original position and nest construction, which aided in identification.

Net Surveys. Net collecting was performed at sites 1, 4, 6, 7, and 8 in 2011 and 2012, and at 1, 2, 3, 4, 5, 6, and 7 in 2013 (Table 1). Different sites were sampled in 2013 based on bloom. Site 8, for instance, has very little blooming in the spring but is very rich in summer, while site 2 is the opposite.

In 2011, seven 2-d collecting trips were made every 2–3 wk, during which each site was sampled twice, once early in the day (loosely defined as 10:00–1:00) and once later (1:00–5:00). The dates of these trips were 25–26 May, 6–7 June, 25–26 June, 16–17 July, 8–9 August, 30–31 August, and 17–18 September.

Each sample was timed at 15 min of sweeping flowers for foraging bees. Upon arriving at the site, two or three collectors spread out in search of flowers in bloom. When a patch of flowers was encountered, the timer started and the collector walked through, sweeping the flowers with broad, quick strokes of the net as he or she moved. The timer was stopped and the net checked for bees if a bee was seen to be captured, if a different flower species was encountered, or at regular intervals if neither of these things occurred. Most captured bees were put in plastic vials, separated by the flower on which they were collected, and euthanized via freezing, to be pinned later. Bees that are easily identifiable on sight (for example, *Osmia lignaria* Say) were recorded and released, after being marked with a spot of nail polish on the thorax to avoid recapture. The timer was also stopped while moving between flowers, so the sample reflects only the diversity of bees present on flowers, not flower density. Collectors continued moving from flower to flower until they had a combined collecting effort of 15 min (with three collectors, this equated to 5 min per person). Care was taken not to sweep the same flower more than once in the same sample if enough other flowers were available.

Weather conditions proved to be an unpredictable, often detrimental factor on the 2011 collecting trips, especially late in the season, and the number of Megachilidae captured was unsatisfactory. To mitigate this problem, as well as focus more collecting effort in June and July (when Mickel and Hodson's class was held), J.D.G. lived on-site at the UMN Biological Station and Laboratories from 30 May 2012 to 5 August 2012. Except for the week of 10–16 June, which was cool and rainy, and 15–21 July, 15 net samples were performed per week. Net collecting protocol was the same as it was in 2011, except the time per sample was reduced to 10 min since there was only one collector working. A 10-min sample with one collector working typically

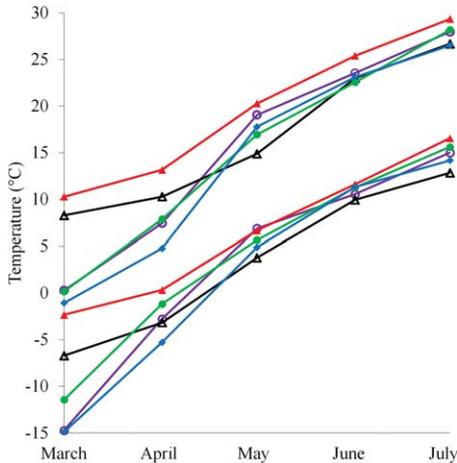


Fig. 4. Average monthly high and low temperatures in Park Rapids, MN, ≈ 15 km south of Itasca State Park. Blue filled diamonds = 2013, red filled triangles = 2012, green filled circles = 2011, black open triangles = 1938, purple open circles = 1937. Note that 2012 was much warmer than any other year, and 2013 was cooler. 1938 also had an exceptionally warm spring, but experienced a 4-d cold snap in May which pulled the average temperature down (data not shown). Source: NOAA National Climatic Data Center.

took between 45 and 90 min including handling and walking time, depending on flower density and the number of bees found. Sites were sampled evenly so that at no time was one site much more heavily sampled than the others, or too heavily skewed toward early or late samples.

