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The native snail shell–nesting bee *Osmia conjuncta* (Hymenoptera: Megachilidae) exploits a local abundance of exotic *Cepaea* snails (Stylommatophora: Helicidae), choosing empty shells by size, colour, and microhabitat

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Abstract

For solitary bees, nesting substrate availability may limit population growth. Here, we investigate the nesting of a locally abundant bee *Osmia conjuncta* Cresson (Hymenoptera: Megachilidae) in empty shells of exotic snails, *Cepaea* spp. Held (Stylommatophora: Helicidae), in Hamilton, Ontario, Canada. A literature review determined that *O. conjuncta* was sparsely distributed throughout its range, with a low relative abundance except for the Niagara Region of Ontario, Canada, three sites in Maryland, United States of America, and the focal study area in Hamilton, Ontario. The Hamilton field survey of five grassland bee communities found *O. conjuncta* to be the most abundant bee species. The average relative abundance was 18.7%, peaking at 87% on one spring day. A survey of 1088 empty snail shells was done at the site with the highest *O. conjuncta* abundance. It revealed an average of 10.87 empty shells per square metre with cocoons in 9.8% of the shells, averaging 1.6 cocoons per nest. The relation between shell traits and cocoon presence indicated that *O. conjuncta* preferred nesting in shells with intermediate size and colour and avoided shells near shrubs. The presence of *Cepaea* snails increased *O. conjuncta* populations with potential consequences to the local bee assemblage.

Introduction

Bees (Hymenoptera: Apoidea: Anthophila) require two limiting resources: food and nesting site (Westerfelt *et al.* 2018; Heneberg *et al.* 2020). Nesting strategies among solitary bees include excavating their own nests, taking over the nests of other bees, and using preexisting cavities (Cane *et al.* 2007; Antoine and Forrest 2021). The type of nest is specific to each species (Michener 2007). Within the family Megachilidae, species of mason bees exhibit preferences for preexisting cavities in dead wood, hollow plant stems, rocks, old insect burrows, and even empty snail shells (Michener 2007). The majority of known snail shell–nesting species are found in Asia, Europe, and Africa (Müller *et al.* 2018). However, despite limited observations that indicate four species of North American Megachilidae nest in snail shells (Rau 1937; Michener 1939; Neff and Simpson 1992; Cane *et al.* 2007), little is known about the nesting habits of North American snail shell–nesting bees.

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In Europe, snail shell–nesting bees and snails can be diverse and abundant. In one study in northeastern Spain, 15 snail shell–nesting bee and wasp species and an additional eight parasitic species were found in a collection of 6000 shells from nine species of snails (Bogusch *et al.* 2020a). In another study in a calcareous grassland in Germany, five species of snail shell–nesting bees made one–fifth of the flower visits over the season, with three species of bees found in the experimentally deployed shells of four snail species (Hopfenmuller *et al.* 2020). In naturally occurring snail shells, occupancy rates by snail shell–nesting bees vary among sites and snail species. In both deployed and naturally occurring empty shell studies in Europe and Africa, shell occupancy varies across site and shell species from as low as 2.4% (Bogusch *et al.* 2020a) to as high as 92% (Gess and Gess 2008). Shell occupancy can differ among habitats (Bogusch *et al.* 2020a; Hopfenmuller *et al.* 2020). A community of snail shell–nesting bees may share a preference for a single shell species, or a bee species may demonstrate specific preferences for the species of shell (Bogusch *et al.* 2020b; Heneberg *et al.* 2020; Hostinska *et al.* 2021). The differences among snail species in size at maturity, shape, and abundance have been implicated as potential factors in shell choice (Heneberg *et al.* 2020; Hostinska *et al.* 2021). Microhabitat and shell age since snail death are argued to affect choice; shells are argued to be less favoured if they are more exposed to predators or contain decaying flesh (Bogusch *et al.* 2020a; Heneberg *et al.* 2020). However, whereas several studies demonstrate that bees have preferences for the species of shells they nest in or the sites the shells are found in (Gess and Gess 2008; Bogusch *et al.* 2020a, 2020b; Heneberg *et al.* 2020; Hopfenmuller *et al.* 2020; Hostinska *et al.* 2021), whether bees choose shells based on within-species characteristics of individual shells or the microhabitat of the shell remains unknown.

The use of the locally abundant shells of the exotic grove snail, *Cepaea* spp. Held (Stylommatophora: Helicidae), by the native Eastern snail shell mason bee, *Osmia* (*Diceratosmia*) *conjuncta* Cresson (Hymenoptera: Megachilidae), in southern Ontario, Canada, provided a system in which to explore bee preferences for shell characteristics. *Osmia conjuncta* was characterised as a snail shell–nesting bee in an anecdotal account by Rau (1937). The author was given a mason bee, later identified as *O. conjuncta*, by two naturalists in St. Louis, Missouri, United States of America, who reported that the bee emerged from a snail shell. The snail was identified as *Pyramidula solitaria* [sic], presumably a misspelling of *P. solitaria*, which is now considered *Anguispira kochi* Linnaeus Pfeiffer (Stylommatophora: Discidae) (MolluscaBase 2021). *Osmia conjuncta* was described as well established locally, such that several bees visiting flowers could be collected within an hour (Rau 1937). In the decades since Rau's (1937) description, the paper remains the sole account of *O. conjuncta* nesting.

Although the ecology of *O. conjuncta* is little known, its life cycle is expected to be similar to that of other species in the genus *Osmia*. *Osmia conjuncta* males have been observed to emerge in high numbers in early spring, followed by females in late spring and summer (Rutgers-Kelly 2005). Both male and female *O. conjuncta* are large (9 mm) and robust (Ascher and Pickering 2020), and the species is speculated to be polylectic (Griswold and Rightmyer 2017) – that is, a generalist on pollen sources. Typically, *Osmia* live less than one year, are univoltine, and lay between two and 50 eggs, depending on the species (Sgolastra *et al.* 2016, 2018). *Osmia* lay eggs in spring or early summer that develop into adults within the nest by late summer or early fall. Adult bees in cocoons remain in diapause and overwinter inside the nest, emerging in early spring (Sgolastra *et al.* 2016; Seidelmann and Rolke 2019).

The range of *O. conjuncta* extends from the southern edge of Canada as far south as Florida and as far west as Kansas, United States of America, with southern Ontario, Canada, comprising the northernmost range of the species (Griswold and Rightmyer 2017). The Canadian National Collection of Insects, Arachnids & Nematodes (Ottawa, Ontario) has only 18 specimens of *O. conjuncta* collected in Ontario between 1896 and 2011, although material collected in the

Ottawa region in 2014–2015 included 66 specimens of *O. conjuncta* (S. Cardinal, personal communication), suggesting the species has been historically sparse but may be becoming more abundant. Surprisingly, *O. conjuncta* is locally abundant in surveys in the Niagara Region of southern Ontario, composing 9.5% of a 2003 bee survey (Richards *et al.* 2011) and 16.1% of the community overall from 2003 to 2013 (Onuferko *et al.* 2018). Richards *et al.* (2011) hypothesised that the high relative abundance of *O. conjuncta* in the Niagara Region was caused by a high number of invasive European grove snails present in the study site, thereby increasing the supply of nesting substrate. Despite this possible association, no one has yet reported that *O. conjuncta* nests in grove snail shells.

Grove snails are native to Western Europe and have been introduced into North America multiple times since the mid-19th century (Örstan 2010; Layton *et al.* 2019). Although no quantitative studies of grove snail distribution in North America have been undertaken, in Europe, the grove snail has a clustered distribution in which dense colonies can be found within areas where numbers are low or absent (Lamotte 1959; Rosin *et al.* 2017). In Europe, grove snail shells are used as a nesting substrate by other *Osmia* species, with reports of *O. bicornis* Linnaeus and *O. rufohirta* Latreille nesting in empty shells of *Cepaea nemoralis* Linnaeus and *C. hortensis* Muller (Raw 1972).

