

**Wybrane aspekty biologii murarki ogrodowej  
(*Osmia rufa*)  
w różnych środowiskach**

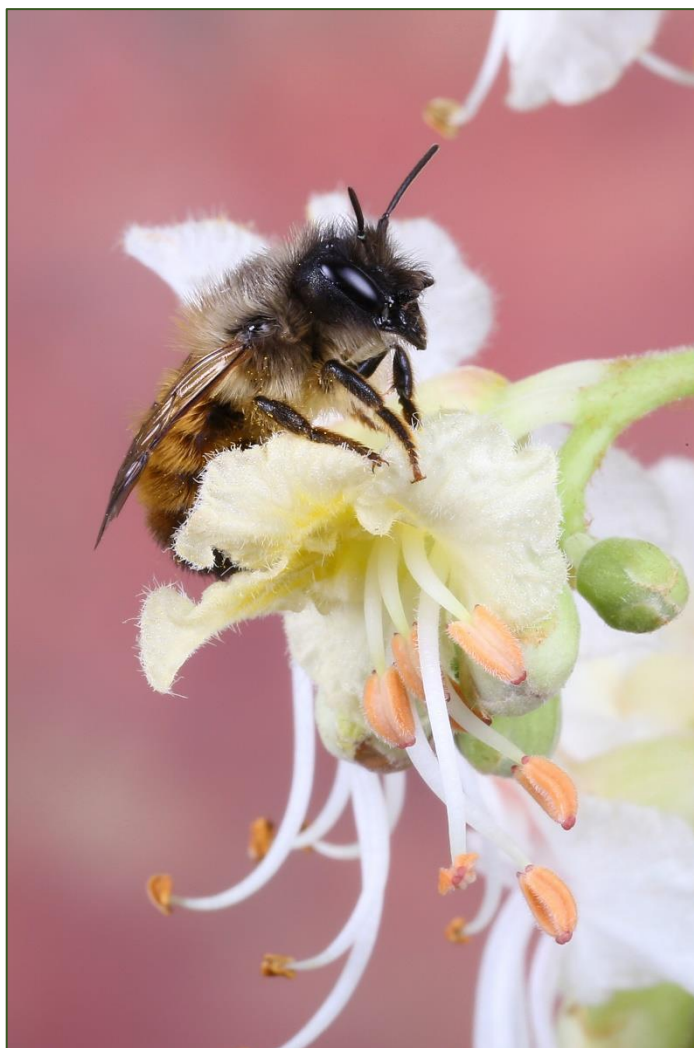
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**Selected aspects of the red mason bee  
(*Osmia rufa*) biology  
in various environments**

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## SPIS PUBLIKACJI

1. Splitt, A., Schulz, M., & Skorka, P. (2022). Current state of knowledge on the biology and breeding of the solitary bee–*Osmia bicornis*. *Journal of Apicultural Research*, 61(2), 163-179. DOI: 10.1080/00218839.2021.1957610
2. Łoś, A., Skórka, P., Strachecka, A., Winiarczyk, S., Adaszek, Ł., Winiarczyk, M., & Wolski, D. (2020). The associations among the breeding performance of *Osmia bicornis* L. (Hymenoptera: Megachilidae), burden of pathogens and nest parasites along urbanisation gradient. *Science of the Total Environment*, 710, 135520. DOI: 10.1016/j.scitotenv.2019.135520
3. Splitt A., Pustkowiak S., Borański M., Strachecka A., Skórka P. Sex and the city: *Osmia bicornis* have more numerous and bigger female offspring in urban environment. *Landscape and Urban Planning* [w trakcie procesu redakcyjnego w czasopiśmie *Landscape and Urban Planning*]
4. Splitt, A., Skórka, P., Strachecka, A., Borański, M., & Teper, D. (2021). Keep trees for bees: Pollen collection by *Osmia bicornis* along the urbanization gradient. *Urban Forestry & Urban Greening*, 64, 127250. DOI: 10.1016/j.ufug.2021.127250

## **PODZIĘKOWANIA**

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## STRESZCZENIE

Urbanizacja to szereg procesów prowadzących do zmiany środowiska przyrodniczego, w tym do zaniku naturalnych siedlisk, i bezpośrednio oraz pośrednio wpływających na biologię organizmów. Uwzględniając dotychczas opublikowane dane wskazujące na istnienie licznych, potencjalnie szkodliwych dla organizmów żywych, czynników antropogenicznych w środowisku miejskim, postawiłam hipotezę, że wzrastający stopień urbanizacji wpływa negatywnie na pszczoły samotnice, murarki ogrodowe (*Osmia bicornis*, syn. *O. rufa*). Przewidywałam, że wraz ze wzrostem stopnia urbanizacji zwiększa się liczebność patogenów w gnieździe, obniża się liczba potomnego pokolenia, zmienia się proporcja płci potomstwa w kierunku przewagi samców oraz zmniejsza się wielkość dorosłych osobników potomnego pokolenia. Ponadto oczekiwałam, że spektrum roślin, z których samice tworzą pakiety pyłkowe różni się pomiędzy lokalizacjami w gradiencie urbanizacji.

Badania prowadzone w ramach dysertacji doktorskiej miały na celu określenie wpływu środowisk o różnym stopniu urbanizacji (miejskiego, podmiejskiego oraz rolniczego) na lokalne populacje murarki ogrodowej. W cyklu artykułów będących podstawą rozprawy doktorskiej przedstawiono szczegółowy przegląd literatury na temat biologii *O. bicornis* (**publikacja 1**). W gradiencie urbanizacji zidentyfikowano mikrobiom gniazd oraz zbadano obecność materiału genetycznego pasożytów *Nosema apis* i *N. ceranae*, a także oceniono poziom sukcesu reprodukcyjnego na podstawie parametrów populacyjnych (obecność zdrowego potomstwa w stosunku do liczby niewykorzystanych pakietów pyłkowych, obumarłych larw i komór lęgowych zajętych przez pasożyty) (**publikacja 2**). Przeprowadzono analizę proporcji płci oraz zweryfikowano różnice wielkości kokonów oraz masy samic i samców w pokoleniu potomnym (**publikacja 3**). Przeprowadzono także analizę palinologiczną pakietów pyłkowych z komór lęgowych gniazd murarek (**publikacja 4**). Dane przeanalizowano różnymi metodami w środowisku R.

W żadnej lokalizacji wśród bakterii i grzybów zidentyfikowanych za pomocą techniki MALDI-TOF MS nie stwierdzono obecności szczepów patogennych dla pszczół. W potomnym pokoleniu murarek ogrodowych nie wykryto też obecności materiału genetycznego pasożytów *N. apis* ani *N. ceranae*. Tereny podmiejskie odznaczały się największą liczebnością pasożytów. Największy sukces reprodukcyjny osiągnęły

miejskie populacje murarki ogrodowej. W potomstwie murarek w środowisku miejskim samice były większe i liczniejsze niż w pozostałych lokalizacjach. Murarki ogrodowe, szczególnie na terenach miejskich, zbierały pyłek głównie z pospolitych gatunków drzew, takich jak dęby, klony, kasztanowce i wiązy, podczas gdy pszczoły z terenów podmiejskich i wiejskich zbierały pyłek także z roślin zielnych i krzewów.

Wyniki sugerują, że środowisko miejskie, wbrew postawionej hipotezie, nie wywiera negatywnego wpływu na populację murarek ogrodowych. Niemniej, wymagane są dalsze badania w innych lokalizacjach i w większym zakresie gradientu urbanizacji.

## SUMMARY

Urbanisation is a phenomenon causing changes of the environment, including the disappearance of natural habitats, which further directly and indirectly affects the biology of organisms. Most of the existing literature indicates potentially harmful influence of the numerous anthropogenic factors in the urban environment on living organisms. Therefore, I hypothesised that the increasing level of urbanisation negatively affects red mason bee (*Osmia bicornis*, syn. *O. rufa*). I assumed that with the increasing level of urbanisation, the manifestation of pathogens in the nest is increasing, the number of the offspring is decreasing, males are prevailing in the offspring sex ratio and the size of the adult offspring is decreasing. Furthermore, I expected that the spectrum of plants from which females form pollen loads differs among locations in the urbanisation gradient.

The research aimed to determine the influence of environments along urbanization gradient (urban, suburban and rural) on the red mason bees. A detailed literature review on the biology of *O. bicornis* was done (**manuscript 1**). The nest microbiome was identified along with the verification of the genetic material of the parasites *Nosema apis* and *N. ceranae* presence. The level of reproductive success was assessed on a basis of the population parameters (presence of healthy offspring in relation to the number of unused pollen loads, dead larvae and brood chambers with parasites) (**manuscript 2**). Analysis of the offspring sex ratio and verification of cocoon and bee size differences were conducted (**manuscript 3**). Palynological analysis of the pollen loads composition was done (**manuscript 4**). The data were analysed using various methods in R.

The presence of bee-pathogenic bacteria and fungi was not described with the MALDI-TOF MS technique in any of the urbanisation gradient localizations. Also, the presence of genetic material of neither *N. apis* nor *N. ceranae* parasites were detected in the red mason bees' offspring. The greatest manifestation of parasites occurred in suburban areas. The greatest reproductive success was achieved by urban populations of the red mason bees. Offspring females were larger and more numerous in the urban environments, than in the other locations. *Osmia bicornis*, particularly in urban areas, repeatedly collected pollen from common trees such as oaks, maples,



chestnut and elm, while bees in suburban and rural areas also collected pollen from herbaceous plants and shrubs.

The results suggest that the urban environment, contrary to the hypothesis, does not have a negative impact on the biology of red mason bees. However, further research in other cities and in an extended urbanisation gradient is required.

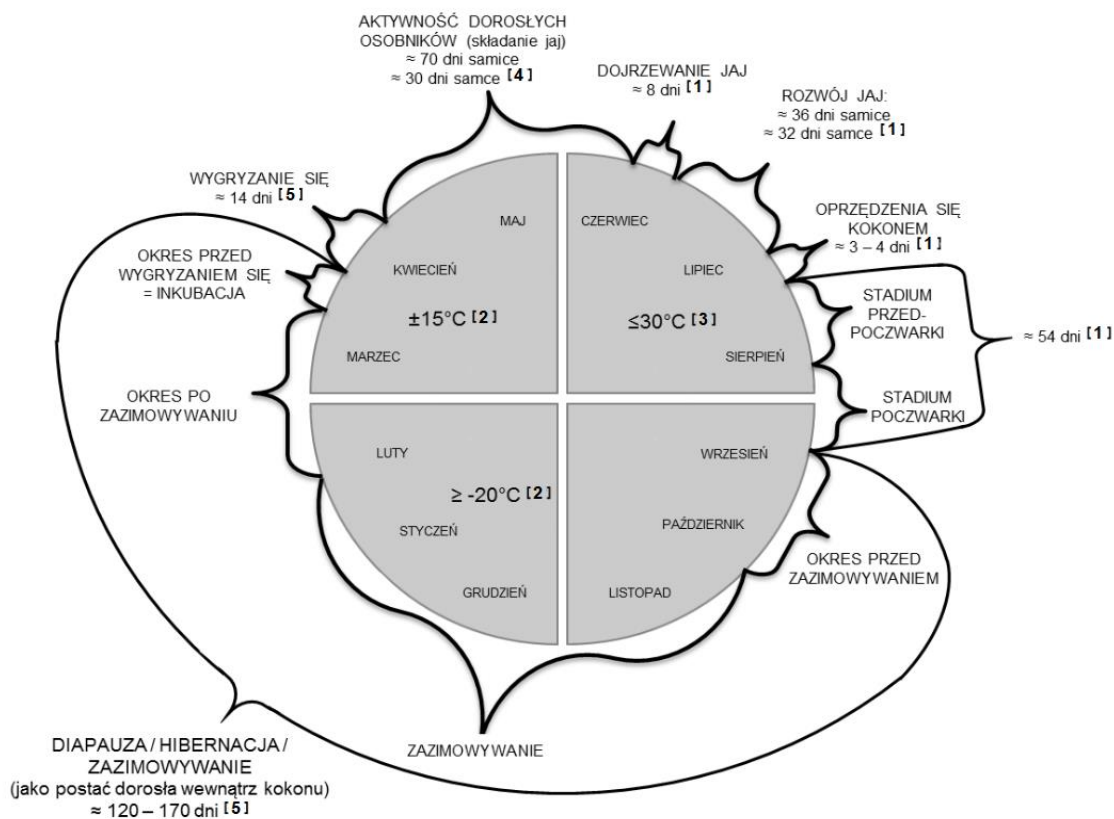
## WSTĘP

Urbanizacja to proces wyrażający się w rozwoju miast, wzroście ich liczby, powiększaniu obszarów miejskich i udziału ludności miejskiej w całości zaludnienia (Szymańska, 2008). Jest to dynamiczne zjawisko kształtujące środowisko wraz ze znajdującymi się w nim organizmami. Ponad 50% powierzchni lądów na Ziemi zostało już przekształcone na potrzeby człowieka (Sun i in., 2022). Aktualne prognozy wskazują na to, że urbanizacja wciąż będzie postępowała i wywierała duży wpływ na populacje organizmów oraz na funkcjonowanie ekosystemów (McKinney, 2008; Darrel Jenerette i Potere, 2010; Gu i in., 2017). Jednym z najważniejszych zjawisk związanych z funkcjonowaniem ekosystemów i utrzymaniem bioróżnorodności jest zapylenie. Ponad 87% roślin wymaga zapylenia przez zwierzęta, którymi w strefie klimatu umiarkowanego są głównie owady (Ollerton i in., 2011). Przeważająca liczba badań przedstawia szkodliwy wpływ urbanizacji na różnorodność i liczebność owadów (Holway i Suarez, 2006; McKinney, 2008; Jones i Leather, 2013; Rosin i in., 2016; Meyer i in., 2020). Jednak wpływ środowiska miejskiego na owady zapyłające jest niejednoznaczny. Cardoso i Gonçalves (2018), Birdshire i in. (2020) oraz Pereira i in. (2021) stwierdzili, że liczebność oraz różnorodność pszczół maleje wraz z postępującą urbanizacją. Natomiast, Wilson i Jamieson (2019) oraz Fitch i in. (2019) przedstawili pozytywny wpływ urbanizacji na obce gatunki pszczół, podkreślając przy tym jednocześnie negatywne oddziaływanie na gatunki rodzime. Kearns i Oliveras (2009) stwierdzili większą różnorodność dzikich pszczół w centrach miast niż na ich obrzeżach. Zostało to potwierdzone przez Banaszak-Cibicką i Żmihorskiego (2012) i wyjaśnione wysoką temperaturą w mieście, niską wilgotnością powietrza, czyli zjawiskiem zwanym „miejską wyspą ciepła”. Na brak negatywnego wpływu urbanizacji na kondycję owadów zapyłających może wskazywać także mniejsza asymetria skrzydeł niektórych pszczół samotnic odłowionych w centrum miasta (Banaszak-Cibicka i in., 2017). Te sprzeczne wyniki badań sugerują, że wpływ urbanizacji na biologię pszczół wymaga prowadzenia dalszej, wnikliwej weryfikacji.

Jako obiekt badań wpływu urbanizacji na pszczoły w dysertacji wykorzystano murarkę ogrodową (*Osmia bicornis* (Linnaeus, 1758), syn. *O. rufa*). Jest to polilektyczny, wczesnowiosenny gatunek z nadrodziny pszczół powszechnie występujący we wszystkich krajach Europy, w Afryce Północnej (Algierii, Maroku,

Tunezji), w południowo-zachodniej Azji (na Cyprze, w Izraelu, Iranie, Syrii i Turcji) oraz w północnej Azji (Kirgistanie, Kazachstanie, Turkmenistanie, a nawet w Japonii) (Romankova, 1984; Banaszak i Romasenko, 2001; Praz i in., 2008; Bees, Wasps & Ants Recording Society 2020; Müller, 2022).

Murarka ogrodowa wykorzystuje jako miejsca do gniazdowania m.in. puste łodygi roślin. Samice zakładają około 30 komór lęgowych dla potomstwa (Sedivy i Dorn, 2014), średnio po 15 w jednym kanale gniazdowym (Giejdasz i in., 2016). Ze względu na filopatrię, czyli przywiązanie do miejsca gniazdowania (Raw, 1972; Ivanov, 2006), niewielki zasięg lotu (około 300-400 m [Gathmann i Tscharntke, 2002; Zurbuchen i in., 2010]) oraz dobre poznanie cyklu życiowego (Ryc.1) możliwe jest utrzymywanie *O. bicornis* w sztucznych konstrukcjach gniazdowych. Z tego powodu, pszczoła ta jest coraz liczniejsza zarówno w środowiskach rolniczych, jak i zurbanizowanych (m.in. Everaars i in., 2011; Holzschuh i in., 2012; Brittain i in., 2013; Güler, 2020; Belien i in., 2021; Eeraerts i in., 2021; Van der Meersch i in., 2021; Yourstone i in., 2021).



**Ryc.1.** Cykl życia murarki ogrodowej (opracowanie własne na podstawie: [1] Giejdasz i Wilkaniec, 2002; [2] Krunic i Stanisavljević, 2006; [3] Radmacher i Strohm, 2010; [4] Raw, 1972; [5] Van der Steen i De Ruijter, 1991).

Jednym z dobrze poznanych zagrożeń ograniczających rozwój populacji murarki ogrodowej są inwazje pasożytów takich jak *Cacoxenus indagator* Loew, 1858, *Chaetodactylus osmiae* (Dufour, 1839), *Anthrax anthrax* (Schrank, 1781) i *Monodontomerus obscurus* Westwood, 1833 (Krunić i in., 2005). Według Seidelmann i in. (2010) pasożyty powodują ponad 70% śmiertelność larw *O. bicornis* w warunkach naturalnych. Pszczoły te mogą być także zainfekowane przez protisty np. *Nosema* spp. (Ravoet i in., 2014; Bramke i in., 2019; Müller i in., 2019), grzyby oraz bakterie (Krunić i in., 2005; Keller i in., 2013; Sedivy i Dorn, 2014). Poważnym zagrożeniem dla murarki ogrodowej mogą być także niedobory pożywienia lub jego nieodpowiedni skład. Spadek kondycji pszczół samotnic może być spowodowany przez krótkotrwałe rozbieżności czasowe pomiędzy pojawem pszczół a kwitnieniem roślin (Schenk i in., 2018). Dieta larw murarek zawierająca pyłek od niektórych gatunków roślin (np. z rodziny jaskrowatych) może powodować karłowatość pszczół, a nawet być przyczyną ich śmiertelności (Sedivy i in., 2011; Eckhardt i in., 2014).