The original intention was to collect only in 2011 and 2012, to correspond with the 2 yr of museum specimen data. However, while beginning data analysis, species richness of the current collection was much lower than it was historically, and many of the missing species were early spring-flying bees such as *Osmia*. The spring of 2012 was exceptionally warm (Fig. 4), raising the possibility that these species were missing not because they did not exist, but because their flight period had already ended by the time collecting began.

Therefore, three more weeks of net collecting were performed from 26 May to 15 June of 2013. The spring of 2013 was exceptionally cold (Fig. 4), allowing collections at the same calendar time as previous years, yet much earlier phenologically. Methods were the same as in 2012, except that the time per sample was reduced to 5 min. This was done to allow coverage of more sites in a single day, as there were often fewer flowers blooming per site than is typical for summer. If enough flowers were available, then two consecutive samples were performed at the same site, effectively generating a 10-min sample. Sites were still sampled evenly, however, based on the number of 5-min samples taken.

Specimen Processing. All bees collected were pinned and labeled with the date and approximate time of collection, GPS coordinates of the study site,

and the plant they were collected on. All bees were identified to species using the keys in Mitchell (1962), Sandhouse (1939), and on DiscoverLife (*Coelioxys*, *Hoplitis*, *Heriades*, *Megachile*: Andrus and Droege; *Osmia*: Griswold, Ikerd, Droege, and Pascarella; accessed 2013). Existing identifications on museum specimens were either verified or corrected before analysis. Terry Griswold and Molly Rightmyer assisted in the identification of some particularly difficult specimens. All specimens will be accessioned in the University of Minnesota Insect Collection.

Data Analysis. EstimateS (Colwell 2013) was used to calculate Fisher log series alpha statistic for species richness, and the exponential Shannon and inverse Simpson indices for species diversity. Fisher alpha was chosen because as a pure richness measure with no evenness component, it is insensitive to collector bias and small sample sizes (Magurran 1988), thus allowing a more conservative analysis of potentially biased data and a “reality check” for more sensitive measures. Chi-squared tests were performed to verify that the data fit the log series distribution, following methods in Magurran (1988). The exponential Shannon and inverse Simpson indices were chosen as standard measures of diversity, including an evenness component. The Shannon index is more affected by differences in species richness, while the Simpson index is more affected by evenness, and is more sensitive to bias and small sample sizes.

Data were loaded into EstimateS as sample-based, with species arranged in rows and “samples” being days of collection arranged in columns. For the purpose of data analysis, a sample henceforth refers to one calendar date and all the bees collected on that date. The 1937–1938 data were 30 species by 50 samples, and the 2011–2013 data were 23 species by 53 samples. The number of individuals of a species captured on a given day was entered for each point on the grid.

In addition to the full dataset, a separate analysis was performed using only H.E. Milliron’s collection from 1938 and a subset of the 2012 net collection. This was done to control for the number of collectors, as there were many more people working on the historical collection. Milliron collected 69 Megachilidae over 10 d. The 10 d in 2012 closest to Milliron’s dates and with at least three bees collected were selected for comparison. The 2012 subset had 55 total individuals.

To obtain standard deviations of the richness and diversity indices, these were computed 100 times, randomizing samples with replacement for each run, with a subsample size equal to the total number of samples (50 or 53). It should be noted that randomizing with replacement naturally produces datasets with lower species richness and diversity than the full dataset, as some samples will be selected more than once and others not at all. However, general trends are preserved. *P* values were obtained by running *t*-tests in R statistical software (R Core Team 2012).

EstimateS was also used to generate species accumulation curves for both datasets. A species accumulation curve shows the rate of discovery of new species as a function of collecting effort. Eventually, the curve

will reach an asymptote where additional effort reveals no new species; this indicates that sampling was sufficient to capture all species present in the area. Neither curve for the Itasca data reached an asymptote, so the curves were extrapolated to 150 samples to allow estimation of the total species richness, including undiscovered species. Colwell (2013) does not recommend extrapolation beyond this point, as the variance increases greatly. The curves were generated separately from the diversity indices, without randomization, based on recommendations in the EstimateS User's Guide and personal communication with Colwell.