Grove snails are considered moderately abundant across southern Ontario and have dense colonies in some human-disturbed open areas (Singh 1981; Örstan *et al.* 2011; Hoxha *et al.* 2019), but an extensive survey of snail communities across the Great Lakes region found only three specimens of *C. nemoralis* (Nekola 2003). In their native range, *Cepaea* spp. are also associated with human disturbance (Ozgo and Bogucki 2011; Rosin *et al.* 2017). Densities of live snails in their native range in southwestern England vary from 0.010 to 1.93/m² (Perry and Arthur 1991), and another study in that region found a median empty-to-living shell ratio of 2.03 (Richardson 1979). In their exotic range in North America, counts of live grove snails include 1.1/m² in Maryland, United States of America (Örstan *et al.* 2011), 2.5/m² in London, Ontario (Mensink and Henry 2011), and 50 to 100/m² in Lexington, Virginia, United States of America (Stine 1989). However, several extensive studies of snail communities in eastern North America found few (Nekola 2003; Hodges and McKinney 2018) or no (McMillan *et al.* 2003; Beier *et al.* 2012; Utz *et al.* 2018) specimens of *Cepaea*.

Sites with abundant snail shells used by a snail shell–nesting bee species are necessary to study nesting preferences and shell selection. Similar to the Niagara study (Richards *et al.* 2011), a multiyear study of five sites in Hamilton, Ontario, Canada (S. Irazuzta, unpublished data) found high numbers of *O. conjuncta* and the presence of empty grove snail shells. Furthermore, an undergraduate course in 2017 found *O. conjuncta* adults in diapause in some grove snail shells collected from the Hamilton site (S. Irazuzta, unpublished data), indicating that *O. conjuncta* nests in grove snail shells. In the present study, we bring together three lines of evidence to examine *O. conjuncta*, its population, and its use of exotic snail shells as a nesting resource. The literature of eastern North American bee community surveys was used to assess the patterns of relative abundance for *O. conjuncta* across its range. Irazuzta's findings (unpublished data) were used to estimate *O. conjuncta*'s relative abundance over the flight season of assemblages from five grassland sites over three years. In a field study at one of Irazuzta's grassland sites, the "Created Prairie," empty snail shells from 99 randomly placed 1-m² quadrats were collected, counted, measured, and opened; the presence and number of bee cocoons found inside were recorded. We asked the following questions: (1) How abundant were *O. conjuncta* adults in Hamilton communities, and how did those numbers compare to other North American bee communities? (2) What were the quantities and qualities of grove snail shells as a nesting resource? and (3) What was the occupancy of empty shells by *O. conjuncta* nests, and was shell choice associated with shell characteristics?

Methods

Literature review

Initially, Google scholar, Web of Science, and JSTOR were searched with the keywords (“*Osmia*” “*conjuncta*”) to find surveys of bee communities reporting *O. conjuncta*. After, the same databases and the *Journal of the Kansas Entomological Society* were searched with the keywords (“bee” + “community” + “survey”, “bee” + “communities” + “survey”, and “bee” + “community” + “survey” + “hymenoptera”) to find surveys of bee communities in eastern North America that may not have included *O. conjuncta*. We also found other bee community surveys from references in other studies. We cast a broad net for range, seasonality, and methodology. However, the studies included in the review are limited to those that used lethal collection methods and identified most individuals to species. Use of pan traps was most common in the included studies, but studies that used netting, malaise traps, vane traps, and mixes of these methods were also included. We included studies that either deliberately avoided catching bumblebees or acknowledged that their traps would not collect a representative sample of larger bees. This review gave a broad picture of the relative abundance of *O. conjuncta* in communities east to Maine, north to southern Manitoba, west to Kansas and Texas, and south to Florida.

From these papers, theses, and reports, we extracted the collection methodology, total number of bees, number of *O. conjuncta*, estimated or provided survey location, year(s) surveyed, and seasons surveyed (spring: March–May; early summer: June–July; late summer: August–September; fall: October–November; and winter: December–February; Supplementary material, Table S1). Site descriptions were used to identify coarsely classified locations by habitat type and human influence, but no analysis was done because many surveys had multiple values for these variables (Supplementary material, Table S1). Location data were used to identify the Environmental Protection Agency ecoregion (Environmental Protection Agency 2022) in which the study was done. For most of the surveys that were carried out over multiple sites within a region or over multiple years, the total numbers of *O. conjuncta* and total numbers of bees were used and the midpoints for site locations and years were estimated. For a few studies that had location differences we judged to be significant, we considered each data set as a separate entry (Pindar 2013; Normandin *et al.* 2017; Parys *et al.* 2020). The data set was then evaluated for evidence of bias created by collection season and methodology. Because *O. conjuncta* is active in spring and early summer (Rutgers-Kelly 2005), the two studies that did no collection in spring or early summer and caught no *O. conjuncta* were dropped. An analysis of the literature studies found that the relative abundance of *O. conjuncta* was unaffected by season (spring only: $n = 6$; summer only: $n = 17$; and spring and summer: $n = 67$), year, latitude and longitude, or use of pan traps as a collection technique (pan traps used: $n = 63$; pan traps not used: $n = 11$; Supplementary material, Table S2). These analyses did not include the Niagara Region study (Onuferko *et al.* 2018) because it is a known outlier. Motten’s (1986) study was omitted from the analyses in Supplementary material, Table S2 because it was found to have high leverage, but it is included in the literature review. No other studies were excluded, and the remaining 74 studies were included in the literature review data set. *Osmia conjuncta* relative abundance was calculated as a percentage of the community ($100 \times \text{number of } O. conjuncta / \text{total number of bees}$).

The approximately 99 000-bee, multiyear, multisite survey of Maryland and vicinity from the Native Bee Inventory and Monitoring Lab of the United States Geological Survey (Kammerer *et al.* 2020) has been included as a single observation in the literature survey, following the protocol above. To examine fine-scale spatial variation in *O. conjuncta* relative abundance, we mapped the variation in *O. conjuncta* relative abundance from this data set. In the Native Bee Inventory and Monitoring Lab data, the majority of collections at a single site were done only for a single year and many were sampled only once. To obtain data comparable to the other literature observations, for each site, only those years for which the first sampling date was before August were included. For each site, surveys from multiple years were pooled together.

For relative abundance in the Niagara Region, we used the cumulative value for multiple sites and multiple years reported in Onuferko *et al.* (2018). For the Hamilton-area communities, the relative abundance of *O. conjuncta* in each of the five sites in 2014, 2015, and 2016 were obtained as described below.