W dzikich populacjach murarki ogrodowej liczebność samców jest wyższa niż samic (Sedivy i Dorn, 2014). Fitch i in. (2019) zauważyli także, że w populacjach dzikich pszczół liczebność samców wzrasta wraz ze wzrostem urbanizacji środowiska. Zajdel i in. (2021) stwierdzili, że nie ma znaczących różnic w liczbie potomstwa *O. bicornis* i jego śmiertelności pomiędzy środowiskami w gradiencie urbanizacji.

Szczegółowy przegląd literatury dotyczącej biologii murarki ogrodowej przedstawiono w **publikacji 1** z cyklu czterech artykułów będących podstawą rozprawy doktorskiej.

Przeprowadzone przeze mnie badania miały na celu poszerzenie wiedzy na temat wpływu urbanizacji na biologię *O. bicornis*. Badania te są jednymi z pierwszych, które określają relacje między parametrami populacyjnymi murarki ogrodowej w różnych warunkach środowiskowych. Pozwala to, przynajmniej częściowo, uzupełnić informacje o wpływie antropogenicznych zmian środowiska przyrodniczego na ten gatunek.

## HIPOTEZA I CEL BADAŃ

Uwzględniając dotychczasową wiedzę literaturową wskazującą na przeważnie negatywny wpływ środowiska miejskiego na owady, postawiono hipotezy, że środowisko miejskie:

- sprzyja wysokiej liczebności patogenów i pasożytów w gnieździe murarek ogrodowych, obniża liczebność i szanse rozwoju pokolenia potomnego,
- przesuwa stosunek płci potomstwa w kierunku samców oraz wpływa negatywnie na wielkość samców i samic w pokoleniu potomnym,
- wpływa na preferencje w wyborze roślin, z których samice tworzą pakiety pyłkowe.

Celem badań było określenie różnorodności patogenów oraz poziomu sukcesu reprodukcyjnego (**publikacja 2**), rozkładu płci w pokoleniu potomnym, wielkości potomnych kokonów oraz masy samic i samców (**publikacja 3**), jakościowego składu pakietów pyłkowych (**publikacja 4**), w populacjach murarki ogrodowych utrzymywanych w środowiskach o różnym poziomie gradientu urbanizacji: rolniczym, podmiejskim oraz w centrum miasta.

## MATERIAŁY I METODY

Przed rozpoczęciem badań dokonano szczegółowego przeglądu literatury dotyczącej biologii murarki ogrodowej (**publikacja 1**).

W celu zrealizowania badań zmapowano wybrane lokalizacje w regionie lubelskim. Wykorzystano oprogramowanie QGIS oraz ortofotomapy dostępne w Geoportalu (<https://www.geoportal.gov.pl>). Kategoryzacji gradientu urbanizacji dokonano zgodnie z koncepcją Fortel i in. (2014) wprowadzając modyfikacje uwzględniające warunki lokalne. Jako dodatkowy wskaźnik poziomu urbanizacji zastosowano także odległość od centrum miasta (Lublin). W wytypowanych do badań lokalizacjach określono stosunek powierzchni infrastruktury zabudowanej (budynki, drogi, chodniki itp.) do powierzchni terenów zielonych (zadrzewienia, skwery, trawniki, zieleńce itd.) w obszarze o promieniu 500 m od konstrukcji gniazdowej. Wytypowano następujące kategorie gradientu urbanizacji:

- środowisko miejskie (90% infrastruktury zabudowanej):
  - U1 - 51°14'N, 22°32'E,
  - U2 - 51°14'N, 22°30'E,
- środowisko podmiejskie (55-65% infrastruktury zabudowanej):
  - S1 - 51°14'N, 22°29'E,
  - S2 - 51°17'N, 22°42'E,
- oraz środowisko rolnicze (do 20% infrastruktury zabudowanej):
  - R - 51°30'N, 22°55'E.

W każdej lokalizacji usytuowano konstrukcję gniazdową o wymiarach 45 cm x 45 cm x 45 cm na drewnianej ramie 0,5 m nad ziemią z siatką ochronną (oczka o średnicy 2 cm).

Jako materiał gniazdowy zastosowano 700 skośnie ściętych rurek trzciniowych. W każdej konstrukcji gniazdowej umieszczono 500 losowo wybranych kokonów murarki ogrodowej z populacji utrzymywanej przez pracowników Zakładu Pszczelnictwa Instytutu Ogrodnictwa - Państwowego Instytutu Badawczego. Po zakończeniu sezonu wegetacyjnego (na przełomie października i listopada) materiał gniazdowy z każdej lokalizacji został przeniesiony z drewnianych konstrukcji do kartonowych pojemników z otworami wentylacyjnymi, które następnie przechowywano w 4°C.

Z każdej lokalizacji z losowo wybranych, zasiedlonych gniazd pobrano materiał do:

- **(A)** identyfikacji mikroorganizmów techniką MALDI-TOF MS, **(B)** badań PCR pod kątem obecności pasożytów *Nosema apis* (Zander, 1909) i *N. ceranae* (Fries, Feng, da Silva, Slemenda & Pieniazek, 1996), **(C)** zebrania danych do oceny sukcesu reprodukcyjnego populacji, w tym liczebności i składu gatunkowego pasożytów gniazdowych (**publikacja 2**);
- **(D)** analizy danych dotyczących rozkładu płci w pokoleniu potomnym, **(E)** długości i szerokości kokonów oraz masy kokonów i pszczoł (**publikacja 3**);
- **(F)** przeprowadzenia analizy palinologicznej składu pakietów pyłkowych (**publikacja 4**).

## **A. Identyfikacja mikroorganizmów techniką MALDI-TOF MS**

Do oddzielnych próbek typu Eppendorf pobrano po 10 próbek: niewykorzystanych pakietów pyłku, obumarłych larw oraz losowo wybranych kokonów, a następnie wyjętych z kokonów dorosłych osobników. Z każdego typu materiału wykonano zawiesiny, które poddano analizie mikrobiologicznej wykorzystując podłoża: lab-agar (5%), McConkey Lab-Agar, Chapman Lab-Agar, Sabouraud Dextrose Lab-Agar (Biocorp). Podłoża inkubowano w 36°C przez 48 godzin. Bakterie i grzyby były pasażowane i inkubowane na kolejnych pożywkach, do czasu wyizolowania czystych kultur. Z każdej kultury wykonano supernatant, który naniesiono na metalową płytkę (Ancorchip™ 800/384, Bruker) i pozostawiono do wyschnięcia w temperaturze pokojowej. Następnie na płytkę naniesiono 1 µl roztworu matrycy (kwas cyjano-4-hydroksycynamonowy, Bruker) i próbkę pozostawiono do wyschnięcia w temperaturze pokojowej. Metalową płytkę z próbkami umieszczono w komorze do analizy MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry). Automatyczny pomiar i analizę porównawczą z widmami referencyjnymi przeprowadzono za pomocą oprogramowania Ultraflex extreme Mass Spectrometer i MALDI Biotyper 3.0 (Bruker). Badanie zostało zrealizowane w Katedrze Epizootiologii i Klinice Chorób Zakaźnych, Wydziału Medycyny Weterynaryjnej Uniwersytetu Przyrodniczego w Lublinie.

## **B. Badania pod kątem obecności materiału genetycznego pasożytów *Nosema apis* i *N. ceranae***

Losowo pobrano po 5 potomnych samców i samic wyjętych bezpośrednio z kokonów. Z odwłoków pszczoł wyizolowano DNA za pomocą DNeasy Blood and Tissue Kit® (Qiagen) zgodnie z instrukcją producenta. W celu wykrycia obecności *N. apis* i *N. ceranae*, przeprowadzono reakcję PCR ze specyficznymi starterami (Ptaszyńska i in., 2016). Elektroforezę przeprowadzono w 2% żelu agarozowym, zabarwionym bromkiem etydyny (5 V/cm przez 30 minut). Uzyskane prążki analizowano na zdjęciach wykonanych w świetle UV za pomocą kamery CCD z wykorzystaniem programu Scion Image. Badanie zrealizowano w Instytucie Biologicznych Podstaw Produkcji Zwierzęcej Wydziału Biologii, Nauk o Zwierzętach i Biogospodarki Uniwersytetu Przyrodniczego w Lublinie.



### C. Określenie poziomu sukcesu reprodukcyjnego populacji

Losowo wybrano 50 rurek. Każdą rurkę ostrożnie otwarto i spisano liczbę oraz zawartość wszystkich komór lęgowych, którą zróżnicowano na:

I. Niezawierające żywego potomstwa, tj.:

- a) niewykorzystane pakiety pyłkowe,
- b) nierozwinięte, obumarłe larwy pszczół,
- c) dorosłe martwe osobniki znajdujące się poza kokonem,
- d) pasożyty;

II. Kokony zawierające zdrowe osobniki murarki ogrodowej.

Na podstawie tych danych oszacowano poziom sukcesu reprodukcyjnego dla poszczególnych populacji wykorzystując równanie:

$$\frac{II}{II + [(a)+b)+c)+d]} * 100\% = \frac{II}{II + I} * 100\% = \text{poziom sukcesu reprodukcyjnego}$$

Do określenia prawdopodobieństw: przeżycia, obecności pasożytów gniazdowych, obecności obumarłych larw oraz niewykorzystanych pakietów pyłkowych w zależności od lokalizacji użyto uogólnionego modelu liniowego mieszanego (GLMM) z rozkładem dwumianowym i funkcją wiążącą typu logit. W modelu identyfikator rurki został użyty jako czynnik losowy. Ponadto, przeprowadzono analizę na poziomie rurki gniazdowej i określono liczebność żywych pszczół, liczbę pasożytów gniazdowych, liczbę obumarłych larw oraz liczbę niewykorzystanych pakietów pyłkowych w zależności od lokalizacji. W analizach tych wykorzystano uogólniony model liniowy (GLM) z rozkładem Poissona i logarytmiczną funkcją wiążącą.

## **D. i E. Pomiary kokonów oraz analiza rozkładu płci w pokoleniu potomnym**

Przed otwarciem 50 losowo wybranych, zasiedlonych rurek gniazdowych elektroniczną suwmiarką zmierzono ich średnicę zewnętrzną, wewnętrzną oraz długość od otworu wejściowego do kolanka. Po zapisaniu liczby i zawartości komór lęgowych z otwartych rurek (patrz ppkt. C metodyki) wyjęto wszystkie kokony. Suwmiarką zmierzono długość i szerokość każdego kokonu, a następnie każdy oczyszczony kokon zawierający w środku imago murarki zważono. Po zważeniu kokony otwierano, by wykorzystując dymorfizm płciowy zidentyfikować samce i samice murarki ogrodowej. Pszczoły delikatnie wyjmowano z kokonów i również zważono. Badanie wykonano w Instytucie Biologicznych Podstaw Produkcji Zwierzęcej Wydziału Biologii, Nauk o Zwierzętach i Biogospodarki Uniwersytetu Przyrodniczego w Lublinie.

Do określenia proporcji płci w lokalizacjach (przedstawianego jako odsetek samic) wykorzystano tylko dane dotyczące kokonów z określoną płcią pszczoł (wykluczono komory / kokony zajęte przez pasożyty, martwe larwy lub puste komory). Objętość kokonów policzono korzystając ze wzoru na objętość elipsoidy obrotowej wydłużonej  $V = (4/3)\pi ab^2$ . W analizach uwzględniono średnicę wewnętrzną rurek gniazdowych. Proporcja płci potomstwa w każdej rurce w badanych lokalizacjach była analizowana przy użyciu uogólnionego mieszanego modelu liniowego z rozkładem dwumianowym i funkcją wiążącą typu logit. W modelu identyfikator rurki został użyty jako czynnik losowy. Różnice w masie i objętości kokonów dla różnych płci i w różnych lokalizacjach były analizowane przy pomocy mieszanych modeli liniowych (LMM).

## **F. Analiza palinologiczna**

Z każdej lokalizacji, z losowo wybranych komór gniazdowych, pobrano do próbek typu Eppendorf po 10 próbek pakietów pyłkowych. Zostały one poddane mikroskopowej analizie palinologicznej. Aby określić względną częstotliwość ziaren pyłku w każdym pakiecie pyłkowym, dodano wodę destylowaną (5:1 objętościowo) i mieszano przez 1 godzinę (do całkowitego zdyspergowania) w celu przeniesienia 15  $\mu$ l jednorodnego roztworu pyłku na szkiełko mikroskopowe z kroplą 30  $\mu$ l wody destylowanej. Szkiełko wysuszono, a następnie zabezpieczono szkiełkiem nakrywkowym i żelatyną glicerolową, jako środkiem utrwalającym. Dla każdego szkiełka mikroskopowego zidentyfikowano ponad 300 kolejnych ziaren pyłku

do rodzaju, gatunku, rodziny lub typu budowy. Oceny dokonano za pomocą atlasów pyłku, baz palinologicznych (m.in. paldat.org) oraz własnego zbioru preparatów referencyjnych z wykorzystaniem klasyfikacji Zandera. Analiza została przeprowadzona w Zakładzie Pszczelnictwa Instytutu Ogrodnictwa – Państwowym Instytucie Badawczym.

Do analizy danych palinologicznych zastosowano kanoniczną analizę korespondencji, aby dowiedzieć się, czy występują różnice w składzie taksonów roślinnych między lokalizacjami gradientu urbanizacji. Ponadto, wykorzystano analizę ko-korespondencji (Co-CA) do wykazania, czy występowanie taksonów roślin w pakietach pyłku może być wyjaśnione składem gatunkowym roślin w badanych lokalizacjach. Zastosowano także model liniowy, aby sprawdzić, czy różnorodność taksonów (wskaźnik Simpsona) i liczba taksonów roślinnych różnią się między lokalizacjami w gradiencie urbanizacji.

## WYNIKI

W materiale biologicznym pobranym z gniazd murarki ogrodowej wykryto głównie bakterie z rodzaju *Pseudomonas* oraz *Sphingomonas*. Częściowa kanoniczna analiza korespondencji wykazała, że istnieją różnice w składzie taksonomicznym kultur bakterii i grzybów pomiędzy środowiskami. Dalsza analiza wykazała, że liczba taksonów mikroorganizmów nie była związana z lokalizacją (GLMM, Wald  $\chi^2 = 8,401$ ;  $df = 4$ ;  $P = 0,08$ ), ale była związana z rodzajem badanego materiału biologicznego (GLMM, Wald  $\chi^2 = 8,888$ ;  $df = 4$ ;  $P = 0,031$ ) oraz z interakcją między tymi dwiema zmiennymi (GLMM, Wald  $\chi^2 = 68,492$ ;  $df = 12$ ;  $P < 0,001$ ). Testy post-hoc Tukeya wskazały, że nie ma różnic w liczbie taksonów bakterii i grzybów między lokalizacjami w próbkach pszczoł i obumarłych larw, ale są różnice w próbkach kokonów i pyłku. W próbkach kokonów liczba taksonów bakterii i grzybów była najwyższa w lokalizacjach U1, S1 i S2, natomiast w próbkach pyłku liczba mikroorganizmów była najwyższa w lokalizacji R i S1.

W odwłokach murarek ogrodowych nie wykryto materiału genetycznego *N. apis* i *N. ceranae*.

Badane lokalizacje różniły się liczbą komór w rurkach gniazdowych, przy czym najwięcej komór budowały murarki ogrodowe w lokalizacji U1 i R (GLMM  $df = 4$ ; Wald  $\chi^2 = 17,852$ ;  $P = 0,001$ ). Analizy wykazały, że lokalizacja ma istotny wpływ na poziom sukcesu reprodukcyjnego (GLMM  $F_{4, 2194} = 37,385$ ;  $P < 0,001$ ), obecność pyłku niewykorzystanego przez larwy (GLMM  $F_{4, 2192} = 11,345$ ;  $P < 0,001$ ), zamieranie larw (GLMM  $F_{4, 2194} = 19,094$ ;  $P < 0,001$ ) oraz obecność pasożytów (GLMM  $F_{4, 2194} = 18,756$ ;  $P < 0,001$ ). W lokalizacjach U1 i U2 poziom sukcesu reprodukcyjnego był najwyższy, a najniższe było prawdopodobieństwo obumarcia larw, niewykorzystania pakietów pyłku przez larwy i występowania pasożytów. Po uwzględnieniu różnic w lokalizacji i liczbie komór, a także interakcji między tymi czynnikami stwierdzono ujemną zależność między liczbą pasożytów, a liczbą zdrowych pszczoł (GLMM  $df = 1$ ; Wald  $\chi^2 = 18,943$ ;  $P < 0,001$ ).

Powyższe wyniki szczegółowo przedstawiono w **publikacji 2** z cyklu czterech artykułów będących podstawą rozprawy doktorskiej.

Rozkład płci w potomstwie *O. bicornis* różnił się pomiędzy obiema lokalizacjami miejskimi a rolniczą. Samice przeważały w lokalizacjach U1 (GLMM  $\beta_{U1} = 1,513$ ;  $P < 0,01$ ) i U2 (GLMM  $\beta_{U2} = 1,123$ ;  $P < 0,05$ ), a samce w lokalizacji R (GLMM  $\beta_R = -5,514$ ;  $P < 0,001$ ). Średnica wewnętrzna rurki gniazdowej miała wpływ na rozkład płci potomstwa - im większa średnica, tym więcej potomnych samic w rurce gniazdowej (GLMM  $\beta_{\text{diameter}} = 0,693$ ;  $P < 0,001$ ).

Płeć potomstwa była silnie skorelowana z masą i objętością kokonów, a także z masą potomstwa. Samce mają średnio mniejszą masę ciała niż samice. Zarówno masa kokonów, jak i potomstwa była wyższa w lokalizacji S2 niż w R (LMM – masa kokonu:  $\beta_{S2} = 0,014$ ;  $P < 0,001$ ; LMM – masa potomstwa:  $\beta_{S2} = 0,013$ ;  $P < 0,001$ ). Kokony miały większą objętość w lokalizacjach U1 (LMM  $\beta_{U1} = 46,911$ ;  $P < 0,001$ ) i U2 (LMM  $\beta_{U2} = 29,819$ ;  $P < 0,001$ ). Stwierdzono interakcję między płcią potomstwa a lokalizacją. Różnice pomiędzy samcami a samicami dotyczące masy i objętości kokonów oraz masy samych pszczoł były największe w lokalizacji S2 (LMM – masa kokonu:  $\beta_{\text{male} \times S2} = -0,018$ ;  $P < 0,001$ ; LMM – objętość kokonu:  $\beta_{S2} = -25,090$ ;  $P < 0,001$ ; LMM – masa potomstwa:  $\beta_{S2} = -0,015$ ;  $P < 0,001$ ). Różnice pomiędzy objętością kokonów samców i samic były największe w lokalizacji U1 (LMM  $\beta_{U2} = -13,936$ ;  $P < 0,05$ ).