Results

Net Surveys. Net collecting in 2011–2013 produced a collection comparable to that of 1937–1938. Twenty-one Megachilidae were collected in 2011, 143 in 2012, and 25 in 2013, for a total of 189 bees, compared with 174 from 1937 to 1938. Twenty-three species were found in 2011–2013 and 30 species in 1937–1938. Eleven species in the 1937–1938 collection were not found in 2011–2013, and four species not present in the 1937–1938 collection were found in 2011–2013. Of these four species, three (*Coelioxys modesta* Smith, *Osmia albiventris* Cresson, and *O. lignaria*) were new species not previously collected from Itasca State Park; the other one (*Hoplitis truncata* Cresson) is in the University of Minnesota Insect Collection from Itasca State Park, only not from 1937 or 1938. Two additional species (*Coelioxys porterae* Cockerell and *C. sodalis* Cresson) are in the University of Minnesota Insect Collection from Itasca State Park, but are not present in either the 1937–1938 or 2011–2013 collections. Mickel and Milliron's 1938 collection and the 2012 subset both had 18 species, nine of which were shared. The collections are summarized in Table 2. Complete data are available in Supp Tables 1 and 2 [online only].

Richness and Diversity Indices. Neither dataset differed significantly from the species distribution expected of the log series (Table 3), allowing the use of Fisher alpha as a meaningful measure of species richness. There were highly significant differences between 1937–1938 and 2011–2013 for all three indices examined (Table 4). In all cases, the 2011–2013 collection had lower species richness and species diversity than the 1937–1938 collection.

There was no significant difference in Fisher alpha between Milliron's collection and the 2012 subset ($t = 0.66$, $df = 17.98$, $P = 0.52$). However, the exponential Shannon index ($t = 3.86$, $df = 17.84$, $P = 0.001$) and the inverse Simpson ($t = 4.00$, $df = 14.88$, $P = 0.001$) were both significantly lower for the 2012 subset.

Species Accumulation Curves. Neither the species accumulation curve for 1937–1938 nor for 2011–2013 visibly reached an asymptote by the time all samples were included. This indicates that sampling was inadequate to capture the true species richness of Itasca State Park at the time of collection. However, after extrapolating the curves to 150 samples, both reached

Table 2. Abundance of Megachilidae species in the 1937–1938 collection (A) and the 2011–2013 collection (B)

Species	A	B
<i>Ashmeadiella buconis</i> Say	1	0
<i>Coelioxys alternata</i> Say	1	1
<i>Coelioxys funeraria</i> Smith	1	0
<i>Coelioxys modesta</i> Smith	0	1
<i>Coelioxys moesta</i> Cresson	1	1
<i>Coelioxys rufitarsis</i> Smith	2	0
<i>Heriades carinata</i> Cresson	2	23
<i>Heriades variolosa</i> Cresson	1	11
<i>Hoplitis albifrons</i> Kirby	3	4
<i>Hoplitis pilosifrons</i> Cresson	5	1
<i>Hoplitis producta</i> Cresson	3	3
<i>Hoplitis spoliata</i> Provancher	4	2
<i>Hoplitis truncata</i> Cresson	0	2
<i>Megachile campanulae</i> Robertson	2	4
<i>Megachile frigida</i> Smith	10	0
<i>Megachile gemula</i> Cresson	2	3
<i>Megachile inermis</i> Provancher	11	18
<i>Megachile latimanus</i> Say	16	0
<i>Megachile melanophaea</i> Smith	2	5
<i>Megachile montivaga</i> Cresson	2	0
<i>Megachile pugnata</i> Say	3	13
<i>Megachile relativa</i> Cresson	26	52
<i>Osmia albiventris</i> Cresson	0	2
<i>Osmia atriventris</i> Cresson	24	14
<i>Osmia bucephala</i> Cresson	3	3
<i>Osmia collinsiae</i> Robertson	5	0
<i>Osmia conjuncta</i> Cresson	2	0
<i>Osmia distincta</i> Cresson	7	0
<i>Osmia lignaria</i> Say	0	12
<i>Osmia proxima</i> Cresson	18	1
<i>Osmia simillima</i> Smith	8	7
<i>Osmia tersula</i> Cockerell	3	6
<i>Osmia virga</i> Sandhouse	4	0
<i>Stelis lateralis</i> Cresson	2	0
Total species	30	23
Total individuals	174	189