Bee survey of the Hamilton, Ontario, communities

The *O. conjuncta* relative abundances reported here are part of a larger study (S. Irazuzta, unpublished data). In this study, bee surveys were conducted at five sites in Hamilton, Ontario, from spring to fall of 2014–2016 on a biweekly basis. There were 186 samples across all three years, with sampling taking place between 3 June and 24 September 2014, 30 April and 15 September 2015, and 12 May and 4 October 2016. In this study, four sites were located within the McMaster University property at 1105 Lower Lions Club Road, Hamilton, Ontario, each 0.3–0.8 km apart: “Created Prairie” (43.2463° N, 79.9524° W), “Old Field Adjacent” (43.2484° N, 79.9496° W), “Old Field North” (43.2515° N, 79.9465° W), and “Wet Meadow” (43.2495° N, 79.9510° W). A fifth site was located 6.5 km away on Hamilton Conservation Authority land: “HCA Meadow” (43.2160° N, 80.0174° W). All the study sites were considered old fields (Cramer *et al.* 2008), but they varied in their historic use, floral characteristics, size, and degree of naturalisation. The Created Prairie was the only site to undergo restoration, with all other sites experiencing naturalisation processes for at least 30 years. This 4-ha area, formerly dominated by buckthorn, *Rhamnus cathartica* Linnaeus (Rhamnaceae), and orchard grass, *Dactylis glomerata* Linnaeus (Poaceae), was restored to a tallgrass prairie in 2014 by removing invasive buckthorn trees, spraying Roundup® herbicide to control other nonnative species, and sowing seeds of native tallgrass prairie species. A controlled burn was conducted in 2017 to aid in the restoration. A plant survey, carried out in 2020 by N. Stegman and S. Irazuzta, identified plant species at each site using common dichotomous keys (Newcomb and Morrison 1989; Chamberlain 2018). The survey found the Created Prairie had 55 plant species, of which 37 were native. The top five most important species, as measured by constancy and weighted by percent cover (Roberts 2019), were Indian grass, *Sorghastrum nutans* Linnaeus (Poaceae), bird’s-foot trefoil, *Lotus corniculatus* Linnaeus (Fabaceae), meadow sedge, *Carex granularis* Muhlenberg ex. Willdenow (Cyperaceae), redtop grass, *Agrostis gigantea* Roth (Poaceae), and switchgrass, *Panicum virgatum* Linnaeus (Poaceae) (S. Irazuzta, unpublished data).

Vegetation cover and density varied among sites, with the Created Prairie and Old Field Adjacent sites having the most open ground, 3% and 9% bare ground, respectively, due to those sites’ harsher clay soil conditions. The HCA Meadow site had been previously identified as a prairie site, but in the recent survey, the vegetation was found to be typical of a cultural meadow, with no open ground and dense vegetation cover dominated by nonnative Kentucky bluegrass, *Poa pratensis* Linnaeus (Poaceae), white sweet clover, *Melilotus albus* Medikus (Fabaceae), and tall fescue, *Festuca arundinacea* Schreber (Poaceae), along with native tall goldenrod, *Solidago altissima* Linnaeus (Asteraceae). The Wet Meadow site was unique in having wet soil, with standing water during spring and summer most years. The Wet Meadow site had large patches of tall goldenrod, *S. altissima*, and thickets of grey dogwood, *Cornus racemosa* Lamarck (Cornaceae), as well as typical nonnative pasture grasses such as redtop grass, *A. gigantea*. The Old Field North was the smallest site (0.8 ha), partly surrounded by forest habitat, with no bare soil and dense vegetation primarily composed of wild bergamot, *Monarda fistulosa* Linnaeus (Lamiaceae), tall goldenrod, *S. altissima*, and thickets of black raspberry, *Rubus occidentalis* Linnaeus (Rosaceae).

Bee surveys followed standard pan trap sampling methods (Droege 2018). Fluorescent yellow, blue, and white pan traps were arranged in a trapline of 30 bee bowls placed in a 50 × 50-m “×” shape in the middle of each survey site. Sampling was conducted on days with predicted winds of less than 20 km/h, no rain, and temperatures between 15 and 30 °C. Pan traps were set out in the morning before 09:00 hours and collected after 17:00 hours, local time. The traps were set at each

site in alternating order (site 1–5 or site 5–1) to balance out the collection effort (time each pan trap was out) and starting times over the season between sites. Collected insects from all 30 pan traps at each site were pooled to create a single sample for each site and date. Pooled samples were kept in sealed vials with 70% alcohol until processed in the lab. Bees were pinned, dried, labelled, and identified to species in most cases, although some ambiguous specimens were classified only to genus or morphospecies. All bees sampled were identified under a dissection microscope. Bee identification was carried out by S. Irazuzta, using keys to Ontario bee genera (Packer *et al.* 2007), the online keys to species in discoverlife.org (Ascher and Pickering 2020), and Mitchell (1962). Specific keys were cross-referenced for species of some genera, such as *Megachile* (Sheffield *et al.* 2011), *Osmia* (Griswold and Rightmyer 2017), *Dufourea* (Gibbs *et al.* 2014), *Bombus* (Colla *et al.* 2011), and for the metallic species of the genus *Lasioglossum* (*Dialictus*) (Gibbs 2011). All bee specimens are maintained in the McMaster University Insect Collection.

Empty snail shell collection and environmental measurements

From October to December 2020, empty snail shells were collected from the Created Prairie site. We established 11 north–south transects: one in the adjacent forest to the east (“Forest”), six in the centre of the restoration area (“Centre”), and four at the northern edge of the restored area (“Edge”). Transects were each 90 m long, parallel to each other, and 10 m apart. Along each transect, a 1-m² quadrat was chosen randomly within each 10-m length along the transect to give nine quadrats per transect, for a total of 99 quadrats.

For each quadrat, we measured vegetation cover, the presence or absence of grass, forbs, and woody shrubs, and snail shell density. We quantified vegetation cover using the Daubenmire cover class method, which estimates cover between less than 5%, 6–25%, 26–50%, 51–75%, 76–95%, and over 96% cover (Bonham *et al.* 2004). Each quadrat was searched for 1–2 minutes. Shells with live snails and completely shattered shells were not collected.

Measuring snail morphology and assessing cocoon presence

Snail shells were stored at room temperature in paper bags from time of collection to March 2021. From January to March 2021, one researcher measured all the shell traits. Shell traits included size, colour, and location of any damage. A ruler accurate to 1 mm was used to measure shell height (top to bottom), shell width (across the top from edge to edge), and aperture width (width of the shell opening; Fig. 1A). Shell damage was classed by location, either at the aperture, on the top, or on the bottom. Shell colour was assessed by band- and ground-colour value, as follows: (1) all white shells, (2) light-toned bands on white ground colour, (3) medium-toned bands on white ground colour, (4) dark-toned bands on white ground colour, and (5) very dark bands on yellow ground colour. The lighter colours of many empty snail shells were likely due to photodegradation of the shell pigmentation (Richardson 1979; Ménez 2002). Consistent with this mechanism, we observed that shells with living snails had shells with intensely dark bands and yellow ground-coloured shells (band colour 5), whereas completely white shells (band colour 1) had thinner shells and were more fragile. We did not attempt to assess the genetically determined banding pattern (Richardson 1979). The few shells that did not fall into this scale, such as shells with pink or orange ground colour, were not included in the statistical analysis of colour. For some shells, the shell colour assessment was not made, and those data are missing.

A *post hoc* standardisation of the colour assessment was created by having the same researcher classify a sample of 39 empty shells into the five categories described above and then evaluate the band colour of each shell for value and chroma in the 10YR hue in the Munsell colour system (Pastilha *et al.* 2019), using colour standards generating by R package “munsell” version 0.5.0 (Wickham 2018; Fig. 1B). The band-colour class was strongly correlated with Munsell value (variation from light to dark), but not with Munsell chroma (colour intensity).