Szczegółową analizę rozkładu płci, masy i wielkości potomnych kokonów oraz masy samic i samców przedstawiono w **publikacji 3** z cyklu czterech artykułów będących podstawą rozprawy doktorskiej.

Analizy palinologiczne wykazały, że murarka ogrodowa wykorzystywała ograniczoną liczbę głównych taksonów roślin pożytkowych do tworzenia pakietów pyłkowych dla potomstwa. W lokalizacjach U1 i U2 pszczoły zbierały pyłek głównie z roślin wiatropylnych, podczas gdy w pozostałych lokalizacjach tworzyły pakiety głównie z roślin owadopylnych. Różnorodność ( $F = 2,571$ ;  $df = 4, 22$ ;  $P = 0,06$ ) i liczba taksonów roślin ( $F = 4,252$ ;  $df = 4, 22$ ;  $P = 0,011$ ) w pakietach pyłku wzrastała od lokalizacji U1, U2, przez S1, S2 do R. Kanoniczna analiza korespondencji ( $F = 2,54$ ;  $df = 4, 22$ ;  $P < 0,001$ ) wskazała na to, że skład zbieranego przez pszczoły pyłku różnił się w poszczególnych lokalizacjach. W pakietach murarek ogrodowych dominował pyłek pochodzący z dębów (*Quercus* spp.) (U1, R), klonów (*Acer* spp.) (U1, U2, R), kasztanowców (*Aesculus* spp.) (U1), orzecha włoskiego (*Juglans regia*) (U1, S1, R)

i wiązów (*Ulmus* spp.) (S1). Murarki ogrodowe z lokalizacji S1 i S2 gromadziły także w znacznych ilościach pyłek roślin z rodziny jaskrowatych (Ranunculaceae), wilczomleczowatych (Euphorbiaceae) i makowatych (Papaveraceae), a w niewielkich ilościach pyłek roślin z rodziny różowatych (Rosaceae) i kapustowatych (Brassicaceae). Analiza ko-korespondencji wykazała, że skład taksonów w pyłku w dużym stopniu odzwierciedla występowanie poszczególnych roślin w otoczeniu gniazd. Wyniki te szczegółowo przedstawiono w **publikacji 4** z cyklu czterech artykułów będących podstawą rozprawy doktorskiej.

## WNIOSKI

W żadnej lokalizacji gradientu urbanizacji wśród bakterii i grzybów zidentyfikowanych za pomocą techniki MALDI-TOF MS nie stwierdzono obecności szczepów patogennych dla pszczoł. Nosemoza nie jest zagrożeniem dla potomnego pokolenia murarek w badanych lokalizacjach. Różnice między lokalizacjami pod względem obecności pasożytów wskazują, że środowisko miejskie stwarza dogodne warunki bytowania dla murarek ogrodowych. W środowisku miejskim możliwe jest osiągnięcie wysokiego poziomu sukcesu reprodukcyjnego, podczas gdy presja pasożytów ogranicza rozwój populacji w innych typach środowisk gradientu urbanizacji. Można zatem stwierdzić, że środowisko miejskie jest filtrem środowiskowym dla pasożytów murarki.

Rozkład płci w potomstwie *O. bicornis* jest zależny od środowiska. Większa proporcja samic, których produkcja jest kosztowna, w środowisku miejskim wskazuje na to, że stwarza ono dogodne warunki do rozwoju populacji murarki ogrodowej. Może na to również wskazywać większa objętość kokonu i małe różnice masy pomiędzy samicami, a samcami w środowisku miejskim.

Wyniki potwierdziły, że istnieją powtarzające się wzorce wyborów różnego typu pyłku u murarek ogrodowych, obserwowane niezależnie od typu środowiska. Pszczoły zbierały pyłek z aktualnie pylących, dostępnych roślin znajdujących się blisko ich gniazd. Warto jednak zauważyć, że mimo lokalnych różnic w składzie taksonomicznym, niezależnie od środowiska, w pakietach pyłku występował pyłek pochodzący z pylących wiosną drzew i krzewów, takich jak dąb, klon czy wierzba. W jednym pakiecie pyłkowym tworzonym przez murarki ogrodowe można oczekiwać od 2 do 4 taksonów jako głównych zasobów, przy jednoczesnym niewielkim (mniej niż 5% objętości) dodatku pyłków z innych roślin. Zaobserwowano, że liczba głównych typów pyłków jest większa w środowiskach rolniczych w porównaniu do miejskich. Na tej podstawie celowym wydaje się utrzymywanie oraz moderowanie składu gatunkowego zieleni – zwłaszcza miejskiej – w taki sposób, aby wpływać pozytywnie na dietę owadów zapylających. Uzupełnianie krajobrazu (zwłaszcza miejskiego) drzewami, np. wierzbami i dębami, może zapewnić dobry dostęp do bazy pokarmowej dla pszczoł aktywnych wiosną, a jednocześnie może mieć duże znaczenie dla innych organizmów.

## PODSUMOWANIE

Środowisko miejskie (o wysokim poziomie zurbanizowania), wbrew postawionej hipotezie, nie miało negatywnego wpływu na murarki ogrodowe, ponieważ:

- liczebność i różnorodność pasożytów była tam niewielka, liczebność pokolenia potomnego pszczoł była większa niż w środowisku podmiejskim oraz rolniczym, a poziom sukcesu reprodukcyjnego wysoki,
- samice były tam większe i liczniejsze w pokoleniu potomnym niż w innych typach środowisk,
- środowisko to zapewniało wystarczającą bazę pożytkową.



## LITERATURA

1. Szymańska D. (2008). Urbanizacja na świecie. Wydawnictwo Naukowe PWN.
2. Suni, S., Hall, E., Bahu, E., & Hayes, H. (2022). Urbanization increases floral specialization of pollinators. *Ecology and Evolution*, 12(3), e8619. <https://doi.org/10.1002/ece3.8619>
3. McKinney, M. L. (2008). Effects of urbanization on species richness: a review of plants and animals. *Urban Ecosystems*, 11(2), 161-176. <https://doi.org/10.1007/s11252-007-0045-4>
4. Darrel Jenerette, G., & Potere, D. (2010). Global analysis and simulation of land-use change associated with urbanization. *Landscape Ecology*, 25(5), 657-670. <https://doi.org/10.1007/s10980-010-9457-2>
5. Gu, C., Guan, W., & Liu, H. (2017). Chinese urbanization 2050: SD modeling and process simulation. *Science China Earth Sciences*, 60(6), 1067-1082. <https://doi.org/10.1007/s11430-016-9022-2>
6. Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals?. *Oikos*, 120(3), 321-326. <https://doi.org/10.1111/j.1600-0706.2010.18644.x>
7. Holway, D. A., & Suarez, A. V. (2006). Homogenization of ant communities in mediterranean California: the effects of urbanization and invasion. *Biological Conservation*, 127(3), 319-326. <https://doi.org/10.1016/j.biocon.2005.05.016>
8. Jones, E. L., & Leather, S. R. (2013). Invertebrates in urban areas: a review. *European Journal of Entomology*, 109(4), 463-478. <https://doi.org/10.14411/eje.2012.060>
9. Rosin, Z. M., Skórka, P., Pärt, T., Žmihorski, M., Ekner-Grzyb, A., Kwieciński, Z., & Tryjanowski, P. (2016). Villages and their old farmsteads are hot spots of bird diversity in agricultural landscapes. *Journal of Applied Ecology*, 53(5), 1363-1372. <https://doi.org/10.1111/1365-2664.12715>
10. Meyer, S., Rusterholz, H. P., & Baur, B. (2020). Urbanisation and forest size affect the infestation rates of plant-galling arthropods and damage by herbivorous insects. *European Journal of Entomology*, 117, 34-48. <https://doi.org/10.14411/eje.2020.004>
11. Cardoso, M. C., & Gonçalves, R. B. (2018). Reduction by half: the impact on bees of 34 years of urbanization. *Urban Ecosystems*, 21(5), 943-949. <https://doi.org/10.1007/s11252-018-0773-7>
12. Birdshire, K. R., Carper, A. L., & Briles, C. E. (2020). Bee community response to local and landscape factors along an urban-rural gradient. *Urban Ecosystems*, 23(4), 689-702. <https://doi.org/10.1007/s11252-020-00956-w>
13. Pereira, F. W., Carneiro, L., & Gonçalves, R. B. (2021). More losses than gains in ground-nesting bees over 60 years of urbanization. *Urban Ecosystems*, 24(2), 233-242. <https://doi.org/10.1007/s11252-020-01030-1>
14. Wilson, C. J., & Jamieson, M. A. (2019). The effects of urbanization on bee communities depends on floral resource availability and bee functional traits. *PloS One*, 14(12), e0225852. <https://doi.org/10.1371/journal.pone.0225852>
15. Fitch, G., Wilson, C. J., Glaum, P., Vaidya, C., Simao, M. C., & Jamieson, M. A. (2019). Does urbanization favour exotic bee species? Implications for the conservation of native bees in cities. *Biology Letters*, 15(12), 20190574. <https://doi.org/10.1098/rsbl.2019.0574>

16. Kearns, C. A., & Oliveras, D. M. (2009). Environmental factors affecting bee diversity in urban and remote grassland plots in Boulder, Colorado. *Journal of Insect Conservation*, 13(6), 655-665. <https://doi.org/10.1007/s10841-009-9215-4>
17. Banaszak-Cibicka, W., & Żmihorski, M. (2012). Wild bees along an urban gradient: winners and losers. *Journal of Insect Conservation*, 16(3), 331-343. <https://doi.org/10.1007/s10841-011-9419-2>
18. Banaszak-Cibicka, W., Fliszkiewicz, M., Langowska, A., & Żmihorski, M. (2018). Body size and wing asymmetry in bees along an urbanization gradient. *Apidologie*, 49(3), 297-306. <https://doi.org/10.1007/s13592-017-0554-y>
19. Romankova, T. G. (1984) Bees of the genus *Osmia* Panz. (Hymenoptera, Megachilidae) in the fauna of the Far East. *Entomological Review*, 53, 358–364.
20. Banaszak, J., & Romasenko, L. (2001) Megachilid bees of Europe (Hymenoptera, Apoidea, Megachilidae). 2<sup>nd</sup> ed. (corrected and supplemented). Bydgoszcz University, Bydgoszcz.
21. Praz, C. J., Müller, A., Danforth, B. N., Griswold, T. L., Widmer, A., & Dorn, S. (2008). Phylogeny and biogeography of bees of the tribe *Osmiini* (Hymenoptera: Megachilidae). *Molecular Phylogenetics and Evolution*, 49(1), 185-197. <https://doi.org/10.1016/j.ympev.2008.07.005>
22. Bees, Wasps & Ants Recording Society 2020 (13.05.2022) *Osmia bicornis*. <http://www.bwars.com/bee/megachilidae/osmia-bicornis>
23. Müller, A. (2022), Palaeartic Osmiine Bees, ETH Zürich, <http://blogs.ethz.ch/osmiini>
24. Sedivy, C., & Dorn, S. (2014). Towards a sustainable management of bees of the subgenus *Osmia* (Megachilidae; *Osmia*) as fruit tree pollinators. *Apidologie*, 45(1), 88-105. <https://doi.org/10.1007/s13592-013-0231-8>
25. Giejdasz, K., Fliszkiewicz, M., Bednárová, A., & Krishnan, N. (2016). Reproductive potential and nesting effects of *Osmia rufa* (syn. *bicornis*) female (Hymenoptera: Megachilidae). *Journal of Apicultural Science*, 60(1), 75. <https://doi.org/10.1515/jas-2016-0003>
26. Raw, A. (1972). The behaviour of males of the solitary bee *Osmia rufa* (Megachilidae) searching for females. *Behaviour*, 56(3), 279-285.
27. Ivanov, S. P. (2006). The nesting of *Osmia rufa* (L.) (Hymenoptera, Megachilidae) in the Crimea: Structure and composition of nests. *Entomological Review*, 86(5), 524-533. <https://doi.org/10.1134/S0013873806050046>
28. Gathmann, A., & Tschamntke, T. (2002). Foraging ranges of solitary bees. *Journal of Animal Ecology*, 71(5), 757-764. <https://doi.org/10.1046/j.1365-2656.2002.00641.x>
29. Zurbuchen, A., Landert, L., Klaiber, J., Müller, A., Hein, S., & Dorn, S. (2010). Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. *Biological Conservation*, 143(3), 669-676. <https://doi.org/10.1016/j.biocon.2009.12.003>
30. Everaars, J., Strohbach, M., Gruber, B., & Dormann, C. (2011). Microsite conditions dominate habitat selection of the red mason bee (*Osmia bicornis*, Hymenoptera: Megachilidae) in an urban environment: A case study from Leipzig, Germany. *Landscape and Urban Planning*, 103(1), 15–427. <https://doi.org/10.1016/j.landurbplan.2011.05.008>




31. Holzschuh, A., Dudenhöffer, J. H., & Tschardt, T. (2012). Landscapes with wild bee habitats enhance pollination, fruit set and yield of sweet cherry. *Biological Conservation*, 153, 101-107. <https://doi.org/10.1016/j.biocon.2012.04.032>
32. Brittain, C., Williams, N., Kremen, C., & Klein, A. M. (2013). Synergistic effects of non-*Apis* bees and honey bees for pollination services. *Proceedings of the Royal Society B: Biological Sciences*, 280(1754), 20122767. <https://doi.org/10.1098/rspb.2012.2767>
33. Güler, Y. (2020) The status of the red mason bee in the orchards of Ankara and Çankırı Provinces, Turkey. *Turkish Journal of Entomology*, 44(2), 249-258. <https://doi.org/10.16970/entoted.651043>
34. Belien, T., Raymaekers, S., Eeraerts, M., Mommaerts, V., Claus, G., Bogen, C., ... & Bylemans, D. (2021). Towards integrated pest and pollinator management in intensive pear cultivation: a case study from Belgium. *Insects*, 12(10), 901. <https://doi.org/10.3390/insects12100901>
35. Eeraerts, M., Piot, N., Pisman, M., Claus, G., Meeus, I., & Smagghe, G. (2021). Landscapes with high amounts of mass-flowering fruit crops reduce the reproduction of two solitary bees. *Basic and Applied Ecology*, 56, 122-131. <https://doi.org/10.1016/j.baae.2021.07.005>
36. Van der Meersch, V., Billaud, O., San Cristobal, M., Vialatte, A., & Porcher, E. (2022). Landscape floral resources provided by rapeseed correlate with next-year reproduction of cavity-nesting pollinators in a national participatory monitoring program. *Landscape Ecology*, 37(2), 551-565. <https://doi.org/10.1007/s10980-021-01353-0>
37. Yourstone, J., Karlsson, M., Klatt, B. K., Olsson, O., & Smith, H. G. (2021). Effects of crop and non-crop resources and competition: High importance of trees and oilseed rape for solitary bee reproduction. *Biological Conservation*, 261, 109249. <https://doi.org/10.1016/j.biocon.2021.109249>
38. Giejdasz, K., & Wilkaniec, Z. (2002). Individual development of the red mason bee (*Osmia rufa* L., Megachilidae) under natural and laboratory conditions. *Journal of Apicultural Science*, 46, 51-57.
39. Krunić, M. D., & Stanisavljević, L. Ž. (2006). Supercooling points and diapause termination in overwintering adults of orchard bees *Osmia cornuta* and *O. rufa* (Hymenoptera: Megachilidae). *Bulletin of Entomological Research*, 96(3), 323-326. <https://doi.org/10.1079/BER2006423>
40. Radmacher, S., & Strohm, E. (2010). Factors affecting offspring body size in the solitary bee *Osmia bicornis* (Hymenoptera, Megachilidae). *Apidologie*, 41(2), 169-177. <https://doi.org/10.1051/apido/2009064>
41. Van der Steen, J., & De Ruijter, A. (1991) The management of *Osmia rufa* L. for pollination of seed crops in greenhouses. In *Proceedings of Experimental and Applied Entomology*, 2, 137-141.
42. Krunić, M., Stanisavljević, L., Pinzauti, M., & Felicioli, A. (2005). The accompanying fauna of *Osmia cornuta* and *Osmia rufa* and effective measures of protection. *Bulletin of Insectology*, 58(2), 141-152.
43. Seidelmann, K., Ulbrich, K., & Mielenz, N. (2010). Conditional sex allocation in the red mason bee, *Osmia rufa*. *Behavioral Ecology and Sociobiology*, 64(3), 337-347. <https://doi.org/10.1007/s00265-009-0850-2>

44. Ravoet, J., De Smet, L., Meeus, I., Smagghe, G., Wenseleers, T., & de Graaf, D. C. (2014). Widespread occurrence of honey bee pathogens in solitary bees. *Journal of Invertebrate Pathology*, 122, 55-58. <https://doi.org/10.1016/j.jip.2014.08.007>
45. Bramke, K., Müller, U., McMahon, D. P., & Rolff, J. (2019). Exposure of larvae of the solitary bee *Osmia bicornis* to the honey bee pathogen *Nosema ceranae* affects life history. *Insects*, 10(11), 380. <https://doi.org/10.3390/insects10110380>
46. Müller, U., McMahon, D. P., & Rolff, J. (2019). Exposure of the wild bee *Osmia bicornis* to the honey bee pathogen *Nosema ceranae*. *Agricultural and Forest Entomology*, 21(4), 363-371. <https://doi.org/10.1111/afe.12338>
47. Keller, A., Grimmer, G., & Steffan-Dewenter, I. (2013). Diverse microbiota identified in whole intact nest chambers of the red mason bee *Osmia bicornis* (Linnaeus 1758). *PLoS One*, 8(10), e78296. <https://doi.org/10.1371/journal.pone.0078296>
48. Schenk, M., Krauss, J., & Holzschuh, A. (2018). Desynchronizations in bee–plant interactions cause severe fitness losses in solitary bees. *Journal of Animal Ecology*, 87(1), 139-149. <https://doi.org/10.1111/1365-2656.12694>
49. Sedivy, C., Müller, A., & Dorn, S. (2011). Closely related pollen generalist bees differ in their ability to develop on the same pollen diet: evidence for physiological adaptations to digest pollen. *Functional Ecology*, 25(3), 718-725. <https://doi.org/10.1111/j.1365-2435.2010.01828.x>
50. Eckhardt, M., Haider, M., Dorn, S., & Müller, A. (2014). Pollen mixing in pollen generalist solitary bees: a possible strategy to complement or mitigate unfavourable pollen properties?. *Journal of Animal Ecology*, 83(3), 588-597. <https://doi.org/10.1111/1365-2656.12168>
51. Fitch, G., Glaum, P., Simao, M. C., Vaidya, C., Matthijs, J., Iuliano, B., & Perfecto, I. (2019). Changes in adult sex ratio in wild bee communities are linked to urbanization. *Scientific Reports*, 9(1), 3767. <https://doi.org/10.1038/s41598-019-39601-8>
52. Zajdel, B., Borański, M., Kucharska, K., & Teper, D. (2021). Reproduction and accompanying fauna of red mason bee *Osmia rufa* L. (syn. *Osmia bicornis* L.) in areas with different levels of urbanization. *Journal of Apicultural Science*, 65(1), 123-137. <https://doi.org/10.2478/JAS-2021-0009>
53. Fortel, L., Henry, M., Guilbaud, L., Guirao, A. L., Kuhlmann, M., Mouret, H., ... & Vaissière, B. E. (2014). Decreasing abundance, increasing diversity and changing structure of the wild bee community (Hymenoptera: Anthophila) along an urbanization gradient. *PloS One*, 9(8), e104679. <https://doi.org/10.1371/journal.pone.0104679>
54. Ptaszyńska, A. A., Borsuk, G., Zdybicka-Barabas, A., Cytryńska, M., & Małek, W. (2016). Are commercial probiotics and prebiotics effective in the treatment and prevention of honeybee noseosis C?. *Parasitology Research*, 115(1), 397-406. <https://doi.org/10.1007/s00436-015-4761-z>

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## Current state of knowledge on the biology and breeding of the solitary bee – *Osmia bicornis*

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### ABSTRACT

This review gathers the majority of foregoing information about the red mason bee (*Osmia bicornis*, syn. *rufa*) (Apoidea: Megachilidae) and elaborates on the following issues: taxonomy and morphology, distribution, reproduction and sex ratios, nesting, life cycle, and daily activity, metabolic changes, accompanying organisms (including parasites), flower preferences and pollination, and breeding. To make forthcoming studies about *O. bicornis* bees more comprehensive, the authors also identified knowledge gaps.