Eleven species are unique to 1937–1938, and four are unique to 2011–2013 (but see Results).

clear asymptotes. The asymptote, representing the estimated true species richness, in 1937–1938 was ≈ 31 species, and in 2011–2013 it was ≈ 26 species (Fig. 5).

The 2011–2013 curve lies clearly below the 1937–1938 curve at all points. The difference is obviously significant close to the reference sample, as the 95% confidence intervals do not overlap. However, the variance of these curves naturally increases with further extrapolation, causing the confidence intervals to expand and overlap at the far end of the graph. Determining statistical significance of extrapolated species accumulation curves with overlapping confidence intervals is a difficult problem for which there is currently no formal method (Colwell et al. 2012). However, the difference at the far right does not appear significant, as the mean true species richness for 1937–1938 is well within the upper 95% confidence limit for 2011–2013.

Species accumulation curves for Milliron's collection and the 2012 subset are nearly identical and the confidence intervals completely overlap (data not shown).

Trap Nests. A total of 674 solitary bee cells were completed in 194 different trap nest tunnels over the years 2010–2012. Of these cells, 475 bees were suc-

Table 3. Chi-squared tests to verify goodness of fit with the log series

Class	Individuals upper boundary	Species observed	Species expected	χ^2	<i>P</i>
(A) 1937–1938 collection					
1	2.5	13	14.5079	0.1567	
2	4.5	7	4.9932	0.8066	
3	8.5	4	4.6005	0.0784	
4	16.5	3	3.4566	0.0603	
5	32.5	3	1.8509	0.7134	
		30	29.4542	1.8154	0.7697
(B) 2011–2013 collection					
1	2.5	8	9.8179	0.3366	
2	4.5	5	3.5432	0.599	
3	8.5	3	3.4806	0.0664	
4	16.5	4	2.9677	0.3591	
5	32.5	2	2.0307	0.0005	
6	64.5	1	0.9371	0.0042	
		23	22.7771	1.3657	0.928

Under this distribution, there are expected to be many rare species with only one or two individuals in the collection, and a few common species with many individuals.

cessfully reared to the adult stage. Eight species of Megachilidae were identified (*Coelioxys alternata* Say, *Heriades carinata* Cresson, *Hoplitis albifrons* Kirby, *Megachile pugnata* Say, *Megachile relativa* Cresson, *O. albiventris*, *O. lignaria*, and *Osmia tersula* Cockerell). *O. tersula* and *O. lignaria* were extremely abundant, making up 40.7 and 26.8% of all nests founded, respectively (40.2 and 41.7% of individual cells).

Only 26 bees were reared from the 2010 trap nests, all *O. tersula* (Supp Table 3 [online only]). When seven trap nests were set up at various sites throughout the park in 2011, there was a dramatic increase in both the number of nests founded and the number of bee species reared (Supp Table 4 [online only]). *M. pugnata* was very common that year, founding 36.9% of all 2011 nests (16.3% of individual cells). 2012 saw similar trends, only there was a dramatic drop in the abundance of *M. pugnata* despite the addition of a second nest at site 6, where they were most common in 2011 (Supp Table 5 [online only]). There was also a very high rate of parasitism of *O. tersula* by *Sapyga martinii* Smith (Hymenoptera: Sapygidae) compared with previous years (47.4% total mortality versus 17.7% and 38.1% in 2011 and 2010; most mortality of *O. tersula* was due to *S. martinii*).