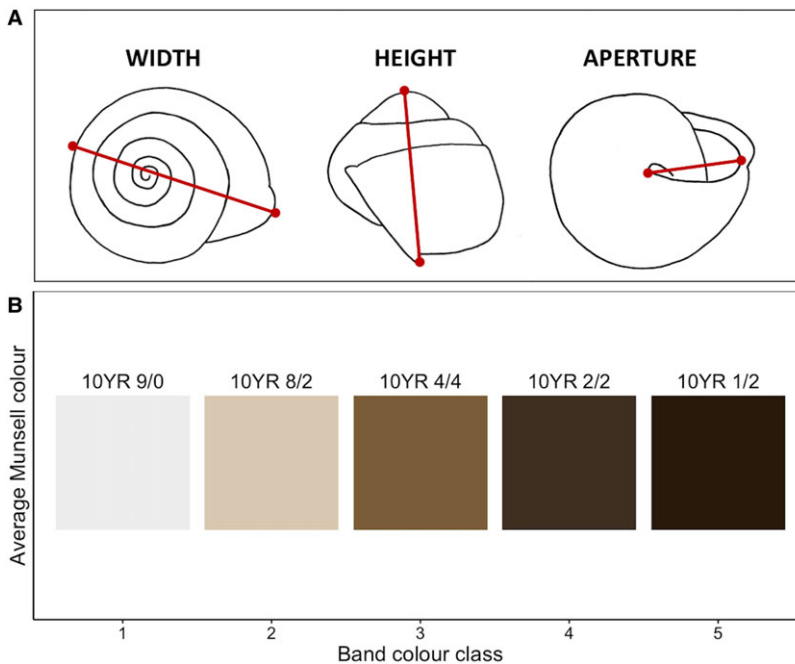


Fig. 1. Measurement of snail size and colour: **A**, measurement of snail size and **B**, average snail band colour for each colour class. The correlation between band colour class and Munsell value (dark to light) was $r = -0.917$, $P < 0.0001$.

This set of 39 snail shells was identified to genus as *Cepaea* spp., using published keys (Grimm *et al.* 2009). The two *Cepaea* species in the local region, *C. nemoralis* Linnaeus and *C. hortensis* Muller (Stylommatophora: Helicidae), are highly similar in appearance but for the darker aperture lip colour of *C. nemoralis*. All the intensely coloured shells were identified as *C. nemoralis*. However, for faded shells, it was not possible to distinguish the lip colour and determine species-level identification, so snail shells were conservatively identified to *Cepaea* spp. rather than as *C. nemoralis*.

After measurement, each shell was carefully broken open with a mortar and pestle to reveal the contents. The contents were examined for bee cocoons or remnants of cocoons. All cocoons or remnants within empty snail shells were assumed to be *O. conjuncta*. Some cocoons were observed to contain bees overwintering inside the shell as adults in diapause. However, many cocoons were empty, implying they were from previous years or had been preyed on. All contents were photographed. In a 2017 undergraduate field course, separate from the bee survey, empty snail shells at Created Prairie were collected and opened. The cocoons found inside contained adult bees in diapause and were identified by S. Irazuzta as *O. conjuncta*, using the online keys at discoverlife.org (Ascher and Pickering 2020) and other published keys (Griswold and Rightmyer 2017). In a separate study in 2022, cocoons were removed from empty snail shells collected in Created Prairie in fall 2021, kept in petri dishes at 2 °C until March, and then placed in a greenhouse under shade until bees emerged. The emerging bees were collected (N. Bacon, personal communication) and identified by N. Stegman as *O. conjuncta*, using online keys (Ascher and Pickering 2020) and voucher specimens in the McMaster University Insect Collection.

Data analysis

Data analysis was conducted using R statistical software, version 4.2.2 (R Core Team 2022), running under the R-Studio shell, version 2022.12.0.353 (Posit Team 2022). Maps were created using the “mapview” package, version 2.11.0 (Appelhans *et al.* 2022) with package “sf”, version 1.0–12 (Pebesma 2018), used to convert and manipulate spatial data. All other figures were created using the package “ggplot2”, version 3.4.0 (Wickham 2016). To test whether *O. conjuncta* was more abundant in the Niagara Region and Hamilton, Ontario sites than had been found in other community surveys in eastern North America, we performed an analysis of variance (function `lm()` in R) on relative abundance that contrasted the Niagara Region and Hamilton surveys with other eastern North America bee communities.

To determine if the numbers of shells in a quadrat followed a Poisson distribution, indicating randomness, or differed significantly, indicating clustering, we did a Poisson goodness-of-fit test using R package “energy,” version 1.7-11 (Rizzo and Szekely 2022).

Multivariate Poisson regressions (function `glm()` in R) were used to explore whether shell morphology and environmental variables were strongly associated with cocoon counts per shell. Multivariate linear regressions were used to explore whether environmental variables were strongly associated with the number of shells per quadrat. In each analysis, several candidate models were designed and guided by functional hypotheses, and models were then chosen through Akaike information criteria for small sample sizes (AICc) using package “AICcmodavg” in R, version 2.3-1 (Mazerolle 2023). When the choice by AICc was ambiguous because at least one model was found with a Δ AICc less than 2, the model with fewest parameters and statistical significance of all predictors was preferred. To obtain overall model significance, the chosen model was tested over an intercept-only model using function `anova(model1, model2, test = ChiSquare)`.

To find variables associated with the number of cocoons per shell, we first determined the best shell size model, then the best shell size, shell colour, and damage model, followed by the best model that incorporated size and environmental variables, and finally the best model that included shell, colour, and environmental variables. Because of missing site and colour data for some shells, we were careful to compare models using the same data sets. The best size predictor for number of pupae was a quadratic function of height with an intermediate optimum, and the best shell predictors were quadratic functions of height and colour. The best shell and environmental predictors were quadratic height, quadratic colour, the categorical variable *Site* (Edge, Centre, or Forest), and the presence/absence variable *Shrubs*.

For the number of shells per quadrat, independent variables were *Percent Cover* (continuous), *Site* (categorical with values Edge, Centre, or Forest), *Shrubs*, *Grasses*, and *Forbs* (present or absent in a quadrat). The three models with lowest AICc varied within a Δ AICc of 2. The preferred model predicted higher shell density in quadrats with lower plant cover, shrubs present, and grasses present.

Results

The review of bee community surveys found *O. conjuncta* in 25 out of the 74 community surveys. Although *O. conjuncta* was broadly distributed across eastern North America (Supplementary material, Table S1; Fig. 2A), the species was absent or found in low relative abundance (< 2%) in all communities except the Niagara Region and Hamilton. In the literature review, relative abundance of *O. conjuncta* was consistently low, averaging 0.117% of the community over all 74 communities and 0.345% in the communities with *O. conjuncta* present. The highest relative abundance of *O. conjuncta* in the literature review was 1.76% of the community. In the large, fine-scale Native Bee Inventory and Monitoring Lab survey of Maryland and vicinity, *O. conjuncta* was absent from 1020 sites, present at low relative abundance (< 2%) in