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### Introduction

During recent years, there has been a growing interest in research on solitary bees such as the red mason bee (*Osmia bicornis*, syn. *rufa*) – an efficient pollinator (i.e., Brittain et al., 2013; Holzschuh et al., 2012; Wilkaniec & Radajewska, 1996). With the current problem of worldwide pollinator deficits (e.g., Gruber et al., 2011; Potts et al., 2016; Senapathi et al., 2015; Thomson, 2001), the high pollination efficiency of red mason bee and the chances associated with employing it in commercial crops look promising (i.e., Gruber et al., 2011; Hansted et al., 2014; Holzschuh et al., 2012; Schindler & Peters, 2011; Sedivy & Dorn, 2014). The information about solitary bees is rather dispersed and requires systematic review (Gruber et al., 2011) because the number of available data has increased in recent decades (Figure 1). This review compiles and organizes the foregoing knowledge about *O. bicornis*.

### Taxonomy and morphology

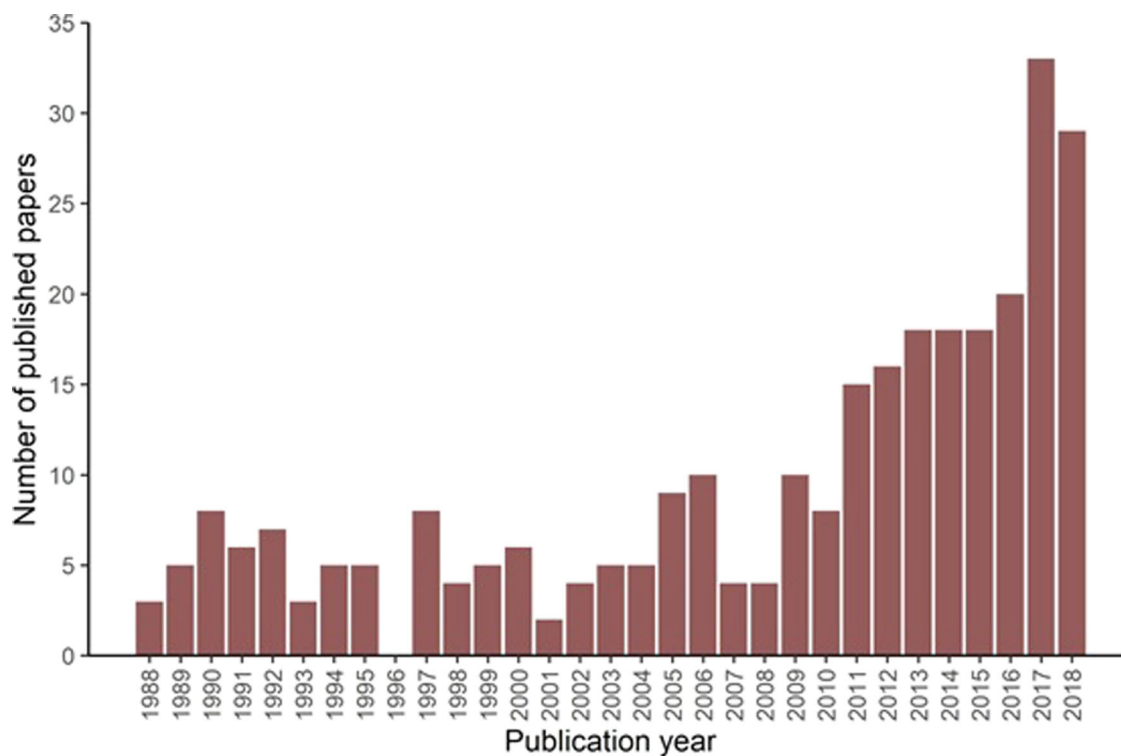
Genus *Osmia* is part of the Megachilidae family from the bee superfamily (Apoidea), which includes 300 species (Michener, 2007). The latest phylogenetic studies suggested that the Megachilidae solitary living group of bees evolved not further than 50 million years ago (Branstetter et al., 2017; Murray et al., 2018).

Red mason bee has two simultaneously used species names: *O. bicornis* and *O. rufa*. In 1758 in the

10<sup>th</sup> edition of Linnaeus' "Systema Naturae" *Apis rufa* and *A. bicornis* were described but, as it appeared later, they were the same species. In subsequent years, many characteristics distinguishing red mason bee from other insects of the *Apis* genus were found and its name was finally changed to *Osmia* (Panzer, 1806). The continued multiplicity of the species name might be a result of the existence of three subspecies within the red mason bee species: *O. bicornis bicornis* (syn. *O. rufa rufa*, *O. rufa bicornis*) (Linnaeus, 1758), *O. globosa* (Scopoli, 1763 via Higgins, 1963), *O. fracticornis* (Pérez, 1895). *Osmia bicornis* was established as a holotype. NCBI:txid1437190 (2018) taxonomy database suggested that only *O. bicornis* is the proper species name. However, according to scientists from the Integrated Taxonomic Information System (ITIS), *O. rufa* (L.) should be used (Ruggiero & Ascher, 2015). On the website of ITIS dedicated to *O. rufa* following information can be found: "See Day (1979) & Ungricht et al. (2008) on an *Apis rufa* vs. *A. bicornis* controversy (both from Linnaeus 1758, p. 175, they are now conspecific in *Osmia*). Kirby (1802) ("First Reviser" under ICZN Art. 24.2.2) used *bicornis* over *rufa*. Dalla Torre (1896) reversed this, and most works since have followed, using *rufa*. An ICZN [International Commission on Zoological Nomenclature] petition & ruling would be required to use *rufa* over *bicornis*, and while this possibility is being considered ITIS will await further information".

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The review was designed and written by AS (90%) in consultation with MS (5%) and supervision and adjustments of PS (5%). All photos were taken and edited by MS. All the authors accepted the final version of the manuscript.



**Figure 1.** Number of published articles on *Osmia bicornis*, syn. *O. rufa* indexed in Web of Science. Search was conducted on 2nd February 2019 in Web of Sciences set on all databases and keywords specified as “*Osmia rufa* OR *Osmia bicornis*”. Time span specified to 1988–2018.

Therefore, we recommend always using both names (at least once in the manuscript’s abstract or included in keywords) and providing one of them as a synonym in brackets.

The descriptions of the *O. bicornis* appearance are present in many identification keys (e.g., Banaszak & Romasenko, 1998; Michener, 2007). The pollen is collected on the scopa, on the ventral surface of the abdomen, not on the corbiculae (pollen baskets) or the last pair of legs as it is in most bees. *Osmia rufa* and many other European species are conspicuous because of abundant red/orange hair (Banaszak & Romasenko, 1998). This coloration may be a result of mimicry which adapts bees to conditions occurring in a particular environment (Catling, 2015).

*Osmia bicornis* is long-haired (rarely forming metasomal fasciae) and robust, 8–12 mm long with the anterior face of the shagreen first metasomal terga. The antennae of the males usually reach the propodeum, and the proboscis does not reach beyond the middle of the thorax in repose (Banaszak & Romasenko, 1998; Michener, 2007). Differences between the sexes of *O. bicornis* are presented in Table 1.

Some red mason bees have an innate tendency to use the left or right antenna more often and that asymmetry also manifests in other individual features (Rogers et al., 2016). For example, left-antenna individuals are more aggressive than right-antenna ones, whereas they are symmetrical in non-aggressive contacts, latency to the first contact, or body wiping.





Frasnelli and Vallortigara (2017) affirmed that *O. bicornis* detects scents in the same way with both antennae (while e.g., honey bees have more sensitive right sides) and the preference of usage of one antenna above the other is an individual quality. There is an asymmetry between the left and right wing that may result from the influence of some environmental factors (Szentgyörgyi et al., 2017).

## Distribution

*Osmia bicornis* can be found throughout most of the Palearctic region. The species occurs in all European countries (Banaszak & Romasenko, 1998; Müller, 2019; Romankova, 1984). In northern Asia, the presence of red mason bee was recorded in Kyrgyzstan, Kazakhstan, Turkmenistan, and even in Japan. *Osmia bicornis* is a widespread species in the Mediterranean Region and occurs in Northern Africa in Algeria, Morocco, Tunisia, whereas Cyprus, Israel, Iran, Syria, and Turkey are limitations for its natural distribution (Banaszak & Romasenko, 1998; Müller, 2019; Praz et al., 2008).

The climatic layout of *O. bicornis* is diversified, extending from the temperate zone to the subtropical. The lowest temperature at which *O. bicornis* can still survive is  $-20^{\circ}\text{C}$  during its hibernation (Krunić & Stanisavljević, 2006b). According to Radmacher and Strohm (2011) at  $30^{\circ}\text{C}$  insect development is disturbed. All factors driving the ambit of distribution may be important indicators of the impacts of

**Table 1.** Differences between the sexes of the red mason bee (*Osmia bicornis*, syn. *O. rufa*) (Banaszak & Romasenko, 1998; Michener, 2007; Seidelmann, 2014; Wilkaniec et al., 2004).

	Female	Male
		
Body length	10–12 mm	8–10 mm
Body weight	45–140 mg	20–100 mg (Figure 2D)
Wing area	29.4–66.2 mm <sup>2</sup>	17.3–48.8 mm <sup>2</sup>
Wing length	7.6–11.3 mm	5.3–9.0 mm
Thorax mass	5.9–18.6 mg	2.2–11.4 mg
Head		
	Black with strongly developed mandibles and with a small, usually bare and shiny depression immediately behind the lower end of the eye (below the posterior margin of the eye)	Black with white hair in front; malar space as long as the width of the scape; a small shining depression in genal area below and behind the lowermost point of the eye margin
Sting	Provided with a thin chaeta free of burrs	Lack of sting
Other	Rust-coloured ventral brush for the collection and carrying of pollen The lower lateral parts of the clypeus protrude forward as strong angles or horns, and the base of the labrum is not hidden	Long antennae (bent backwards, they reach the trunk) The absence of a lateral tooth on the sixth metasomal tergum
Life length	Approximately 46 days on average in natural conditions (Radmacher, 2011), up to 10 weeks (70 days) (Raw, 1972)	Under laboratory conditions, they live 15 days on average (up to 27) (Giejdasz & Wilkaniec, 2002); in natural habitat approximately 4–6 weeks (32 days) (Raw, 1972)

climate change and urbanization processes on bee populations.

### Reproduction and sex ratio

*Osmia bicornis* ends the hibernation process gradually when the ambient temperature fluctuates at approximately 15 °C for a few days in a row (Krunić & Stanisavljević, 2006a). The daily rhythms of emergence are triggered by temperature cycles (Beer et al., 2019). Red mason bee is a protandrous species (males emerge from cocoons before females) (Sedivy & Dorn, 2014). The whole generation begins its adult activity within 14 days from the moment of the first individual emerging (Van der Steen & De Ruijter, 1991).

Each male has its own home range, within which it performs patrol flights in search of females. The area covers ground from 3 to 30 m<sup>2</sup>, may change temporarily and the ranges of several males may partially or even totally overlap as males do not defend their home ranges (Seidelmann, 1999b). Females just after emerging are ready-to-mate and begin to attract males (Biliński & Teper, 2004; Wasielewski et al., 2011). There is also a female-attracting substance on the antennae of males, but

they do not have any special glands, so the origin of the substance is unknown (Conrad, Vidkjaer, et al., 2017). Depending on the temperature of the environment, the composition of the male fragrance changes along with the female scent preferences (Conrad, Stöcker, et al., 2017). Conrad et al. (2018) hypothesized that females from different locations might be selective towards males with a local fragrance bouquet, probably to avoid outbreeding depression. Before copulation males persuade females with pre-copulatory behavior. When a male starts pre-copulatory courtship, his rivals leave the couple alone until the female decides whether she wants to copulate. Throughout the pre-copulatory courtship, the male stimulates the female by standing on her back, embracing her mesothorax with his first and second pair of legs and then with thorax vibration. The male also rubs himself against the female and moves his forward pointing antennae repeatedly over the female's laterally pointing antennae and swings his forelegs over the female's compound eyes. After a male pre-copulatory behavior the female might reject him by physically pushing him off her back or accept copulation with him. Females prefer copulation with males larger than average whose pre-copulatory vibration duration is



longer than others (Conrad & Ayasse, 2015). Males' vibrations are thought to be a signal of vigor, metabolic activity, health, and descent (Conrad & Ayasse, 2015, 2019; Conrad et al., 2010). If accepted, the male moves backward on the female's back and inserts his genitalia into the female's genital chamber while using his antennae to produce a tremolo (continuous drumming) on the female's face, and copulation lasts for several minutes (Conrad et al., 2010; Seidelmann, 1999b) (Table 2).

The female's monandry is induced by up to 13 minutes of the post-copulatory display (Raw, 1976; Seidelmann, 2014). No anti-aphrodisiac is detectable even on freshly mated females (Seidelmann & Rolke, 2019). The protection from remating depends on the cooperation of the inseminated female and the level of her appeasement. Females that copulated and received the ejaculate but have not been satisfied by the post-copulatory display proceed to mate with a different male (Seidelmann, 1999b, 2014). Every single male can participate in 10 matings (but no more than four daily), whereas three matings seem optimal for females (Fliszkiewicz & Wilkaniec, 2009; Seidelmann, 2015). After using up their spermatozoa males are not interested in sexual intercourses because their set of spermatozoa cannot be replenished after a few ejaculations (Boomsma et al., 2005; Paxton, 2005; Seidelmann, 2014). High degrees of selection and competition among the males (52% to 86% of the males do not mate at all, while others mate repeatedly) forced the development of additional protection against females' polyandry (Seidelmann, 2015). Males of *O. bicornis* may use secretions of their accessory reproductive glands and a specialized vasa deferential segment to make a "mating plug" at the opening of females' ductus receptaculi and entire vagina. This plug does not disqualify the female from further copulations. It only prevents different males' ejaculates from mixing and ensures the priority of the first mating male's spermatozoa (Seidelmann, 2014, 2015). The female does not absorb the mating plug but ejects it without changes in its size and structure one day after copulation (Seidelmann, 2015).