No bee species were reared from the trap nests that were not detected in the net surveys. However, the abundance of *O. tersula* and *O. lignaria* in the trap nests does suggest that these species are much more

common in Itasca State Park than indicated by the net data.

Discussion

At first glance, these results seem to suggest a decline in Megachilidae at Itasca State Park sometime in the last 75 yr. All richness and diversity indices examined were significantly lower in 2011–2013 than in the past, including Fisher alpha, the most conservative measure used and least sensitive to bias. The species accumulation curves were also highly significantly different around the reference sample. Both the number of bees collected and the number of days sampled were similar between collections, even slightly higher in 2011–2013, although person-hours may have been higher in 1937–1938 due to the greater number of collectors.

Table 4. Mean Fisher’s alpha, exponential Shannon, and inverse Simpson indices from 100 randomized runs with replacement

	Fisher’s α	exp Shannon	inv Simpson
1937–1938	8.87 (1.15)	16.55 (1.26)	11.89 (1.30)
2011–2013	5.88 (0.77)	11.67 (1.25)	7.98 (1.13)
df	84.90	100.55	97.21
<i>t</i>	15.41	19.72	16.25
<i>P</i>	<0.001	<0.001	<0.001

Standard deviations are given in parentheses. *P* values reflect significant differences between the 1937–1938 and 2011–2013 datasets for all indices examined.

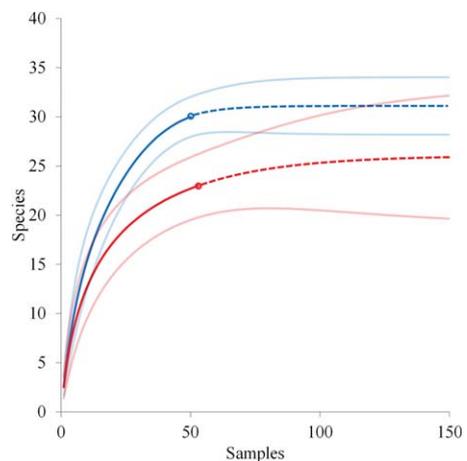


Fig. 5. Species accumulation curves for the 1937–38 collection (upper, blue line) and the 2011–13 collection (lower, red line). 95% confidence intervals are shown in pale blue and pale red, respectively. Open circles mark the reference sample (50 and 53, respectively); extrapolation beyond this point is shown by dotted lines.

Collecting in the spring of 2013 failed to find any of the missing early species, despite excellent phenological timing. Collecting in 2012 began just after *Taraxacum officinale*, an important early spring nectar source, had finished blooming, while collecting in 2013 ended near this same point and began much earlier. This suggests that the missing species were not absent simply due to collecting too late.

However, when the species accumulation curves are extrapolated to their asymptotes, the results are inconclusive. The 2011–2013 curve slowly converges on the 1937–1938 curve, and the variance is much wider, obscuring any differences. It cannot be said with certainty whether species richness of Megachilidae decreased in the last 75 yr because some species have disappeared, or only because those species were somehow not sampled. It should be noted that the weather was poor for much of the spring of 2013 and only 25 Megachilidae were collected. It may be that further sampling in better years would reveal the missing species.

A consistent problem with old museum collections is collector bias. With so much of Mickel and Milliron's actual collection methods left up to hints and speculation, bias cannot be ruled out as a confounding factor. The original collectors may have deliberately biased the collection toward rare species by ignoring common species once several individuals were captured, perhaps to make a synoptic set. This type of bias would be mitigated by the sheer number of collectors working at once (59 students plus Mickel, Milliron, Dodge, Pritchard, and possibly Hodson).