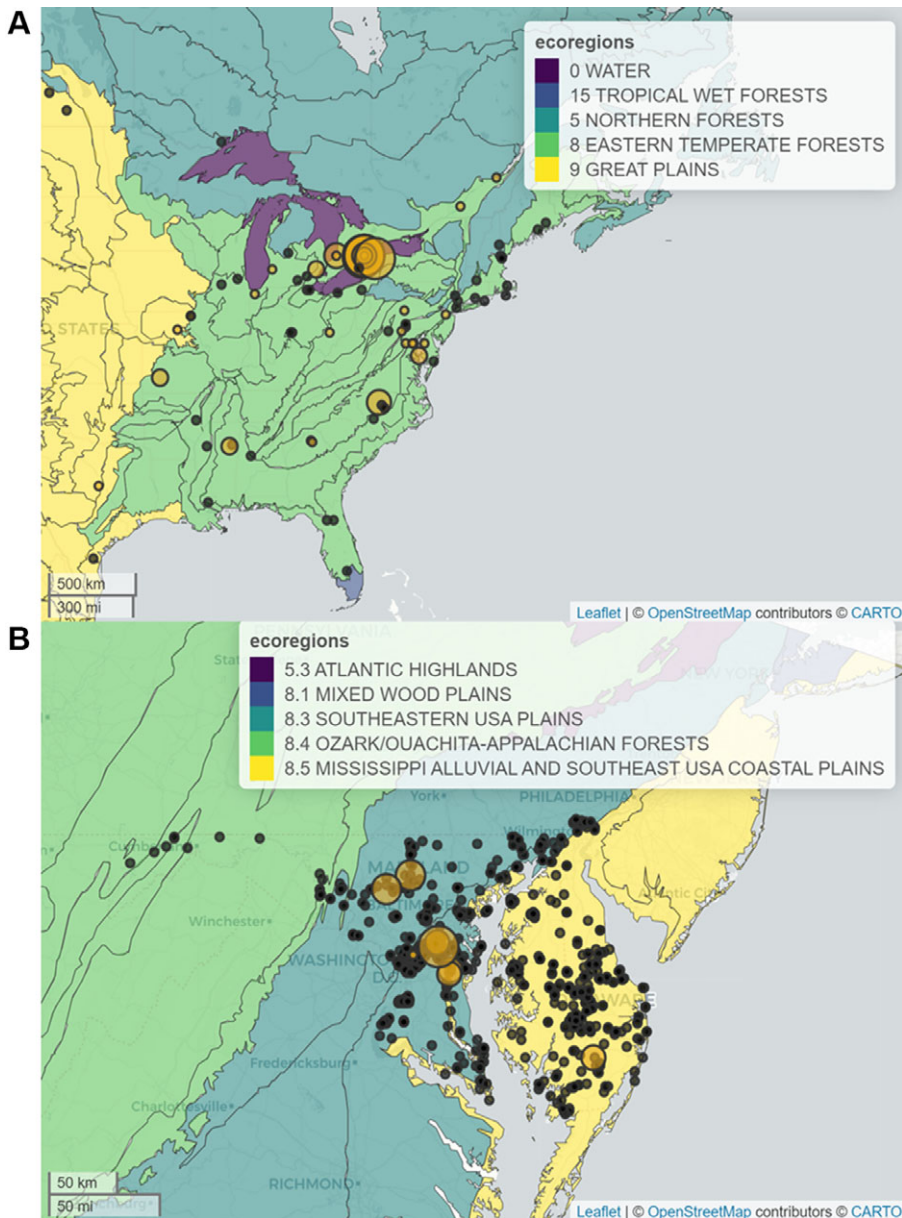


Fig. 2. Map of *O. conjuncta* relative abundance (percentage of the total number of bees that are *O. conjuncta*): **A**, in eastern North America from a literature review of 74 bee community surveys, Onuferko *et al.* (2018), and Irazuzta's findings (unpublished data; Supplementary material, Table S1); and **B**, in Maryland and nearby regions, United States of America, from the Native Bee Inventory and Monitoring Lab (BIML) of the United States Geological Survey (Kammerer *et al.* 2020). The map shading in each panel indicates the ecoregion in which the community was surveyed. Communities without *O. conjuncta* are indicated by small black dots. Communities with *O. conjuncta* are indicated by orange-shaded circles that are graduated by relative abundance of *O. conjuncta* (intervals for relative abundance %: <0.2, <0.5, <1, <2, <5, >5).

six sites, abundant (2.5% and 4.8%) in two sites that were surveyed once in June, and highly abundant (20%, or 10 *O. conjuncta* among 50 bees total) from a single day of sampling in April (Fig. 2B). The high abundances in the Niagara Region and Hamilton, which averaged 18.6%, with a minimum of 0.35% and a maximum of 54.2%, were markedly different from the relative

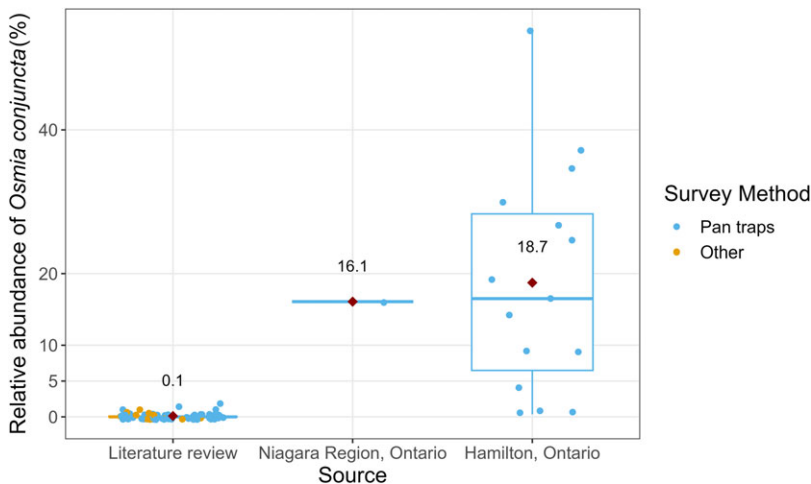


Fig. 3. Relative abundances (%) of *O. conjuncta* from three difference sources: the literature review of 74 bee community surveys in eastern North America; a long-term survey in the Niagara Region, Ontario, Canada (Onuferko *et al.* 2018); and five sites in Hamilton, Ontario, Canada (Irazuzta, unpublished data). The Niagara Region results were reported as the pooled data from 10 years of surveys at five sites. Hamilton results are separated into results for five sites in each of three years. For each group, the average is indicated by text above the corresponding diamond symbols. Colour of the symbols indicates whether pan traps or other survey methods were used to collect bees. Many studies used multiple methods (see Supplementary material, Table S1).

abundances of *O. conjuncta* in communities identified in the general literature review (analysis of variance, $F_{1,90} = 112.8$, $P < 0.0001$; Fig. 3).

The relative abundance of *O. conjuncta* in the bee community in Hamilton from 2014 to 2016 varied over the flight season. Males and females differed in abundance and phenology, including emergence times and active periods (Fig. 4). *Osmia conjuncta* were most abundant in Created Prairie and Old Field Adjacent, with spring relative abundance reaching as high as 87% on 7 May 2015 at Created Prairie. In contrast, Old Field North and Wet Meadow both had low *O. conjuncta* relative abundance, with no *O. conjuncta* found in 2016 at Old Field North (Fig. 4A). Female absolute abundance appeared to vary more between years than male absolute abundance did (Fig. 4B and C). Over the three years, 186 samples contained a total of 8391 bees, of which 1910 were *O. conjuncta*, with 1276 males and 634 females. Total *O. conjuncta* abundance was 15.4% in 2014, 27.5% in 2015, and 19.9% in 2016. Peak relative abundance of *O. conjuncta* shifted between years (Fig. 4A; Supplementary material, Table S3). Emergence of males in early spring varied across years as did the persistence of females into late summer. Male abundance in early spring was an order of magnitude greater than female abundance over the same period (Fig. 4B and C). However, males were no longer present by 16 June in 2014, 2 June in 2015, and 30 May in 2016, whereas females persisted in low numbers until 11 August in 2014, 17 July in 2015, and 11 July in 2016. Subsequently, female sex ratio changed dramatically from spring through late summer (Supplementary material, Table S3).