*Osmia bicornis* females may collect from 1400 to over 5650 spermatozoa in the spermatheca (Fliszkiewicz & Wilkaniec, 2009) to fertilize only those eggs from which daughters emerge, whereas sons will develop from unfertilized ones (Sedivy & Dorn, 2014; Seidelmann, 2015). *Osmia bicornis* first lays mainly female eggs, whereas, with age and decreasing nesting conditions, it switches to laying eggs with male descendants (Giejdasz et al., 2016; Ivanov, 2006). *Osmia* specimens live in collectives/populations/aggregations estimated between approximately 50 to 250 individuals in one place or 15 females per 1000 m<sup>2</sup> on average (Steffan-Dewenter & Schiele,

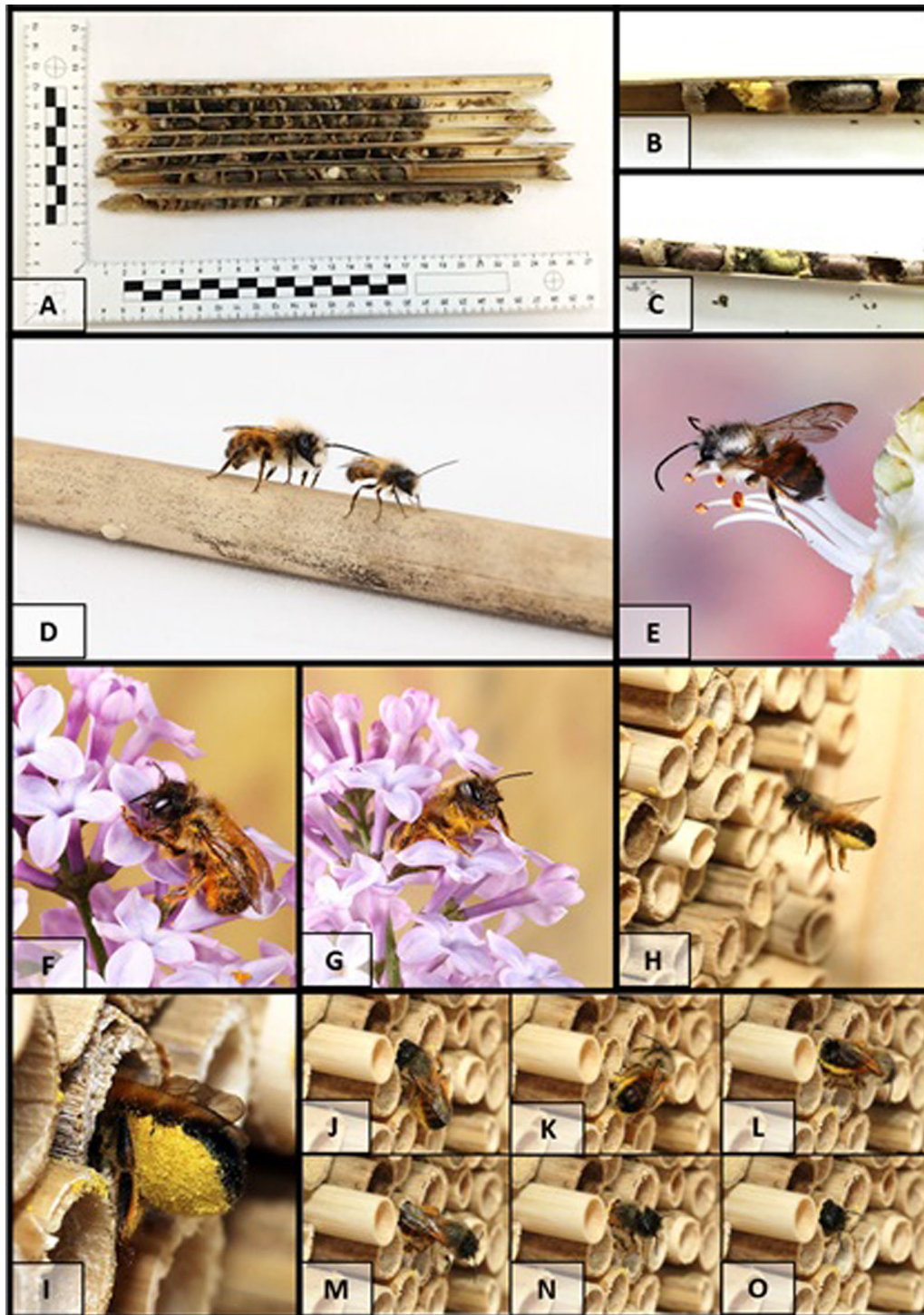
2004). Virgin females may also be found in such aggregations. They have lower chances to establish nests; therefore, they often usurp other females' nesting tubes and produce sons (Groulx & Forrest, 2018; Sedivy & Dorn, 2014). This is caused by an inadequate number of males in a paternal generation, and the surplus of sons will compensate male shortage in the next season. *Osmia bicornis* reproductive success is associated with the environment and its microclimates (presence of parasites, temperature, shading of the nest, and weather during the season) (Fliszkiewicz et al., 2015). Sedivy and Dorn (2014) found that in natural conditions, the *Osmia* population is usually male-biased. Fliszkiewicz et al. (2013) showed that the predominance of females in the population causes a reduction in the number of spermatozoa in the females' spermathecae. Females match the sex of the laid egg to the nesting conditions and the availability of food (Ivanov, 2006). Male eggs are usually deposited in tubes with a diameter of 5–6 mm, whereas female eggs occur mostly in larger tubes with a diameter of 7–8 mm (up to 10) (Ivanov, 2006; Radmacher & Strohm, 2010; Seidelmann et al., 2016). The percentage of daughters increases with the length of the nesting tube (Gruber et al., 2011). The offspring sex ratio is also correlated with the falling efficiency of pollen collection throughout the season and, consequently, can be even three-quarters lower by the end of the nesting season. This is because the eggs from which sons develop require less pollen than daughter eggs (Radmacher & Strohm, 2010; Seidelmann, 2006). Smaller females have more sons, whereas larger females tend to have more daughters (Seidelmann et al., 2010). When a parasite invasion threatens females, they prefer to stay close to the nest, which is associated with collecting smaller portions of pollen and hence the advantage of male offspring in the next generation (Seidelmann, 2006). Male eggs are laid closer to the opening of the tube. This is connected either with the phenomenon of protandry (the appearance of males earlier in the season than females), the higher risk of parasite infestation, or with lower costs of producing male eggs than females (Gruber et al., 2011; Seidelmann, 2006; Seidelmann et al., 2010).

## Nesting

Females usually show philopatry (nest side fidelity) to their emerging place (Ivanov, 2006; Raw, 1972). Female bees are not discouraged from establishing a nest, even if there are discernible threats in its surrounding (e.g., rodent urine, dead bees) (Kierat, Filipiak, et al., 2017). The clay used for the construction of the septa is carried in the mandibles. To begin

**Table 2.** A set of species found in *Osmia bicornis*, syn *O. rufa* nests.

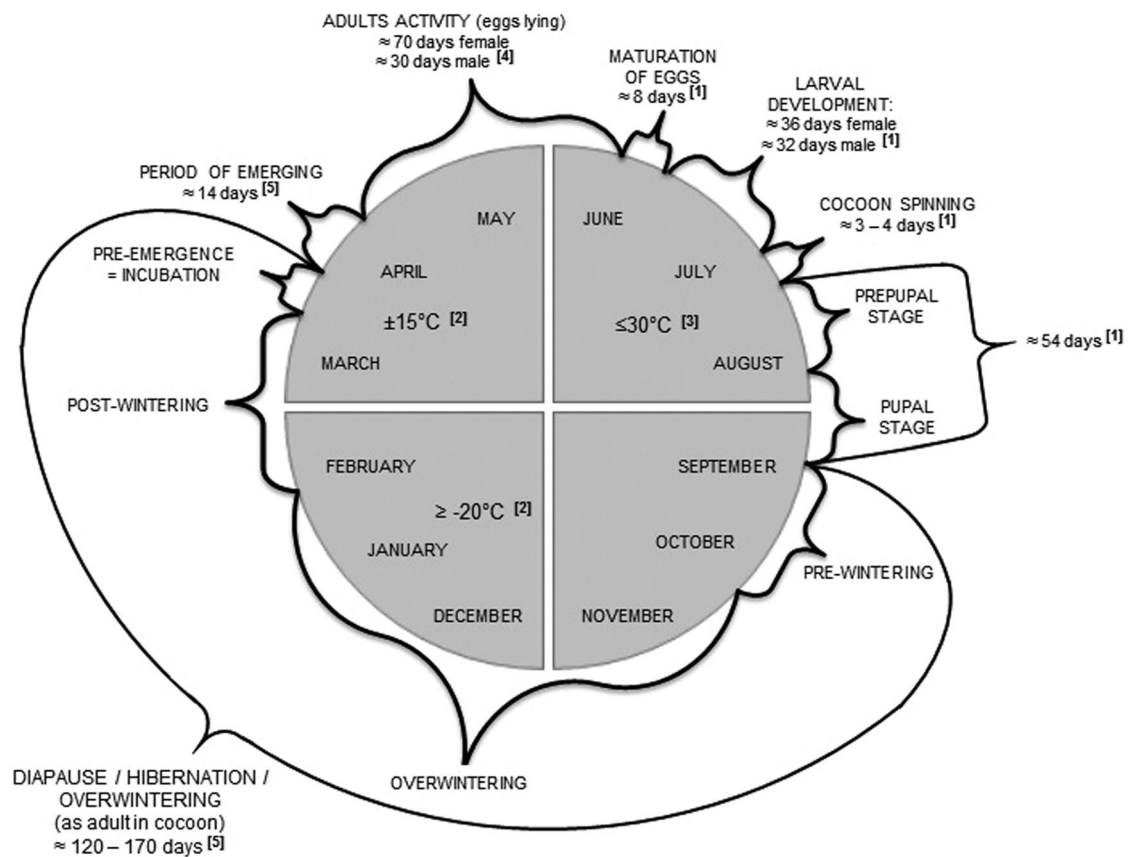
Species	Taxonomy rank (class: order: family)	Reference
<b>Cleptoparasites</b>		
<i>Cacoxenus indagator</i>	Insecta: Diptera: Drosophilidae	Bosch and Kemp (2002), Fliszkiewicz, Kuśnierczak, et al. (2012), Krunić et al. (2005), Sedivy and Dorn (2014), Seidelmann (2006), Steffan-Dewenter (2002), Steffan-Dewenter and Leschke (2003), Tschardt et al. (1998), Zajdel et al. (2014)
<i>Chaetodactylus osmiae</i>	Arachnida (Acari): Sarcopiformes: Chaetodactylidae	Bosch and Kemp (2002), Krunić et al. (2005), Sedivy and Dorn (2014), Seidelmann (2006), Steffan-Dewenter and Leschke (2003), Zajdel et al. (2014)
<i>Chaetodactylus reaumuri</i>	Arachnida (Acari): Sarcopiformes: Chaetodactylidae	Bosch and Kemp (2002)
<i>Chrysis ignita</i> (ruby-tailed wasp)	Insecta: Hymenoptera: Chrysididae	Bosch and Kemp (2002), Fliszkiewicz, Kuśnierczak, et al. (2012), Krunić et al. (2005), Zajdel et al. (2014)
<i>Sapyga clavicornis/Monosapyga clavicornis</i> (wasp)	Insecta: Hymenoptera: Sapygidae	Tschardt et al. (1998)
<i>Sapyga quinquepunctata</i> (solitary wasp)	Insecta: Hymenoptera: Sapygidae	Bosch and Kemp (2002), Fliszkiewicz, Kuśnierczak, et al. (2012)
<b>Cleptobionts</b>		
<i>Camponotus fallax</i> (carpenter ant)	Insecta: Hymenoptera: Formicidae	Krunić et al. (2005)
<i>Formica balcanina</i>	Insecta: Hymenoptera: Formicidae	Krunić et al. (2005)
<i>Formica cunicularia</i>	Insecta: Hymenoptera: Formicidae	Krunić et al. (2005)
<i>Formica rufibarbis</i>	Insecta: Hymenoptera: Formicidae	Krunić et al. (2005)
<i>Tetramorium caespitum</i> (pavement ant)	Insecta: Hymenoptera: Formicidae	Krunić et al. (2005)
<b>Parasitoids</b>		
<i>Anthrax anthrax</i> (fly)	Insecta: Diptera: Bombyliidae	Fliszkiewicz, Kuśnierczak, et al. (2012), Krunić et al. (2005), Steffan-Dewenter and Leschke (2003), Seidelmann (2006), Tschardt et al. (1998), Zajdel et al. (2014)
<i>Hemipenthes morio</i> (bee fly)	Insecta: Diptera: Bombyliidae	Fliszkiewicz, Kuśnierczak, et al. (2012)
<i>Melittobia acasta</i> (wasp)	Insecta: Hymenoptera: Eulophidae	Bosch and Kemp (2002), Krunić et al. (2005), Tschardt et al. (1998)
<i>Monodontomerus obscurus</i> (wasp)	Insecta: Hymenoptera: Torymidae	Bosch and Kemp (2002), Fliszkiewicz, Kuśnierczak, et al. (2012), Krunić et al. (2005), Sedivy and Dorn (2014), Steffan-Dewenter and Leschke (2003), Tschardt et al. (1998), Zajdel et al. (2014)
<b>Predators</b>		
<i>Dendrocopos</i> sp. (woodpecker)	Aves: Piciformes: Picidae	Krunić et al. (2005)
<i>Motacilla alba</i> (white wagtail)	Aves: Passeriformes: Motacillidae	Krunić et al. (2005)
<i>Parus major</i> (great tit)	Aves: Passeriformes: Paridae	Krunić et al. (2005)
<i>Pica pica</i> (Eurasian/common magpie)	Aves: Passeriformes: Corvidae	Krunić et al. (2005)
<i>Trichodes apiaris</i> (checkered beetle)	Insecta: Coleoptera: Cleridae	Bosch and Kemp (2002), Krunić et al. (2005), Tschardt et al. (1998), Zajdel et al. (2014)
<b>Predator or accidental nest resident</b>		
<i>Chrysopidae</i> sp. (green lacewing)	Insecta: Neuroptera: Chrysopidae	Krunić et al. (2005)
<b>Nest destroyers</b>		
<i>Ancistrocerus</i> sp. (e.g., <i>A. parietum</i> ) (potter wasp)	Insecta: Hymenoptera: Vespidae	Krunić et al. (2005)
<i>Auplopus carbonarius</i> (spider wasp)	Insecta: Hymenoptera: Pompilidae	Zajdel et al. (2014)
<i>Ptinus fur</i> (white-marked spider beetle)	Insecta: Coleoptera: Ptinidae	Bosch and Kemp (2002), Krunić et al. (2005), Zajdel et al. (2014)
<i>Ptinus sexpunctatus</i>	Insecta: Coleoptera: Ptinidae	Bosch and Kemp (2002), Tschardt et al. (1998)
<i>Symmorphus</i> sp. (potter wasp)	Insecta: Hymenoptera: Vespidae	Krunić et al. (2005)
<i>Trogoderma glabrum</i> (glabrous cabinet beetle/coloured cabinet beetle)	Insecta: Coleoptera: Dermestidae	Krunić et al. (2005)
<b>Accidental nest residents</b>		
<i>Anthidium florentinum</i> (leaf-cutter bee)	Insecta: Hymenoptera: Megachilidae	Krunić et al. (2005)
<i>Anthidium melanurum</i> (bee)	Insecta: Hymenoptera: Megachilidae	Krunić et al. (2005)
<i>Bombus terrestris</i> (buff-tailed bumblebee/large earth bumblebee)	Insecta: Hymenoptera: Apidae	Zajdel et al. (2014)
<i>Chalicodoma</i> sp. (e.g., <i>Ch. hungarica</i> ) (bee)	Insecta: Hymenoptera: Megachilidae	Krunić et al. (2005)
<i>Dermestes lardarius</i> (larder beetle)	Insecta: Coleoptera: Dermestidae	Zajdel et al. (2014)
<i>Formica auricularia</i> (common earwig)	Insecta: Dermaptera: Forficulidae	Krunić et al. (2005), Zajdel et al. (2014)
<i>Graphopsocus cruciatus</i>	Insecta: Psocodea: Stenopsocidae	Zajdel et al. (2014)
<i>Lepisma saccharina</i> (silverfish)	Insecta: Zygentoma: Lepismatidae	Krunić et al. (2005), Zajdel et al. (2014)
<i>Liposcelis divinatorius</i> (booklice/barklice)	Insecta: Psocoptera: Liposcelididae	Krunić et al. (2005)
<i>Megachile</i> sp. (e.g., <i>M. pilicrus</i> ) (bee)	Insecta: Hymenoptera: Megachilidae	Krunić et al. (2005)
<i>Megatoma undata</i> (beetle)	Insecta: Coleoptera: Dermestidae	Steffan-Dewenter and Leschke (2003), Tschardt et al. (1998)
<i>Osmia coerulescens</i> (blue mason bee)	Insecta: Hymenoptera: Megachilidae	Krunić et al. (2005)
<i>Plodia interpunctella</i> (Indian-meal moth)	Insecta: Lepidoptera: Pyralidae	Krunić et al. (2005)
<i>Polistes bischoffi</i> (paper wasp)	Insecta: Hymenoptera: Vespidae	Krunić et al. (2005)
<i>Polistes gallicus</i> (paper wasp)	Insecta: Hymenoptera: Vespidae	Krunić et al. (2005)
<i>Tribolium castaneum</i> (red flour beetle)	Insecta: Coleoptera: Tenebrionidae	Zajdel et al. (2014)
<i>Vespula germanica</i> (European wasp/German wasp/German yellowjacket)	Insecta: Hymenoptera: Vespidae	Krunić et al. (2005)
<i>Xylocopa violacea</i> (violet carpenter bee)	Insecta: Hymenoptera: Apidae	Krunić et al. (2005)



**Figure 2.** (A) cut open nesting tubes of reed with visible brood chambers filled with cocoons, (B) an unused pollen package inside a brood chamber in a nesting tube, (C) a single mummified undeveloped larva inside a nesting tube, (D) two *Osmia bicornis* (syn. *O. rufa*) males of different sizes (E), male feeding on an *Aesculus hippocastanum* flower, (F–G) *Osmia rufa* female collecting pollen from *Syringa vulgaris* flowers, (H) *Osmia rufa* female provisioning pollen for a nesting chamber, (I) close-up of *O. bicornis* female's abdomen, (J–O) subsequent stages of entering the nesting tube by *O. bicornis* female.

nesting *O. bicornis* females usually look for plant stems of appropriate diameter. Tubes with diameters below 5 mm seem unsuitable for nesting (Everaars et al., 2011), and that of 8 mm are preferred (Ivanov, 2006). If the inner diameter of the tube exceeds 12 mm, the strict linear arrangement of the cells may be disrupted (Ivanov, 2006). Nesting starts with masoning a septum that functions as a retaining wall at the end of the tube and as the back wall of the first chamber. The

inner surface of the septa is hemispherical and smooth. If it is already hemispherical, then the retaining wall may be absent. The length of brood chambers varies from 10 to 21 mm and it decreases from the first chamber to the last, while the septa thickness simultaneously increases (Ivanov, 2006). The number of chambers depends on the length of the nesting tube (Bąk et al., 2003; Fliszkievicz et al., 2015; Gruber et al., 2011; Ivanov, 2006; Seidelmann et al., 2010).



**Figure 3.** The annual activity cycle of red mason bee (*Osmia bicornis*, syn. *O. rufa*) on a base of (1) Giejdasz and Wilkaniec (2002), (2) Krunic and Stanisavljević (2006b), (3) Radmacher and Strohm (2010), (4) Raw (1972), and (5) Van der Steen and De Ruijter (1991).

Ivanov (2006) found that red mason bee built from 1 to 28 brood chambers per tube and Giejdasz et al. (2016) reported similar numbers (from 5 to 34 breeding chambers per tube; 16 on average). Exemplary reed tubes with bees' cocoons are presented in Figure 2A. The bees establish more nests in the shade than in the sun (Kierat, Szentgyörgyi, Czarnoleski, et al., 2017). The number of chambers falls to even 3–4 per nesting tube in environments contaminated with heavy metals (Moroń et al., 2014). *Osmia bicornis* often leave the last chamber in the tube empty (it is called the vestibulum) probably to protect the offspring from temperature fluctuations and to increase reproductive success when the level of parasitic infestation is high (female distributes offspring among a greater number of nests) or simply due to limited space to build up a final brood chamber (Seidemann, 1999a).

At the bottom of a tube, against the septum, *O. bicornis* females store mixtures of pollen and nectar destined for larval consumption (Figure 2B). The average pollen package contains  $\frac{3}{4}$  of what is needed for the maximum development of descendants (Seidemann, 2018). This is the nutrition value that allows for the successful development of larvae, but not enough to let them achieve maximum dimensions. Ivanov (2006) stated that red mason bee females load more food into each chamber only in

seasons abundant in blooming flowers, instead of building more nesting chambers. One female produces approximately 30 descendants on average; each egg is laid in a separate chamber and stored pollen package (Sedivy & Dorn, 2014).