However, having so many collectors could introduce its own unintentional bias. More people could cover a wider area and sample more plant species, increasing the chance that the current collection effort could miss resampling an important place or flower. While every suitable clearing found in the park was used as a study site, and every flower species found was sampled, it is still possible that something was missed. The western and central areas, in particular, were not sampled in 2011–2013, mainly due to difficulty of access and lack of obvious clearings. Willows (*Salix* spp.), an important resource for spring bees, were not sampled because they had finished blooming by the time collecting began. However, it is unlikely that Milliron and the others could have sampled willows either, as they began collecting at the same time. Various other wildflowers are known from the park but were not found during the collecting period, including most notably *Penstemon* (Scrophulariaceae). *Penstemon* is the preferred pollen source of *Osmia distincta* and *O. proxima*, though both bee species are known to visit other plants such as *Trifolium* and *Rubus* (Crosswhite and Crosswhite 1966), which were abundant and intensively sampled. But if Milliron and the others had found and collected on *Penstemon* or *Salix*, it may have yielded a higher number and diversity of *Osmia*.

Finally, with a small number of collectors in the current effort, there may also be unintentional bias due to small differences in personal collecting habits.

There is little one can do about these problems, aside from using conservative analyses and interpreting results with caution.

When Milliron's collection alone was compared with a similarly sized subset of the 2012 collection, neither Fisher alpha nor the extrapolated species accumulation curves were significantly different. Although the exponential Shannon and inverse Simpson indices were different, these indices should be interpreted with great caution, as they are sensitive to bias and small sample sizes. The lack of significant differences in less sensitive measures suggests that Milliron's collection, and by extension the rest of the 1937–1938 collection, are subject to bias.

Bee collection records for the state of Minnesota were obtained from the DiscoverLife Global Mapper, John Ascher (American Museum of Natural History), Paul Tinerella (Illinois Natural History Survey), Sam Droege (U.S. Geological Survey), and the University of Minnesota Insect Collection to check for recent records of the missing 11 species and *O. proxima* in and around Itasca State Park. Only one *Osmia collinsiae* from Itasca County in 2010 was collected more recently than 1971. This could be evidence of a decline, but is more likely due to a lack of recent collecting effort in northern Minnesota.

Although the results of this study are uncertain, the possibility of a real decline in Megachilidae species richness should be considered. A decline of any sort would be troubling because Itasca State Park has been a protected area since it was established in 1891. The park's immediate surroundings, while not protected, are not heavily developed either. The usual factors blamed for bee declines—agricultural intensification, pesticide misuse, and habitat loss—cannot have played major roles in this case. What, then, could be responsible for a loss of species?

The most likely cause is natural ecological succession. Banaszak et al. (2003) documented a major decline in bee species richness at one of their study sites in Poland, which they attributed to ecological succession. The site, a xerothermic grassland which was once subject to plowing and grazing, has been protected since the establishment of Wielkopolska National Park in 1957. This allowed the protected grassland to be colonized by woody plants, decreasing the number of flowering herbs available for bees. Grixti and Packer (2006) also attributed changes in the bee species community in the Caledon Hills, Ontario, to ecological succession and global warming. While they documented an increase in species richness, rather than a decline, many of the bee species present at their study site 34 yr ago were not rediscovered. Species richness only increased because of an influx of new species.

Over the last 75 yr, certain areas of Itasca State Park have also undergone ecological succession. Aerial photos from 1939 were obtained from the Minnesota Department of Natural Resources (DNR) and compared with 2011 Google Earth satellite images to evaluate changes in tree cover. Five sites have obvious differences. Site 6, an old farm, was probably cropland in 1939, and is now grassland dotted with young pines.



Fig. 6. Site 7 in 1939 (left) and 2011 (right), shown in black and white to facilitate comparison with 1939. 2011 collecting focused on the roadsides, including the old path only visible in 1939. Most of the visible clearings are wetlands. Photo credit (1939): Soil Conservation Service, scanned by DNR; (2011): Google.