Empty snail shells were common in the Created Prairie site. We found 1088 empty snail shells in the 99 1-m² quadrats. All shells appeared to be from *Cepaea* spp. The mode for empty shell numbers per square metre was zero because 17 quadrats had no shells, but the empty shell number per square metre averaged 10.87 with a median of 5, a standard deviation of 15.47, and a maximum of 87 (Fig. 5A). The numbers of shells per quadrat differed from the expected Poisson distribution, indicating clustering of empty shells ($E = 399$, $P < 0.001$). The number of shells with cocoons (Fig. 5B) also showed clustering ($E = 14.3$, $P < 0.001$), although this clustering appeared to be a consequence of the linear relation between the empty shells with cocoons and total number

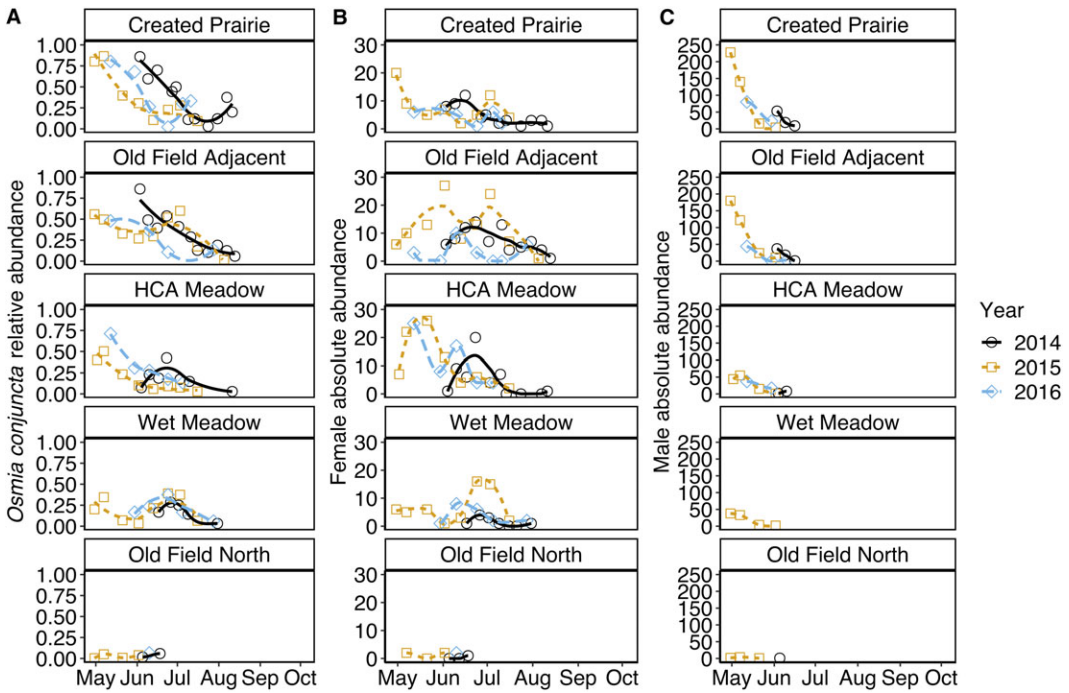


Fig. 4. *Osmia conjuncta* phenology over three consecutive years at five sites in Hamilton, Ontario, Canada: **A**, relative abundance of *O. conjuncta* at each site, absolute abundance of **B**, female and **C**, male *O. conjuncta*. Abundance varied between sites. Males were very abundant in early spring and absent after early June, whereas females were present from spring through August. Start dates varied for each year due to logistics and seasonal variations. Number of sampling units: 186; total bee abundance: 8391; number of *O. conjuncta* males: 1276; and number of females: 634. Supplementary material, Table S3.

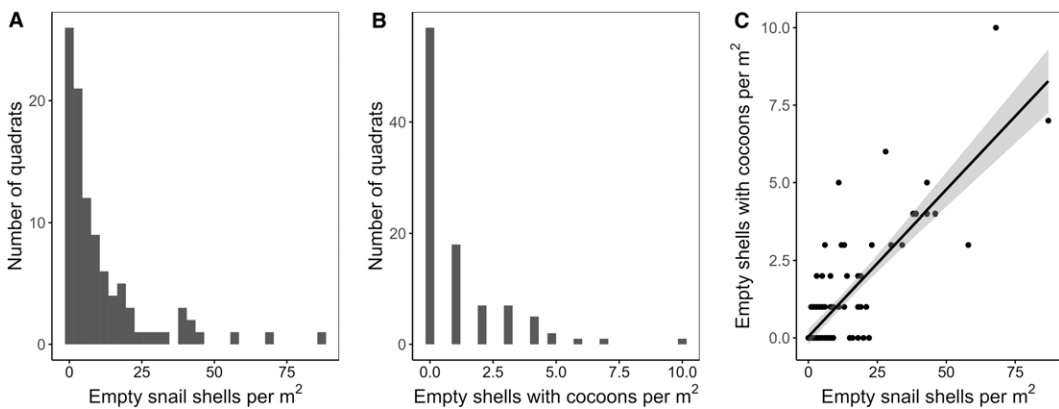


Fig. 5. **A**, The frequency distribution of empty snail shells per square metre from 99 randomly chosen quadrats in the Created Prairie; **B**, the frequency distribution of empty snail shells with cocoons per square metre from 99 randomly chosen quadrats in Created Prairie; and **C**, the relationship between empty snail shells with cocoon and empty snail shells per quadrat.

Table 1. Trait parameters for empty snail shells. Means, standard errors, minima, maxima, and population correlations are given for the continuous traits: height, width, and aperture, and the ordinal trait band colour. Shell state provides the *F*-ratio testing for differences in means between whole and broken shells for each trait. For size traits, the total number of shells equals 1086, the number of broken shells equals 196, and the number of whole shells equals 890. For band colour, the total number of shells equals 952, the number of broken shells equals 186, whole shells equals 766. Significance codes: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

	Height (mm)	Width (mm)	Aperture (mm)	Band colour (rank)
Mean (standard error)				
Min-max				
All shells	14.2 (0.072) 4–22	18.7 (0.090) 5–25	9.52 (0.047) 3–14	2.73 (0.030) 1–5
Whole shells	14.8 (0.062) 4–22	19.5 (0.075) 7–25	9.89 (0.042) 3–14	2.69 (0.033) 1–5
Broken shells	11.5 (0.179) 4–18	15.1 (0.232) 5–22	7.85 (0.124) 3–12	2.90 (0.072) 1–5
Correlations				
Width	0.84***	–	–	–
Aperture	0.77***	0.88***	–	–
Band colour	–0.08*	–0.07*	–0.06	–
Shell state	$F_{1,1084} = 462^{***}$	$F_{1,1084} = 503^{***}$	$F_{1,1084} = 365^{***}$	$F_{1,950} = 7.66^{**}$

of shells per quadrat (Fig. 5C). Shells frequently showed damage, with 196 of 1086 empty snail shells (18.0%) damaged. Damaged snail shells were smaller than whole shells in all three size measures: height, width, and aperture width (Table 1). The shell traits height, width, and aperture width were strongly correlated with each other and weakly correlated with shell colour (Table 1). Shell numbers per square metre showed variation among microhabitats: shell numbers were negatively associated with plant cover but were positively associated with the presence of grasses and shrubs in the quadrat (Table 2; Supplementary material, Table S4). In the univariate analysis, the Edge location was found to have more shells per quadrat.

Of the 1088 collected snail shells, 106 shells (9.8%) had at least one *O. conjuncta* cocoon. The mean number of cocoons in occupied shells was 1.6 cocoons (± 0.083 standard error), and 3.4% of the occupied shells held more than one cocoon. The maximum number of cocoons in a single shell was six. A total of 174 *O. conjuncta* cocoons were found, counting empty husks, live adults, and dead adults.

The empty shell traits that best predicted cocoon number were shell height and shell colour (Table 3; Supplementary material, Table S5). Cocoon number increased with shell height and had an optimal intermediate at approximately 14–18 mm in height (Fig. 6A). Cocoon number decreased with darker shell colour, with an optimal intermediate at colour classes 2 and 3, corresponding to light- and medium-coloured bands (Fig. 6B). Out of the 952 shells with all four traits measured, 547 shells (57%) met both size and colour optima; that is, they were light- to medium-banded (colour class 2 or 3) shells, from 14 to 18 mm in height. Seventy-eight (14.3%) of those shells had cocoons inside.