### Life cycle and daily activity

Giejdasz and Wilkaniec (2002) determined that under natural conditions and an average temperature of  $14^\circ\text{C}$ , eggs matured in 4–9 days (7.6 mean  $\pm$  1.33SD). More than one day after a larva hatches, it starts to eat pollen. *Osmia bicornis* have five larval instars (Krunic & Stanisavljević, 2006a). On average, a female larva feeds for  $35.5 \pm 1.99$ , while the male larvae for  $31.6 \pm 2.41$  days (Giejdasz & Wilkaniec, 2002). Next, prepupae spin four-layered cocoons, which takes between 3 and 6 days to complete (on average  $3.6 \pm 1.09$  for females and  $3.5 \pm 0.96$  for males) (Giejdasz & Wilkaniec, 2002; Krunic & Stanisavljević, 2006a). Under laboratory conditions at a constant temperature of  $28^\circ\text{C}$  and relative humidity (RH) of 70% all stages of larval development were accelerated and took on average, successively: egg maturation  $3.1 \pm 0.36$ , feeding of female larvae  $17.0 \pm 3.21$ , male  $15.3 \pm 3.36$ , cocoon spinning by females took  $2.7 \pm 0.66$ , males  $2.9 \pm 0.89$  days (Giejdasz & Wilkaniec,

2002). If the larva is separated from pollen, it may not find its way back and dies by dehydration (Figure 2C). Mummification occurs in approximately 10–30% of larvae in unpolluted habitats and 55% in polluted ones (Moroń et al., 2014).

Seidelmann (2018) claimed that metamorphosis is induced by a bail-out process when larvae run out of food provisions. Healthy pupae develop within cocoons into adults in August/September (Giejdasz & Wilkaniec, 2002; Radmacher, 2011). If the pupae do not transform into adults before the winter, they will not complete development (Sedivy & Dorn, 2014). In laboratory conditions, an extra burst of pollen delivered to the larvae resulted in body size enlargement approximately by half. The weight of floss used by larvae to form cocoons after receiving a normal portion of pollen is 9.9 mg and can increase to 17.5 mg after providing male larvae with a double portion of pollen and analogously from 17.3 to 22.1 mg for female larvae (Wilkaniec et al., 2004). When the temperature increases to 30 °C or more, the larvae leave the unconsumed portion of pollen and start spinning a cocoon earlier, which results in small adult body sizes. When this temperature sustains at the early stage of diapause (mostly in August/September) 20% of cocooned offspring die, whereas, at 20 °C, the mortality rate is lower than 6% (Giejdasz & Fliszkiewicz, 2016; Radmacher & Strohm, 2010). A rise in UV-B radiation (to approximately 9.24 kJ/m<sup>2</sup>/d and higher) is harmful to individuals in cocoons and might cause deformities, mostly in the wings and mouthparts, and a loss of weight (Wasielewski et al., 2015). Giejdasz and Wilkaniec (2002) determined that under natural conditions, normal pre-pupal and pupal stages have taken together 54.2 ± 2.34 days for females, 52.6 ± 2.03 for males. Under laboratory conditions (28 °C, 70% RH) the combined pre-pupal and pupal stages last 47.2 ± 5.9 days for females, 46.4 ± 5.03 for males. Duration of all of the stages takes approximately 100.8 days for females and 95.5 for males under natural conditions, whereas 70 days for females and 67.6 for males under laboratory conditions. However, adult individuals spend even more time inside nests because they hibernate in cocoons throughout the winter.

Van der Steen and De Ruijter (1991) stated that optimal diapause lasts 120–170 days. Prolonged overwintering shortens life-span of bees (Giejdasz & Wasielewski, 2017). Krunić and Stanisavljević (2006b) stated that emergence is gradual and induced by falling glycerol levels caused by persistent high ambient temperature. *Osmia bicornis* is an early-spring or mid-spring species emerging from February (e.g., Krunić & Stanisavljević, 2006a) to the turn of March and April (e.g., Schenk, Mitesser, et al., 2018). The estimated life cycle of *O. bicornis* is

presented in Figure 3. Schenk, Mitesser, et al. (2018) claimed that spring-emerging solitary bees use a strategic approach and emerge at dates that are the most promising for their fitness expectations. Small bees emerge later than their larger and heavier conspecifics. During this stage, an excessively high temperature may cause bee emergence to fail (Sedivy & Dorn, 2014).

The proper orientation of cocoons in the linearly structured nests is crucial for emergence. The head of a future imago has to be directed towards the entrance; otherwise, a misoriented individual will kill its siblings. On average 29% of male and 10% of female cocoons are misoriented, mostly in tubes with larger diameters (Szentgyörgyi & Woyciechowski, 2013). Kierat, Szentgyörgyi, and Woyciechowski (2017) showed that 82.6% of males and 8% of females whose cocoons were misoriented were able to turn inside their chambers and emerge properly.

Schenk, Krauss, et al. (2018) stated that the timing of the appearance of bees is crucial and must be synchronized with blooming flowers. In laboratory conditions, bees lost fitness and could die just after 6 days of starvation. Females also limited their activity and reproductive output. High temperatures during the starvation period contribute to increased mortality.

The bee's daily rhythms in locomotion are synchronized with light cycles (Beer et al., 2019). Red mason bees show the lowest foraging activity in the morning, returning to the nest with pollen and/or mud only twice per hour, compared with almost six times per hour at noon and over four times in the evening (Bąk et al., 2003). The females actively collect pollen and build brood chambers when the temperature exceeds 13 °C under sunny weather conditions. The highest activity can be observed at temperatures over 25 °C. Bees hide in the tubes in rainy and windy conditions (Bąk et al., 2003). If a cold wave (below 9 °C) surprises the bees they do not succeed in returning to the nest. If the cold weather maintains for several days and is accompanied by frost and snowfall, then all individuals remaining outside die (Krunić & Stanisavljević, 2006a).

### Metabolic changes

Several metabolic processes occur in adults' diapausing inside cocoons. Three stages can be distinguished: pre-wintering (September/October), overwintering (approx. from November until January,) and post-overwintering (February/March). In each period various biochemical parameters have been comprehensively examined.

Through the winter, red mason bees are preparing themselves for spring reproduction, and ovary growth takes place along with vitellogenesis processes. In overwintering red mason bees, the proteins in fat bodies decline, boosting the protein levels in the ovaries (Wasielewski et al., 2011). The activity of enzymatic and non-enzymatic antioxidant and proteolytic systems, the activity of phenoloxidase, the concentrations of aspartate and alanine aminotransferase, and the concentration of alkaline phosphatase were all at constant levels in hemolymph during wintering, and they were usually lower in males than in females (Strachecka et al., 2017). These parameters increased in hemolymph while decreasing in fat bodies in late spring and summer. While the midgut protease activity was low at pre-wintering, its activity increased significantly post-wintering before the emergence of bees from cocoons (Wasielewski et al., 2013). Amylase activity showed high activity during pre-and post-wintering, but its activity was low during overwintering. The level of adiponectin- and resistin-like peptides in females was the highest in the fat body during early pre-wintering, followed by a gradual and significant decline during the overwintering and post-wintering periods (Wasielewski et al., 2014). There was a gradual reduction of the lipid levels in hemolymph. These results strongly suggested the presence of adiponectin- and resistin-like peptides in fat bodies of *O. bicornis*, postulating a dynamic physiological role for these peptides during hibernation. Strachecka et al. (2017) stated that trophocyte sizes and the numbers of lipid droplets in fat bodies were the greatest during pre-wintering. Lipid droplets decreased slowly with age until the emergence of bees from cocoons (Fliszkiewicz, Giejdasz, et al., 2012; Strachecka et al., 2017). According to Strachecka et al. (2017), during all stages of diapausing, the concentrations of triglycerides and glucose systematically decreased in fat body cells, whereas they increased in hemolymph. Lipids and carbohydrates were mobilized during pre-wintering in the fat body and hemolymph, whereas they were used in the midgut (Wasielewski et al., 2013). The researchers also observed that during the winter months, the concentration of lipids and carbohydrates slowly decreased in fat body and hemolymph. Glycogen concentration dramatically dropped during pre-wintering and stayed at very low levels until post-wintering, when it increased with the rise of the ambient temperature.

There are also results obtained from homogenates of whole red mason bee organisms that allow making inferences on some of their metabolic processes.

The total antioxidant status, glutathione, catalase, superoxide dismutase, nonspecific peroxidase, proteases, and protease inhibitors, cholinesterase, and

carboxylesterases are maintained at constant levels, whereas ascorbic acid concentrations decrease during diapause (it is higher in females than males) (Dmochowska et al., 2012; Dmochowska-Ślęzak, Fliszkiewicz, et al., 2015; Dmochowska-Ślęzak, Giejdasz, et al., 2015; Dmochowska-Ślęzak, Zaobidna, et al., 2015; Zaobidna et al., 2014). The evaluated parameters were, of course, significantly higher in active individuals than overwintering ones. Diapausing males' and females' proteolytic enzymes, decomposed gelatin, casein, hemoglobin, and bovine serum albumin. In actively flying bees cholinesterase activity increased with age (Dmochowska et al., 2012; Dmochowska-Ślęzak, Fliszkiewicz, et al., 2015; Dmochowska-Ślęzak, Giejdasz, et al., 2015; Dmochowska-Ślęzak, Zaobidna, et al., 2015; Zaobidna et al., 2014). Dmochowska et al. (2013) verified that the prolongation of diapause during the extended period of low temperatures in early spring changed the values of the biochemical components in bee-body homogenates.

### Organisms occurring in nests of *Osmia bicornis*

A variety of species, such as those compiled in Table 2, may be noted in *O. bicornis* nests. Natural populations and breedings carried out without replacing nesting materials in the following seasons are increasingly more vulnerable to parasites and predators. Fliszkiewicz, Kuśnierczak, et al. (2012) observed that an increasing number of pests in *O. bicornis* nests may cause the weakness or even collapse of the bee populations. Seidelmann et al. (2010) observed that under natural conditions, parasites cause over 70% mortality among *O. bicornis* larvae. Fliszkiewicz, Kuśnierczak, et al. (2012) also claimed that the severity of a parasite invasion depends on the parasite biotope and is the highest in natural habitats (forests).

The accompanying fauna of *O. bicornis* can be categorized into cleptoparasites, cleptobionts, parasitoids, predators, nest destroyers and accidental nest residents (Krunić et al., 2005). Each category has different development and life strategies, and various species among them may jeopardize bee nests on different levels. Representatives of cleptoparasites, cleptobionts and parasitoids might also be called open-cell parasites because they "attack" when the nesting chambers (cells) are not yet closed by the outer septum.

The invasion of cleptoparasites is the most typical phenomenon. Their life tactics involve using the food prepared by another species, and they benefit from its pollen storage. Cleptoparasites seem not to be a direct threat to the bees but they may decrease survival of bee larvae. *Cacoxenus indagator* seems to

be the most ubiquitous cleptoparasite species. Krunic et al. (2005) and literature within claimed that healthy bees can develop on the same portion of pollen along with 2–3 larvae of *C. indagator*. Another common cleptoparasite, *Chaetodactylus osmiae*, can even consume the eggs and subsequent larval stages of the bee in an addition to the pollen. This species is also dangerous because the number of mites rapidly increases and they can be transmitted by the bees to other nesting tubes (Krunic et al., 2005).

A strategy less harmful to bees is presented by cleptobionts, who steal the pollen collected by the mason bees and place it in their own nests. Several species of ants collect pollen from the piles under the bees' nests and from the nesting tubes. When bees stop foraging, the abundance of ants declines. Due to the presence of ants in the vicinity of nests on sandy ground, and lions (*Euroleon nostras*) can be observed. They hunt ants for food, but they do not harm the bees (Krunic et al., 2005).

Parasitoids have strategy based on living in close association with a host and at the host's expense, which ends with killing the host. The parasitoids of the mason bees mostly include species from the Diptera and Hymenoptera orders. Female flies lay their eggs inside the nesting tubes inhabited by bees (Krunic et al., 2005). *Anthrax anthrax* larvae are mostly attracted to the middle of nesting tubes where female gender offspring develop (Felicoli et al., 2017). Flies are parasites of the pupae and can be found inside the bees' cocoons. According to Krunic et al. (2005) *A. anthrax* poses a serious threat to *Osmia* populations in some regions and can be found in up to 95% of their cocoons. When the fly larvae finish feeding on bee larvae, they wander to the end of tubes through encountered septa and cocoons, causing additional damage. *Anthrax anthrax* can also be classified as a nest destroyer (Krunic et al., 2005). Parasitoids, such as chalcid wasps, have different strategies. Female of *Monodontomerus obscurus* perforates nest, cocoon, and integument of a host larva/prepupa, injects a paralyzing fluid, and then lays 3–27 eggs on a bee larva inside a single nesting chamber. *Monodontomerus obscures* larvae feed on the bee and overwinter as prepupae inside the bee cocoon (Krunic et al., 2005) and infestations are difficult to detect (Sedivy & Dorn, 2014). Coleopteran *Trichodes apiarius* can be an example of a similar but strictly predatory strategy. Its larvae hatch from eggs, enter nests of solitary bees, and find eggs or larvae, which they consume. Then, they actively go on to the next chambers and eat all of the bees from a nesting tube.

Populations of solitary *O. bicornis* are also endangered by large predators, such as insectivorous birds

and rodents. They can prey on adult *Osmia* bees, destroy their nests, consume cocoons with larvae and consequently destroy a bee population (Krunic et al., 2005; Sedivy & Dorn, 2014).

Another group accompanying bee populations is the nest destroyers. They are usually species that use the same nesting material as the mason bees. If they build breeding chambers before the bees, then they may damage the offspring of the bees during emergence. Moreover, eumenid wasps may also withdraw the content of the bee nests and then build their own. This group is however not considered a serious threat to bee populations. Other insects using the same nesting material as *O. bicornis* can be called accidental nest residents (Krunic et al., 2005).

Bees have some defensive strategies. Mass-flowering at the beginning of spring modifies the nesting behavior of red mason bee because the availability of pollen encourages them to build larger nests with higher numbers of tubes and fewer brood chambers. The invasion of parasites is facilitated because one female has to supervise many tubes containing offspring (Jauker et al., 2012). Seidelmann (1999a) noticed that females ensured reproductive success by distributing their offspring into more tubes. Seidelmann (2006) reported that the occurrence of open-cell parasites implies a change in the female reproductive strategy. *Osmia bicornis* shifts the offspring sex ratio into smaller sons. In this way, the females reduce time spent outside the nest and provide better protection against parasites. Groulx and Forrest (2018) asserted positive, density-dependent parasitism: the more bees working at the nest, the more attacking parasites. They also noted that it was possible for *O. bicornis* to terminate nesting tubes when they were infested by parasites because the parasite was located in the outermost part of a tube and did not damage bee cocoons during emerging. Parasitism rates collectively increased with the number of days during which the nesting tube was provisioned and decreased with a course of a nesting season. The last chambers built in the season are the least parasitized in comparison with previously sealed chambers (Groulx & Forrest, 2018).

Bees can also be infected by protists, fungi, viruses, and bacteria. Ravoet et al. (2014) stated that solitary bees can be reservoirs for honey bee pathogens. Graystock et al. (2015) called flowers "hotspots" for the transmission of parasites in the environment and among pollinators. Using the same food source is the most likely cause of the spread of pathogens among bees. Indeed, all bees have the potential to be sources or vectors of pollinator diseases, which are spread by them into the environment. The filamentous virus and the *Varroa destructor* macula-like virus thus far considered exclusive to honey bees

can also attack *O. bicornis*. Microsporidia such as *Nosema ceranae*, which usually infects honey bees, may also be contagious to solitary bees (Ravoet et al., 2014). Schoonvaere et al. (2018) found the following viruses in *O. bicornis* materials using RNA screening: *O. cornutanudivirus* (OcNV), arabis mosaic virus (AMV), and cherry leaf roll virus (CLRV).

According to Keller et al. (2013), there are no differences in the bacterial compositions within the first and the last chambers of a tube or among the bacterial floras in the nests built by different individual females. The most common bacteria inside *O. bicornis* nest chambers belong to the phyla Firmicutes (32%), Actinobacteria (27%), and Proteobacteria (26%) among others. There are only a few potential pathogens in the nest bacterial microflora, such as *Bacillus cereus*, *Bacillus thuringensis*, and *Paenibacillus larvae*. There are further threats to *O. bicornis* larvae, i.e., *Photorhabdus luminescens* and *Xenorhabdus nematophila*, which are nematode-associated and recess into the bee body after a parasite attack. The death of red mason bee larvae occurs within 48 h after the bacteria release toxic substances to disintegrate their tissue. Another larval-specific bacterium, *Pseudomonas entomophila*, also kills larvae within a similar timespan (Keller et al., 2013). Chalkbrood caused by several pathogenic fungi species from the *Ascosphaera* genus is also a threat to *Osmia* larvae and may infect them and cause death (Krunić et al., 2005; Sedivy & Dorn, 2014). Not every type of associated fauna has a significant or negative impact on *Osmia* populations, an appropriate set of bacteria is required for proper functioning (Engel et al., 2012).

### Food resources and foraging

Red mason bees visit flowers to provide themselves with nectar and pollen (Figure 2E–G) and collect pollen on the scope to store it for offspring (Figure 2H–O). The food consumed by females determines the chemical composition and constituent substances of *Osmia* eggs (Cane, 2016). During the course of foraging, they cover their bodies with an additional portion of pollen and provide pollination services (Figure 2F–G). *Osmia bicornis* is a polylectic species (pollen generalist without specialization), but may have preferences (e.g., Haider et al., 2014; Krunić & Stanisavljević, 2006a).

The flight range of the red mason bee reaches 300–400 m. Only a few individuals may be seen at further distances from their nests (Gathmann & Tscharrntke, 2002; Yoon et al., 2015; Zurbuchen et al., 2010). Red mason bee prefers to disperse along (not across) the lines of trees (Gruber et al., 2011). Females may visit all flowers within a flight range. According to Coudrain et al. (2016), *O. bicornis*

adapts its foraging strategy as a function of nest proximity to pollen sources. In females of *O. bicornis*, pollen can be found in guts; therefore, it is believed that they are capable of actively ingesting it (Teper, 2007). According to Howell and Alarcón (2007), adult red mason bee females collect pollen and choose flowers filled with nectar. Carbohydrate source with the addition of  $\beta$ -alanine shortens the life of *O. bicornis*, whereas the content of gamma-aminobutyric acid (GABA) induces higher locomotion capabilities (Felicoli et al., 2018). *Osmia bicornis* females prefer to starve rather than eat honeydew (Konrad et al., 2009).