Site 7 had what might have been a homestead in a 4,300-m² clearing, which is now almost completely forested (Fig. 6). And site 1, the UMN Biological Station and Laboratories, appears to be much more densely forested now than it was in 1939 (Fig. 7). The last two sites, 4 and 8, were completely forested in 1939 and must have been cleared sometime later. The fact that site 1 was much more open in the past is especially significant, as this is where the University of Minnesota labs and student cabins are located and likely where Mickel's class did a large part of their collecting.

This situation—the establishment of a protected area and the conversion of formerly disturbed grassland to young forest—bears many similarities to that of Banaszak et al. (2003). Even the time frame is similar, if the farm at site 6 was abandoned sometime in the 1950s. It should be noted, however, that none of the other sites studied by Banaszak et al. (2003) experienced declines such as this. If the decline in species richness at Itasca State Park was due to the

protection of formerly disturbed areas, the lost species may persist in the surrounding, unprotected area.

To summarize, it is useful to review each of the missing species. *Ashmeadiella buconis*, *Coelioxys funeraria*, *Coelioxys rufitarsis*, *Osmia conjuncta*, and *Stelis lateralis* are all uncommon species, both presently and historically, and it is not particularly surprising not to find them. *Megachile latimanus* has no clear explanation for its disappearance. It is a ground-nesting bee, but other, less common ground-nesting species such as *Megachile gemula* and *Megachile melanophaea* were still found; it prefers to visit thistles (*Cirsium* spp.), but these were common and intensively sampled. However, this species, along with *Megachile frigida* and *Megachile montivaga*, are all common in other areas, and should not be considered species of concern. *O. distincta* and *O. proxima* are likely missing or rare because their preferred host plants, *Penstemon* spp., were not found. *O. virga* is another oligolege but its preferred host plants,

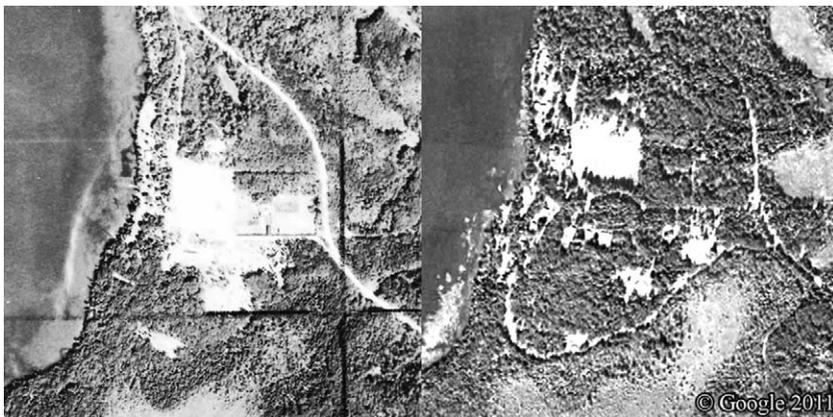


Fig. 7. Site 1 in 1939 (left) and 2011 (right), shown in black and white to facilitate comparison with 1939. 2011 collecting focused along the curved path to the south only visible in 2011. Photo credit (1939): Soil Conservation Service, scanned by DNR; (2011): Google.

Vaccinium spp., were very common and intensively sampled. This species, along with *O. collinsiae*, has no clear explanation for its apparent disappearance, and may warrant special attention.

Of the four new species, *H. truncata* is recorded from the park in 1936, and was only missed by Mickel's class. *C. modesta* and *O. albiventris* were uncommon, and could easily have been missed as well. The absence of *O. lignaria* in the 1937–1938 collection is unusual, however. Perhaps the relatively recent development of this species as a commercial orchard pollinator has facilitated range expansion and population growth. This may be worth further investigation.

In the near future, the complete collections of all bees in 1937–1938 and 2011–2013 will be studied. The inclusion of additional bee taxa and functional guilds will allow for a stronger analysis, and determine if the trends observed in Megachilidae hold true for bees in general, helping to clarify apparent declines and inform decisions regarding conservation practices.

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