The only microhabitat variables associated with cocoon number were *site* and the *presence of shrubs* (Table 3). Surprisingly, bees did not nest more or less in quadrats with more shells. They did nest more in the Edge habitat, which had more empty shells. However, bees were less likely to nest in quadrats with shrubs present, even though the number of shells was greater in quadrats with shrubs (Table 3; Supplementary material, Table S5).

Table 2. Univariate regressions and the best-fitting model to estimate the dependent variable $\log_e(\text{snails}+1)$. The best-fitting model was chosen following Akaike information criteria (AICc; Supplementary material, Table S4). $N = 99$. Significance: † $P < 0.10$, $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Explanatory variables	Values	Coefficients	
		Univariate regressions	Best multivariate regression
Site	Centre	–	–
	Edge	0.51*	–
	Forest	–0.42	–
Cover	Continuous from 0 to 100	–0.01*	–0.014***
Shrubs	Absent	–	–
	Present	1.00***	1.09***
Forbs	Absent	–	–
	Present	0.68*	–
Grasses	Absent	–	–
	Present	0.52†	0.86**

Table 3. Univariate Poisson regressions and the best-fitting model to estimate the dependent variable number of cocoons per snail shell, which varied from 0 to 6. The best fitting model was chosen following Akaike information criteria (AICc; Supplementary material, Table S5). $N = 936$. Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Explanatory variables	Values	Polynomial regression terms	Coefficients	
			Univariate regressions	Best multivariate regression
Shell height	Continuous	Linear	77.7***	65.9***
	from 4–22	Quadratic	–41.5***	–35.0**
Shell colour	Ordinal scores	Linear	–14.0***	–11.5**
	from 1–5	Quadratic	–17.0***	–14.3***
Shell damage	Damaged		–	–
	Whole		1.34***	–
Site	Centre		–	–
	Edge		0.48**	0.68***
	Forest		–1.42	–1.16***
Number of shells per m ²	Continuous from 0 to 87		0.0002	–
Cover	Continuous from 0 to 100		–0.0023	–
Shrubs	Absent		–	–
	Present		–0.36*	–0.65***
Forbs	Absent		–	–
	Present		0.65**	–
Grasses	Absent		–	–
	Present		0.26	–

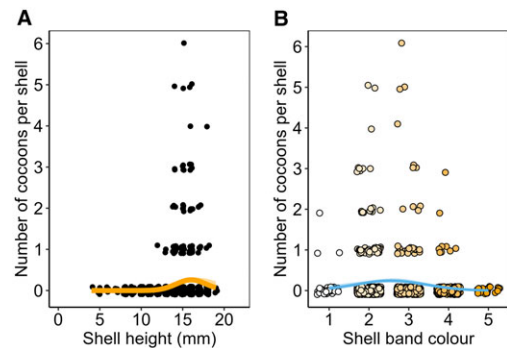


Fig. 6. Scatter plot and predicted relationships from univariate Poisson regressions between **A**, the number of cocoons in an empty snail shell and shell height, and **B**, shell band colour. The number of cocoons ranged from zero to six, with points jittered for clarity. Shell band colour classes are arranged from lightest to darkest value.

Discussion

Here, we present the first quantitative study of a North American snail shell–nesting bee. We confirmed that *O. conjuncta* were using grove snail shells as a nesting substrate, supporting Rau’s (1937) characterisation of *O. conjuncta* as a snail shell–nesting bee. We found that the relative abundance of *O. conjuncta* was sparse across its range, except for a few exceptional sites with high relative abundance, including our focal site. Empty *Cepaea* shells were abundant at the focal study site, and they varied in size, colour, damage, and microhabitat association. The presence of cocoons was related to shell characteristics and microhabitat variation, indicating bee choice of nesting habitat. Taken together, these results support the causal link between *O. conjuncta* abundance and the high density of introduced grove snails suggested by Richards *et al.* (2011).

The density of empty snail shells found in the present study, at 10.87/m², was comparable, if lower than what was found at a European site with high numbers of snail shell–nesting species, where densities ranged from less than 20 to more than 50 empty shells suitable for nesting per square metre (Bogusch *et al.* 2020a). For two North American community studies that collected across multiple habitats in natural areas in southern Illinois (Anderson and Coppolino 2009) and Tennessee (Hodges and McKinney 2018), United States of America, we calculated the density of the macrosnails in the size range preferred by *O. conjuncta* in the study species, using published values for shell length and width (Nekola 2014; Fig. 7). The Illinois study found 294 individuals whose adult size is within the size range and an additional 94 larger snails whose shells could be suitable as juveniles in the 720 m² searched (60 sites, 12 m² per site), a density of 0.54 suitable snails per square metre. In the Tennessee study, considering only the sites in natural areas, 196 individuals of a suitable adult size and an addition 265 potentially suitable as juveniles were collected from 864 m² (48 18-m² sites), a density of 0.53/m². This limited data suggests a relatively low density of macrosnails in eastern North America compared to Europe.

In North America, other grove snail colonies may sometimes be as dense as seen in the present study. The introduced ranges of *C. nemoralis* and *C. hortensis* (Global Biodiversity Information Facility 2022) coincide with the known range of *O. conjuncta*, particularly in eastern North America. However, the clumped distribution of *Cepaea* spp. into colonies (Lamotte 1959), weak competitive ability (Hoxha *et al.* 2019), slow movement (Ozgo and Bogucki 2011), and the barriers to its movement from roads, crops, and coniferous forests (Rosin *et al.* 2017) make predicting whether grove snails will be found in abundance in a given habitat difficult in the absence of direct observation. Despite the known affiliation of grove snails with human disturbance, *O. conjuncta* were not more likely to be found in urban surveys (Supplementary material, Table S1). The only surveys in the literature review where a relative abundance of *O. conjuncta* similar to that in Hamilton (18.7%) and the Niagara Region (16.1%; Onuferko *et al.* 2018) were within the Native Bee Inventory and Monitoring Lab data set (Kammerer *et al.* 2020). Although most sites had no *O. conjuncta* and eight sites had low to moderate abundance, one sample taken in April

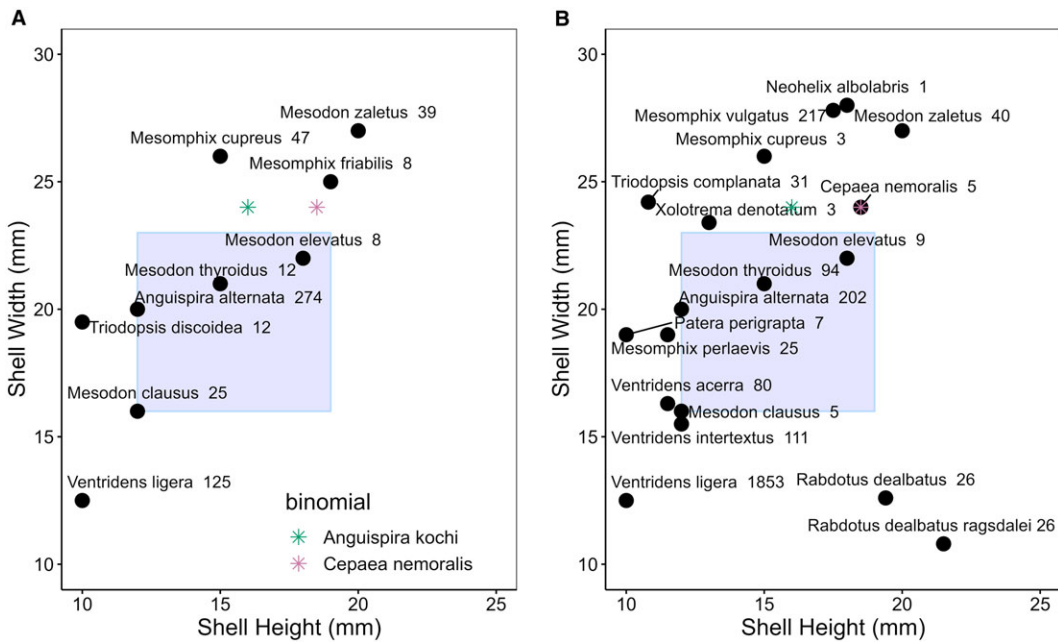


Fig. 7. A scatter plot of shell width versus shell height for species of macrosnails found in two quantitative community studies: **A**, in Illinois, United States of America (Anderson and Coppolino 2009) and **B**, in Tennessee (Hodges and McKinney 2018). The numbers indicate the total number of snails of each species. The shaded rectangle indicates the choice of shell size found in the present study. The sizes of species whose shells are known to be used by *O. conjuncta*, and *Cepaea nemoralis* (grove snail) and *Anguispira kochi* are indicated by stars.