Pollens of different plants can have various nutritional properties, composition, and digestibility, which affect larval development (Radmacher & Strohm, 2010; Roulston & Cane, 2000). The pollen stored by *O. bicornis* appears to be relatively dry, which has been connected to low nectar addition (Haider et al., 2014). The range of weights for a stored pollen can be estimated between 100–250 mg (Radmacher & Strohm, 2010), up to 300 mg (Biliński & Teper, 2004).

The species composition of pollen used by bees is very diverse and, one can distinguish trees such as *Salix*, *Acer*, *Betula*, *Quercus*, herbs: *Ranunculus*, *Juglans*, *Papaver*, *Liquidambar*, Brassicaceae, Cistaceae, Fagaceae, Aceraceae, and Rosaceae as important food resources (Bukovinszky et al., 2017; Coudrain et al., 2016; Haider et al., 2014; Hansted et al., 2014; Krunić & Stanisavljević, 2006a; Radmacher & Strohm, 2010; Ruddle et al., 2018; Schindler & Peters, 2011; Sedivy et al., 2011; Sedivy & Dorn, 2014).

Flowers can also be a source of harmful substances, such as pesticides. Uhl et al. (2016), in an assessment of the LD50 of dimethoate for several bee species, found that females of *O. bicornis* are the most pesticide-resistant bees. Examination of the effect of exposure of *O. bicornis* larvae to a spectrum of field-realistic concentrations (0–10 ppb) of clothianidin revealed no effect of the neonicotinoid on larval development time, overwinter survival, or future adult weight (Nicholls et al., 2017). These authors highlighted that evaluation of the toxicity of a particular pesticide should be based on more than a single “model” species and more detailed studies on solitary bees are needed. Sgolastra et al. (2017) checked whether propiconazole (a triazole fungicide also known as a DMI) had a synergistic influence with a neonicotinoid (clothianidin) on honey bees, *B. terrestris*, and *O. bicornis*. The results for all three insects’ species showed a negative influence of the propiconazole-clothianidin combination. These results underscore the importance of testing pesticide combinations likely to occur in agricultural



environments. Ruddle et al. (2018) found the significant negative effects of exposure to winter oilseed canola grown from thiamethoxam-treated seeds on the nest establishments and adult emergence. Peters et al. (2016) on a basis of research funded by Bayer CropScience AG indicated that Elado® (10 g clothianidin and 2 g beta-cyfluthrin/kg) seed-dressed oilseed canola did not negatively influence *O. bicornis* breedings. Mason bees showed high reproductive output and low parasitization rates. Woodcock et al. (2017) conducted studies funded by Syngenta Ltd. and Bayer CropScience AG to verify winter-sown oilseed canola with seed coatings containing neonicotinoids (clothianidin or thiamethoxam). They found the reproduction of wild bees (*B. terrestris*, *O. bicornis*) negatively correlated with neonicotinoid residues. These findings point to neonicotinoids causing a reduced capacity of bee species to establish new populations in the year following exposure. Further studies are needed to explain these inconsistent results.

### Economic importance and commercial breeding

Red mason bees are considered excellent pollinators. Krunic and Stanisavljević (2006a) stated that pollination provided by 2–4 strong honey bee colonies is equal to that carried out by approximately 500 females of solitary *Osmia* bees, although this amount may vary depending on the plant species and flower density. The pollination effectiveness of the red mason bees is moderated by the landscape structure and this species prefers overgrown edges of fields (Gathmann & Tschardtke, 2002; Gruber et al., 2011; Holzschuh et al., 2012; Steffan-Dewenter, 2002). Pollination effectiveness increases also along with the diversity of other pollinators (Brittain et al., 2013; Holzschuh et al., 2012).

The use of red mason bees for crop pollination resulted in various manners of breeding. The creation of artificial places for nesting *Osmia*, called “houses”, “hotels”, “trap nests” or “nest(ing) boxes” has become very popular since the mid-1970s (Krunic et al., 1995; Krunic & Stanisavljević, 2006b; Maclvor, 2017; Maclvor & Packer, 2015; Sedivy & Dorn, 2014). Attempts are being undertaken at manipulating the development of *O. bicornis* towards emerging in periods other than natural (Fliszkiewicz et al., 2011). However, Dmochowska et al. (2013) stated, the artificial prolongation of diapause reduces *O. bicornis* life expectancy.

There are many ready-made artificial nest structures and numerous do-it-yourself instructions available (Maclvor, 2017). The construction should be placed on a stable stand, secured with grease from

the ground to 10 cm up to protect the nesting materials against cleptobionts. It is advisable to secure the front of the construction with a net (2 cm diameter mesh) or chicken wire to protect the bees and their offspring from birds or rodents attempting to settle in the nest (Krunic et al., 2005; Krunic & Stanisavljević, 2006a). For protection against birds, Krunic et al. (2005) suggested using shiny plastic strips or mirrors that move in the wind. The construction should be set in partial shade rather than in full sun (Kierat et al., 2017). Sedivy and Dorn (2014) stated that the opening should face in an eastern to the south-eastern direction and should not be shaded in the morning. Maclvor (2017) mentioned using wood, bamboo, reeds, hollow and pith-bearing stems, cardboard paper tubes, glass, and plastic as nesting materials. Wilkaniec and Giejdasz (2003) claimed that managing *O. bicornis* is the easiest in disassembled nests made of wood, whereas reeds are almost equally good. In industrial constructions, reusable “lamellar boxes” for solitary bees may be made from thermoformable foil (e.g., based on the protective description of utility model nr UP-RP/W.120675, 2014). The use of lamellar boxes speeds up subsequent breeding procedures and allows the use of additional parasite protections. Annual disinfection (e.g., with heat) is highly effective for eradicating *Ch. osmia* mites (Sedivy & Dorn, 2014). Placing yellow painted lamellar boxes with 0.5 cm deep black colored holes may bluff *A. anthrax* parasitoids into laying their eggs inside the fake nesting holes (Krunic et al., 2005). At the same time, bees use the real nesting holes of the same construction. Nests should not be moved during the period of bee activity. Vibrations and shaking badly affect offspring and may cause adults to abandon the nest (Krunic & Stanisavljević, 2006a; Sedivy & Dorn, 2014). When adults are developed inside cocoons during hibernation, the construction may be stored, and additional breeding procedures may be implemented. To reduce the volume of stored material, cocoons can be removed from nesting tubes. This breeding procedure significantly limits the parasite load. Lamellar boxes make this procedure quicker because all the cocoons from a whole lamella can be taken out at the same time. Any mummified larvae, damaged cocoons, and cocoons or larvae of possible parasites should be removed (Gruber et al., 2011; Krunic et al., 2005). Additionally, cocoons may be disinfected with endosulfan (a 0.007% aqueous solution) for a period of 3 minutes to restrain *Ch. osmia* mite populations and have no negative effect on the bees (Krunic et al., 2005). Sedivy and Dorn (2014) suggested storing cocoons in boxes covered with sawdust. Cocoons should be stored at low temperatures (not higher than 4 °C [Dmochowska et al.,

2013] but not lower than  $-20^{\circ}\text{C}$  [Krunić & Stanisavljević, 2006b]). Krunić and Stanisavljević (2006a) stated to introduce the transition temperature before transferring the cocoons to a nesting construction. Directly before emergence cocoons should be incubated at  $10^{\circ}\text{C}$  and then at approximately  $20^{\circ}\text{C}$  for several days. To obtain wholesale emergence cocoons should be placed outside when the temperature reaches  $20\text{--}24^{\circ}\text{C}$ . The cocoons should be placed near/in a nesting construction. When the bees finish emerging, empty cocoons ought to be removed from the nesting area and destroyed (e.g., burned). This procedure helps to prevent the appearance of parasites that have not been eliminated in the winter selection of cocoons (Krunić et al., 2005).

Becker and Keller (2016) succeeded in implementing techniques for controlled laboratory procedures of solitary bee rearing. Individual freshly masoned nesting tubes should be transferred to the laboratory. After cutting the tubes open, the larva along with their pollen (without separating) should be placed in individual sterile wells of 48-well plates with flat bottoms. Then, the well plates should be loosely topped. Plates should be kept in darkness under constant conditions of  $24^{\circ}\text{C}$  and 60% humidity. This method is flexible and allows a variety of experiments.

## Disclosure statement

All the authors declare no conflicts of interest.

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## References

- Banaszak, J., & Romasenko, L. (1998). *Megachilid bees of Europe (Hymenoptera, Apoidea, Megachilidae)*. Wydawnictwo Bydgoszcz University.
- Bąk, B., Wilde, J. B., & Bratkowski, J. (2003). The monitoring of the flight activity of the red mason bee (*Osmia rufa* L.). *Acta Biologica Universitatis Daugavpiliensis*, 3, 97–100.
- Becker, M. C., & Keller, A. (2016). Laboratory rearing of solitary bees and wasps. *Insect Science*, 23(6), 918–923. <https://doi.org/10.1111/1744-7917.12242>
- Beer, K., Schenk, M., Helfrich-Förster, C., & Holzschuh, A. (2019). The circadian clock uses different environmental time cues to synchronize emergence and locomotion of the solitary bee *Osmia bicornis*. *Scientific Reports*, 9(1), 17711–17748. <https://doi.org/10.1038/s41598-019-54111-3>
- Biliński, M., & Teper, D. (2004). Rearing and utilization of the red mason bee –*Osmia rufa* L. (Hymenoptera, Megachilidae) for orchard pollination. *Journal of Apicultural Science*, 48(2), 69–74.
- Boomsma, J., Baer, B., & Heinze, J. (2005). The evolution of male traits in social insects. *Annual Review of Entomology*, 50, 395–420. <https://doi.org/10.1146/annurev.ento.50.071803.130416>
- Bosch, J., & Kemp, W. P. (2002). Developing and establishing bee species as crop pollinators: The example of *Osmia* spp. (Hymenoptera: Megachilidae) and fruit trees. *Bulletin of Entomological Research*, 92(1), 3–16. <https://doi.org/10.1079/BER2001139>
- Branstetter, M. G., Danforth, B. N., Pitts, J. P., Faircloth, B. C., Ward, P. S., Buffington, M. L., Gates, M. W., Kula, R. R., & Brady, S. G. (2017). Phylogenomic insights into the evolution of stinging wasps and the origins of ants and bees. *Current Biology: CB*, 27(7), 1019–1025. <https://doi.org/10.1016/j.cub.2017.03.027>
- Brittain, C., Williams, N., Kremen, C., & Klein, A. (2013). Synergistic effects of non-*Apis* bees and honey bees for pollination services. *Proceedings. Biological Sciences*, 280(1754), 20122767. <https://doi.org/10.1098/rspb.2012.2767>
- Bukovinszky, T., Rikken, I., Evers, S., Wäckers, F. L., Biesmeijer, J. C., Prins, H. H., & Kleijn, D. (2017). Effects of pollen species composition on the foraging behaviour and offspring performance of the mason bee *Osmia bicornis* (L.). *Basic and Applied Ecology*, 18, 21–30. <https://doi.org/10.1016/j.baae.2016.11.001>
- Cane, J. H. (2016). Adult pollen diet essential for egg maturation by a solitary *Osmia* bee. *Journal of Insect Physiology*, 95, 105–109. <https://doi.org/10.1016/j.jinphys.2016.09.011>
- Catling, P. M. (2015). *Osmia* species (Megachilidae) pollinate *Cypripedium parviflorum* (Orchidaceae) and *Packerapaupercula* (Asteraceae): A localized case of Batesian mimicry? *The Canadian Field-Naturalist*, 129(1), 38–44. <https://doi.org/10.22621/cfn.v129i1.1665>
- Conrad, T., Paxton, R., Assum, G., & Ayasse, M. (2018). Divergence in male sexual odor signal and genetics across populations of the red mason bee, *Osmia bicornis*, in Europe. *PLoS One*, 13(2), e0193153. <https://doi.org/10.1371/journal.pone.0193153>
- Conrad, T., Paxton, R., Barth, F., Francke, W., & Ayasse, M. (2010). Female choice in the red mason bee, *Osmia rufa* (L.) (Megachilidae). *The Journal of Experimental Biology*, 213(Pt 23), 4065–4073. <https://doi.org/10.1242/jeb.038174>
- Conrad, T., Stöcker, C., & Ayasse, M. (2017). The effect of temperature on male mating signals and female choice in the red mason bee, *Osmia bicornis* (L.). *Ecology and Evolution*, 7(21), 8966–8975. <https://doi.org/10.1002/ece3.3331>
- Conrad, T., Vidkjaer, N. H., & Ayasse, M. (2017). The origin of the compounds found on males' antennae of the red mason bee, *Osmia bicornis* (L.). *Chemoecology*, 27(5), 207–216. <https://doi.org/10.1007/s00049-017-0245-1>
- Conrad, T., & Ayasse, M. (2015). The role of vibrations in population divergence in the red mason bee, *Osmia bicornis*. *Current Biology: CB*, 25(21), 2819–2822. <https://doi.org/10.1016/j.cub.2015.08.059>
- Conrad, T., & Ayasse, M. (2019). The differences in the vibrational signals between male *O. bicornis* from three countries in Europe. *Journal of Low Frequency Noise, Vibration and Active Control*, 38(2), 871–878. <https://doi.org/10.1177/1461348418816263>
- Coudrain, V., Rittiner, S., Herzog, F., Tinner, W., & Entling, M. (2016). Landscape distribution of food and nesting sites affect larval diet and nest size, but not abundance

- of *Osmia bicornis*. *Insect Science*, 23(5), 746–753. <https://doi.org/10.1111/1744-7917.12238>
- Day, M. C. (1979). The species of Hymenoptera described by Linnaeus in the genera *Sphex*, *Chrysis*, *Vespa*, *Apis* and *Mutilla*. *Biological Journal of the Linnean Society*, 12(1), 45–84.
- Dmochowska, K., Giejdasz, K., Fliszkiewicz, M., & Żółtowska, K. (2012). Changes in the antioxidative system of the red mason bee (*Osmia rufa*) (Hymenoptera: Megachilidae) induced by artificially elongated diapause. *Journal of Apicultural Science*, 56(1), 99–106. <https://doi.org/10.2478/v10289-012-0011-0>
- Dmochowska, K., Giejdasz, K., Fliszkiewicz, M., & Żółtowska, K. (2013). Prolonged postdiapause: Influence on some indicators of carbohydrate and lipid metabolism of the red mason bee, *Osmia rufa*. *Journal of Insect Science (Online)*, 13(1), 12–77. <https://doi.org/10.1673/031.013.7701>
- Dmochowska-Ślęzak, K., Fliszkiewicz, M., Giejdasz, K., & Żółtowska, K. (2015). The antioxidant system in diapausing and active red mason bee *Osmia bicornis*. *Physiological Entomology*, 40(1), 82–89. <https://doi.org/10.1111/phen.12090>
- Dmochowska-Ślęzak, K., Giejdasz, K., Fliszkiewicz, M., & Żółtowska, K. (2015). Variations in antioxidant defense during the development of the solitary bee *Osmia bicornis*. *Apidologie*, 46(4), 432–444. <https://doi.org/10.1007/s13592-014-0333-y>
- Dmochowska-Slęzak, K., Zaobidna, E., Domeracka, J., Swiatkowska, M., Rusznica, M., & Żółtowska, K. (2015). The activity of cholinesterases in diapausing and flying red mason bees *Osmia bicornis* (Megachilidae). *Folia Biologica*, 63(4), 235–240. [https://doi.org/10.3409/fb63\\_4.235](https://doi.org/10.3409/fb63_4.235)
- Engel, P., Martinson, V., & Moran, N. (2012). Functional diversity within the simple gut microbiota of the honey bee. *Proceedings of the National Academy of Sciences of the United States of America*, 109(27), 11002–11007. <https://doi.org/10.1073/pnas.1202970109>
- Everaars, J., Strohbach, M., Gruber, B., & Dormann, C. (2011). Microsite conditions dominate habitat selection of the red mason bee (*Osmia bicornis*, Hymenoptera: Megachilidae) in an urban environment: A case study from Leipzig, Germany. *Landscape and Urban Planning*, 103(1), 15–427. <https://doi.org/10.1016/j.landurbplan.2011.05.008>
- Felicioli, A., Ambroselli, S., Cilia, G., & Sagona, S. (2017). Parasitization of a wild and reared population of the solitary bee *Osmia cornuta* Latr. by the parasitoid *Anthrax anthrax* Schrank (Diptera, Bombyliidae): Comparison between two types of artificial nest. *Journal of Apicultural Research*, 56(5), 598–605. <https://doi.org/10.1080/00218839.2017.1343019>
- Felicioli, A., Sagona, S., Galloni, M., Bortolotti, L., Bogo, G., Guarnieri, M., & Nepi, M. (2018). Effects of nonprotein amino acids on survival and locomotion of *Osmia bicornis*. *Insect Molecular Biology*, 27(5), 556–563. <https://doi.org/10.1111/imb.12496>
- Fliszkiewicz, M., Giejdasz, K., Wasielewski, O., & Krishnan, N. (2012). Influence of winter temperature and simulated climate change on body mass and fat body depletion during diapause in adults of the solitary bee, *Osmia rufa* (Hymenoptera: Megachilidae). *Environmental Entomology*, 41(6), 1621–1630. <https://doi.org/10.1603/EN12004>
- Fliszkiewicz, M., Giejdasz, K., & Wilkaniec, Z. (2011). The effect of diapause termination time on the successful insemination of *Osmia rufa* L. females. *Journal of Apicultural Science*, 55(2), 79–84.
- Fliszkiewicz, M., Kuśnierczak, A., & Szymaś, B. (2012). The accompanying fauna of solitary bee *Osmia bicornis* (L.) syn. *Osmia rufa* (L.) nests settled in different biotopes. *Journal of Apicultural Science*, 56(1), 51–58. <https://doi.org/10.2478/v10289-012-0006-x>
- Fliszkiewicz, M., Kuśnierczak, A., & Szymaś, B. (2015). Reproduction of the red mason solitary bee *Osmia rufa* (syn. *Osmia bicornis*) (Hymenoptera: Megachilidae) in various habitats. *European Journal of Entomology*, 112(1), 100–105. <https://doi.org/10.14411/eje.2015.005>
- Fliszkiewicz, M., Langowska, A., & Tryjanowski, P. (2013). Effect of manipulated sex ratio on insemination of the red mason bee *Osmia bicornis* L. under net cage conditions. *Journal of Apicultural Science*, 57(2), 73–79. <https://doi.org/10.2478/jas-2013-0018>
- Fliszkiewicz, M., & Wilkaniec, Z. (2009). Potential possibilities of insemination of mason bee (*Osmia rufa* L.) females by a single male in laboratory conditions. *Annals of Warsaw University of Life Sciences–SGGW: Animal Science*, 46, 51–58.
- Frasnelli, E., & Vallortigara, G. (2017). Distribution of antennal olfactory and non-olfactory sensilla in different species of bees. *Symmetry*, 9(8), 135–145. <https://doi.org/10.3390/sym9080135>
- Gathmann, A., & Tschardt, T. (2002). Foraging ranges of solitary bees. *Journal of Animal Ecology*, 71(5), 757–764. <https://doi.org/10.1046/j.1365-2656.2002.00641.x>
- Giejdasz, K., & Fliszkiewicz, M. (2016). Effect of temperature treatment during development of *Osmia rufa* L., on mortality, emergence and longevity of adults. *Journal of Apicultural Science*, 60(2), 221–232. <https://doi.org/10.1515/jas-2016-0029>
- Giejdasz, K., Fliszkiewicz, M., Bednárová, A., & Krishnan, N. (2016). Reproductive potential and nesting effects of *Osmia rufa* (syn. *bicornis*) female (Hymenoptera: Megachilidae). *Journal of Apicultural Science*, 60(1), 75–86. <https://doi.org/10.1515/jas-2016-0003>
- Giejdasz, K., & Wasielewski, O. (2017). Effect of artificial prolonged wintering on emergence and survival of *Osmia rufa* adults. *Journal of Apicultural Science*, 61(1), 127–137. <https://doi.org/10.1515/jas-2017-0011>
- Giejdasz, K., & Wilkaniec, Z. (2002). Individual development of the red mason bee (*Osmia rufa* L., Megachilidae) under natural and laboratory conditions. *Journal of Apicultural Science*, 46(1), 51–57.
- Graystock, P., Goulson, D., & Hughes, W. O. (2015). Parasites in bloom: Flowers aid dispersal and transmission of pollinator parasites within and between bee species. *Proceedings of the Royal Society B: Biological Sciences*, 282(1813), 20151371. <https://doi.org/10.1098/rspb.2015.1371>
- Groulx, A. F., & Forrest, J. R. (2018). Nesting aggregation as a predictor of brood parasitism in mason bees (*Osmia* spp.). *Ecological Entomology*, 43(2), 182–191. <https://doi.org/10.1111/een.12484>
- Gruber, B., Eckel, K., Everaars, J., & Dormann, C. (2011). On managing the red mason bee (*Osmia bicornis*) in apple orchards. *Apidologie*, 42(5), 564–576. <https://doi.org/10.1007/s13592-011-0059-z>
- Haider, M., Dorn, S., Sedivy, C., & Müller, A. (2014). Phylogeny and floral hosts of a predominantly pollen generalist group of mason bees (Megachilidae: Osmiini).