(MDbeaec698 near Jericho Park, Maryland) found 20% relative abundance of *O. conjuncta* (Fig 2B). Field research assessing both snail and bee populations at multiple sites will be needed to determine how grove snails impact the bee's populations through its range.

The native snail species that provide shells used by *O. conjuncta* are largely unknown. Rau (1937) indicated *Anguispira kochi* (Stylommatophora: Discidae) was observed as a shell species used by *O. conjuncta*, but the range of this snail is restricted to the central-midwestern United States of America (Hubricht 1985). In the present study, all shells found were from *Cepaea* snails. A comparison of eastern North American terrestrial snails (Perez *et al.* 2008) against species size characteristics (Nekola 2014) yielded 14 candidate snail species (Supplementary material, Table S6) with shell dimensions consistent with the size preferences observed for *O. conjuncta* in *Cepaea* shells. Two candidate native snail species within the size preferences shown in this study and with ranges overlapping with that of *O. conjuncta* are the white-lip globe snail, *Mesodon thyroidus* Say (Stylommatophora: Polygyridae), and the eastern whitelip snail, *Neohelix albolabris* Say (Stylommatophora: Polygyridae). These species are the second (*M. thyroidus*, $n = 3298$) and third (*N. albolabris*, $n = 2242$) most commonly reported terrestrial snails in the Global Biodiversity Information Facility (2022), with the grove snail, *C. nemoralis* ($n = 7289$) being the most commonly observed.

Despite an abundance of empty shells at the Hamilton site, *O. conjuncta* used only 9.8% of the available empty shells. The majority of *O. conjuncta* were found in a narrow range of empty shell sizes and colours. Nesting frequency also varied with area and vegetation, suggesting the choice of nesting sites by female bees was deliberate and based on both the individual shell traits and microhabitat.

Previous studies on size preferences in snail shell–nesting bees have focused on snail shell species, using reports of adult size as a measure of shell size (Bogusch *et al.* 2020b; Heneberg *et al.* 2020; Hostinska *et al.* 2021) and have found relatively weak preferences for shell species or size. In the present study, we found considerable size variation in shells from the one snail species. *Osmia conjuncta* chose shells from within a 4-mm size range; although the empty shells ranged from 3 to 20 mm in height, 94.5% of shells containing *O. conjuncta* cocoons were between 14 and 17 mm in height, indicating that these bees may strongly prefer shells of intermediate size and that they largely avoid shells outside this range. Preferences in nesting are observed in other *Osmia* species that nest in stem or wood cavities; that is, they select nesting sites based on visual cues outside the nest (Loukola *et al.* 2020) or on nest entrance size (Budriene *et al.* 2004; MacIvor 2016; Rauf *et al.* 2022). Cavity size has been shown to affect nesting likelihood: because brood cells that fit tightly prevent the entry of parasites (MacIvor 2016), cavity size may be of greater importance to *O. conjuncta*, because no plugs were observed at the nest entrance in the present study (unpublished data). The strength of the shell may also play a role in *O. conjuncta* shell choice because smaller shells are more fragile and vulnerable to predation (Jordaens *et al.* 2006; Rosin *et al.* 2011). The observed shell damage was consistent with predator damage (Rosin *et al.* 2013) and was observed more often with small shells, indicating younger snails are more likely to be preyed upon. Although nesting likelihood was not affected by shell damage, that smaller shells were avoided resulted in few damaged shells (2.27%) containing cocoons.

Osmia conjuncta chose to nest in shells of intermediate colour, avoiding both sun-bleached white shells and shells that had dark bands and resembled living shells. Few bees being found in dark-banded shells could result from those shells' snails having died after the flight season or from dark-banded empty shells being too similar to living snails' shells. Recently dead snails' shells may have also been avoided for their poor quality because shells with decaying flesh may promote pathogens, detritivores, and predators (Heneberg *et al.* 2020). Older white shells have a weaker structure because snail shells degrade over time, depending on their size, thickness, and environment (Říhová *et al.* 2018; Heneberg *et al.* 2020). *Osmia conjuncta* shell choice may also be influenced by how the colour of the shell affects its visibility to predators (Surmacki *et al.* 2013) and by temperature (Rosin *et al.* 2018).

Microhabitat variation affected the probability of shell nesting. More shell nesting occurred in the northern Edge area of the Created Prairie site than in the Centre area. In addition, although more snail shells were found near shrubs, *O. conjuncta* were less likely to nest in shells near shrubs. Fewer *O. conjuncta* nesting near shrubs may have been the consequence of shading or the use of shrubs by other animals. Surprisingly, the probability that *O. conjuncta* nested in a shell was not affected by the density of shells in the quadrat; instead, the number of shells with a cocoon in a quadrat increased linearly with the number of shells in that quadrat. This result indicates that *O. conjuncta* neither nest gregariously (Antoine and Forrest 2021) nor avoid other *O. conjuncta*.

Heneberg *et al.* (2020) argues that snail shell–nesting bees are limited by both the abundance and quality of available nests. In the present study, the Created Prairie site contained many shells that met both the size and colour preferences of *O. conjuncta* (57%), but only 14.3% of these shells were used for nesting. Some of those shells were in less-preferred microhabitats, such as near shrubs where they may not have been visible to bees. However, apparently suitable shells may have other characteristics that deter bees from choosing them. Other explanations for this difference may be that the *O. conjuncta* population at Created Prairie is still growing or that the population is constrained by factors beyond nesting substrate availability. The *O. conjuncta* population may be restricted by fungal pathogens, such as chalkbrood (*Ascospaera*), that are known to attack *Osmia* species (LeCroy *et al.* 2023), nest parasitism (Goodell 2003), or competition for floral resources (LeCroy *et al.* 2020; Meeus *et al.* 2021).

The unusually high relative abundance of *O. conjuncta* in early spring at the Created Prairie site prompts further considerations. Such an abundance could have significant effects on other bees if food resources are limiting (Carey *et al.* 2012; LeCroy *et al.* 2020). The numerical superiority of

O. conjuncta could deplete the floral resources from the limited number of native early spring flowering species (Guezen and Forrest 2021). We would predict that *O. conjuncta* may compete more strongly with closely related species that often have similar suites of functional traits, including the 23 *Osmia* species described in eastern Canada (Packer *et al.* 2007). Although the effects of invasive species are well documented, the consequences of an overabundant native bee species have yet to be studied.

The unusually high abundance of *O. conjuncta* in Hamilton and the Niagara Region provide an opportunity for future studies to understand the snail shell preferences, examine the nesting behaviour, and predict the population dynamics of *O. conjuncta*, a species with a unique nesting biology in eastern North America that has not been studied since 1937 (Rau 1937).

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.4039/tce.2023.10>.

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Competing interests. The authors declare they have no competing interests.

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