- Biological Journal of the Linnean Society*, 111(1), 78–91. <https://doi.org/10.1111/bij.12186>
- Hansted, L., Grout, B., Toldam-Andersen, T., & Eilenberg, J. (2014). An assessment of *Osmia rufa* (syn. *bicornis*) as a pollinator of the sour cherry (*Prunus cerasus*) cv. Stevnsbaer in Eastern Denmark. *Journal of Apicultural Research*, 53(1), 177–182. <https://doi.org/10.3896/IBRA.1.53.1.20>
- Holzschuh, A., Dudenhöffer, J., & Tschardt, T. (2012). Landscapes with wild bee habitats enhance pollination, fruit set and yield of sweet cherry. *Biological Conservation*, 153, 101–107. <https://doi.org/10.1016/j.biocon.2012.04.032>
- Howell, A. D., & Alarcón, R. (2007). *Osmia* bees (Hymenoptera: Megachilidae) can detect nectar-rewarding flowers using olfactory cues. *Animal Behaviour*, 74(2), 199–205. <https://doi.org/10.1016/j.anbehav.2006.11.012>
- Ivanov, S. P. (2006). The nesting of *Osmia rufa* (L.) (Hymenoptera, Megachilidae) in the Crimea: Structure and composition of nests. *Entomological Review*, 86(5), 524–533. <https://doi.org/10.1134/S0013873806050046>
- Jauker, F., Peter, F., Wolters, V., & Diekötter, T. (2012). Early reproductive benefits of mass-flowering crops to the solitary bee *Osmia rufa* outweigh post-flowering disadvantages. *Basic and Applied Ecology*, 13(3), 268–276. <https://doi.org/10.1016/j.baec.2012.03.010>
- Keller, A., Grimmer, G., & Steffan-Dewenter, I. (2013). Diverse microbiota identified in whole intact nest chambers of the red mason bee *Osmia bicornis* (Linnaeus 1758). *PLoS One*, 8(10), e78296. <https://doi.org/10.1371/journal.pone.0078296>
- Kierat, J., Filipiak, M., Szentgyörgyi, H., & Woyciechowski, M. (2017). Predation cues in solitary bee nests. *Journal of Insect Behavior*, 30(4), 385–393. <https://doi.org/10.1007/s10905-017-9626-0>
- Kierat, J., Szentgyörgyi, H., Czarnoleski, M., & Woyciechowski, M. (2017). The thermal environment of the nest affects body and cell size in the solitary red mason bee (*Osmia bicornis* L.). *Journal of Thermal Biology*, 68, 39–44. <https://doi.org/10.1016/j.jtherbio.2016.11.008>
- Kierat, J., Szentgyörgyi, H., & Woyciechowski, M. (2017). Orientation inside linear nests by male and female *Osmia bicornis* (Megachilidae). *Journal of Insect Science*, 17(2), 40. <https://doi.org/10.1093/jisesa/iex010>
- Konrad, R., Wäckers, F. L., Romeis, J., & Babendreier, D. (2009). Honeydew feeding in the solitary bee *Osmia bicornis* as affected by aphid species and nectar availability. *Journal of Insect Physiology*, 55(12), 1158–1166. <https://doi.org/10.1016/j.jinsphys.2009.08.012>
- Krunic, M., Pinzauti, M., Felicioli, A., & Stanisavljević, L. (1995). Further observations on *Osmia cornuta* Latr. and *O. rufa* L. as alternative fruit pollinators, domestication and utilization. *Archives of Biological Sciences*, 47(1–2), 59–66.
- Krunic, M., & Stanisavljević, L. (2006a). Population management in the mason bee species *Osmia cornuta* and *O. rufa* for orchard pollination in Serbia (Hymenoptera: Megachilidae). *Entomologia Generalis*, 29(1), 27–38.
- Krunic, M., & Stanisavljević, L. (2006b). Supercooling points and diapause termination in overwintering adults of orchard bees *Osmia cornuta* and *O. rufa* (Hymenoptera: Megachilidae). *Bulletin of Entomological Research*, 96(3), 323–326. <https://doi.org/10.1079/ber2006423>
- Krunic, M., Stanisavljević, L., Pinzauti, M., & Felicioli, A. (2005). The accompanying fauna of *Osmia cornuta* and *Osmia rufa* and effective measures of protection. *Bulletin of Insectology*, 58(2), 141–152.
- MacIvor, J. (2017). Cavity-nest boxes for solitary bees: A century of design and research. *Apidologie*, 48(3), 311–327. <https://doi.org/10.1007/s13592-016-0477-z>
- MacIvor, J., & Packer, L. (2015). ‘Bee hotels’ as tools for native pollinator conservation: A premature verdict? *PLoS One*, 10(3), e0122126. <https://doi.org/10.1371/journal.pone.0122126>
- Michener, C. D. (2007). *The bees of the world*. Johns Hopkins University Press.
- Moroń, D., Szentgyörgyi, H., Skórka, P., Potts, S. G., & Woyciechowski, M. (2014). Survival, reproduction and population growth of the bee pollinator, *Osmia rufa* (Hymenoptera: Megachilidae), along gradients of heavy metal pollution. *Insect Conservation and Diversity*, 7(2), 113–121. <https://doi.org/10.1111/icad.12040>
- Müller, A. (2019, May 21). *Palaeartic Osmiine bees*. ETH Zürich. <https://blogs.ethz.ch/osmiini>
- Murray, E. A., Bossert, S., & Danforth, B. N. (2018). Pollinivory and the diversification dynamics of bees. *Biology Letters*, 14(11), 20180530. <https://doi.org/10.1098/rsbl.2018.0530>
- NCBI:txid1437190. (2018, January 15). <https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Infoandid=1437190andlvl=3andlin=fandkeep=1andsrch-mode=1andunlock>
- Nicholls, E., Fowler, R., Niven, J. E., Gilbert, J. D., & Goulson, D. (2017). Larval exposure to field-realistic concentrations of clothianidin has no effect on development rate, over-winter survival or adult metabolic rate in a solitary bee, *Osmia bicornis*. *PeerJ*, 5, e3417. <https://doi.org/10.7717/peerj.3417>
- Paxton, R. (2005). Male mating behaviour and mating systems of bees: An overview. *Apidologie*, 36(2), 145–156. <https://doi.org/10.1051/apido:2005007>
- Peters, B., Gao, Z., & Zumkier, U. (2016). Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: Effects on red mason bees (*Osmia bicornis*). *Ecotoxicology (London, England)*, 25(9), 1679–1690. <https://doi.org/10.1007/s10646-016-1729-4>
- Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Aizen, M. A., Biesmeijer, J. C., Breeze, T. D., Dicks, L. V., Garibaldi, L. A., Hill, R., Settele, J., & Vanbergen, A. J. (2016). Safeguarding pollinators and their values to human well-being. *Nature*, 540(7632), 220–229. <https://doi.org/10.1038/nature20588>
- Praz, C., Müller, A., Danforth, B., Griswold, T., Widmer, A., & Dorn, S. (2008). Phylogeny and biogeography of bees of the tribe Osmini (Hymenoptera: Megachilidae). *Molecular Phylogenetics and Evolution*, 49(1), 185–197. <https://doi.org/10.1016/j.ympev.2008.07.005>
- Radmacher, S. (2011). *Temperature effects on the development in the solitary bee Osmia bicornis (Hymenoptera, Megachilidae) - Development time, brain microstructure and cognitive abilities* [Doctoral dissertation]. University of Regensburg. <https://epub.uni-regensburg.de/>
- Radmacher, S., & Strohm, E. (2010). Factors affecting offspring body size in the solitary bee *Osmia bicornis* (Hymenoptera, Megachilidae). *Apidologie*, 41(2), 169–177. <https://doi.org/10.1051/apido/2009064>
- Radmacher, S., & Strohm, E. (2011). Effects of constant and fluctuating temperatures on the development of the solitary bee *Osmia bicornis* (Hymenoptera:

- Megachilidae). *Apidologie*, 42(6), 711–720. <https://doi.org/10.1007/s13592-011-0078-9>
- Ravoet, J., De Smet, L., Meeus, I., Smagghe, G., Wenseleers, T., & de Graaf, D. (2014). Widespread occurrence of honey bee pathogens in solitary bees. *Journal of Invertebrate Pathology*, 122, 55–58. <https://doi.org/10.1016/j.jip.2014.08.007>
- Raw, A. (1972). The biology of the solitary bee *Osmia rufa* (L.) (Megachilidae). *Transactions of the Royal Entomological Society of London*, 124(3), 213–229.
- Raw, A. (1976). The behaviour of males of the solitary bee *Osmia rufa* (Megachilidae) searching for females. *Behaviour*, 56(3–4), 279–285. <https://doi.org/10.1163/156853976X00055>
- Rogers, L. J., Frasnelli, E., & Versace, E. (2016). Lateralized antennal control of aggression and sex differences in red mason bees, *Osmia bicornis*. *Scientific Reports*, 6, 29411. <https://doi.org/10.1038/srep29411>
- Romankova, T. G. (1984). Bees of the genus *Osmia* Panz. (Hymenoptera, Megachilidae) in the fauna of the Far East. *Entomological Review*, 53, 358–364.
- Roulston, T., & Cane, J. (2000). Pollen nutritional content and digestibility for animals. *Plant Systematics and Evolution*, 222(1–4), 187–209. <https://doi.org/10.1007/BF00984102>
- Ruddle, N., Elston, C., Klein, O., Hamberger, A., & Thompson, H. (2018). Effects of exposure to winter oil-seed rape grown from thiamethoxam-treated seed on the red mason bee *Osmia bicornis*. *Environmental Toxicology and Chemistry*, 37(4), 1071–1083. <https://doi.org/10.1002/etc.4034>
- Ruggiero, M., & Ascher, J. (2015). ITIS bees: World bee checklist (version Sep 2009). In: Y. Roskov, L. Abucay, T. Orrell, D. Nicolson, T. Kunze, A. Culham, N. Bailly, P. Kirk, T. Bourgoin, R. E. DeWalt, W. Decock, & A. De Wever (Eds.). *Species 2000 and ITIS Catalogue of Life, 2015 Annual Checklist, Species 2000: Naturalis*. <https://www.catalogueoflife.org/annual-checklist/2015/>
- Schenk, M., Krauss, J., & Holzschuh, A. (2018). Desynchronizations in bee-plant interactions cause severe fitness losses in solitary bees. *The Journal of Animal Ecology*, 87(1), 139–149. <https://doi.org/10.1111/1365-2656.12694>
- Schenk, M., Mitesser, O., Hovestadt, T., & Holzschuh, A. (2018). Overwintering temperature and body condition shift emergence dates of spring-emerging solitary bees. *PeerJ*, 6, e4721. <https://doi.org/10.7717/peerj.4721>
- Schindler, M., & Peters, B. (2011). Mason bees *Osmia bicornis* and *Osmia cornuta* as suitable orchard pollinators. *Erwerbs-Obstbau*, 52(3–4), 111–116. <https://doi.org/10.1007/s10341-010-0118-z>
- Schoonvaere, K., Smagghe, G., Francis, F., & de Graaf, D. C. (2018). Study of the metatranscriptome of eight social and solitary wild bee species reveals novel viruses and bee parasites. *Frontiers in Microbiology*, 9, 177. <https://doi.org/10.3389/fmicb.2018.00177>
- Sedivy, C., & Dorn, S. (2014). Towards a sustainable management of bees of the subgenus *Osmia* (Megachilidae; *Osmia*) as fruit tree pollinators. *Apidologie*, 45(1), 88–105. <https://doi.org/10.1007/s13592-013-0231-8>
- Sedivy, C., Müller, A., & Dorn, S. (2011). Closely related pollen generalist bees differ in their ability to develop on the same pollen diet: Evidence for physiological adaptations to digest pollen. *Functional Ecology*, 25(3), 718–725. <https://doi.org/10.1111/j.1365-2435.2010.01828.x>
- Seidelmann, K. (1999a). The function of the vestibulum in nests of a solitary stem-nesting bee, *Osmia rufa* (L.). *Apidologie*, 30(1), 19–29. <https://doi.org/10.1051/apido:19990103>
- Seidelmann, K. (1999b). The race for females: The mating system of the red mason bee, *Osmia rufa* (L.) (Hymenoptera: Megachilidae). *Journal of Insect Behavior*, 12(1), 13–25. <https://doi.org/10.1023/A:1020920929613>
- Seidelmann, K. (2006). Open-cell parasitism shapes maternal investment patterns in the red mason bee *Osmia rufa*. *Behavioral Ecology*, 17(5), 839–848. <https://doi.org/10.1093/beheco/arl017>
- Seidelmann, K. (2014). Optimal progeny body size in a solitary bee, *Osmia bicornis* (Apoidea: Megachilidae). *Ecological Entomology*, 39(5), 656–663. <https://doi.org/10.1111/een.12145>
- Seidelmann, K. (2015). Double insurance of paternity by a novel type of mating plug in a monandrous solitary mason bee *Osmia bicornis* (Hymenoptera: Megachilidae). *Biological Journal of the Linnean Society*, 115(1), 28–37. <https://doi.org/10.1111/bij.12472>
- Seidelmann, K. (2018). Optimal resource allocation, maternal investment, and body size in a solitary bee, *Osmia bicornis*. *Entomologia Experimentalis et Applicata*, 166(9), 790–799. <https://doi.org/10.1111/eea.12711>
- Seidelmann, K., Bienesch, A., & Pröhl, F. (2016). The impact of nest tube dimensions on reproduction parameters in a cavity nesting solitary bee, *Osmia bicornis* (Hymenoptera: Megachilidae). *Apidologie*, 47(1), 114–122. <https://doi.org/10.1007/s13592-015-0380-z>
- Seidelmann, K., & Rolke, D. (2019). Advertisement of unreceptivity—Perfume modifications of mason bee females (*Osmia bicornis* and *O. cornuta*) and a non-existing antiaphrodisiac. *PLoS One*, 14(5), e0215925. <https://doi.org/10.1371/journal.pone.0215925>
- Seidelmann, K., Ulbrich, K., & Mielenz, N. (2010). Conditional sex allocation in the red mason bee, *Osmia rufa*. *Behavioral Ecology and Sociobiology*, 64(3), 337–347. <https://doi.org/10.1007/s00265-009-0850-2>
- Senapathi, D., Biesmeijer, J. C., Breeze, T. D., Kleijn, D., Potts, S. G., & Carvalheiro, L. G. (2015). Pollinator conservation—The difference between managing for pollination services and preserving pollinator diversity. *Current Opinion in Insect Science*, 12, 93–101. <https://doi.org/10.1016/j.cois.2015.11.002>
- Sgolastra, F., Medrzycki, P., Bortolotti, L., Renzi, M. T., Tosi, S., Bogo, G., Teper, D., Porrini, C., Molowny-Horas, R., & Bosch, J. (2017). Synergistic mortality between a neonicotinoid insecticide and an ergosterol-biosynthesis-inhibiting fungicide in three bee species. *Pest Management Science*, 73(6), 1236–1243. <https://doi.org/10.1002/ps.4449>
- Steffan-Dewenter, I. (2002). Landscape context affects trap-nesting bees, wasps, and their natural enemies. *Ecological Entomology*, 27(5), 631–637. <https://doi.org/10.1046/j.1365-2311.2002.00437.x>
- Steffan-Dewenter, I., & Leschke, K. (2003). Effects of habitat management on vegetation and above-ground nesting bees and wasps of orchard meadows in Central Europe. *Biodiversity and Conservation*, 12(9), 1953–1968. <https://doi.org/10.1023/A:1024199513365>
- Steffan-Dewenter, I., & Schiele, S. (2004). Nest-site fidelity, body weight and population size of the red mason bee, *Osmia rufa* (Hymenoptera: Megachilidae), evaluated by mark-recapture experiments. *Entomologia Generalis*,

- 27(2), 123–131. <https://doi.org/10.1127/entom.gen/27/2004/123>
- Strachecka, A., Chobotow, J., Paleolog, J., Łoś, A., Schulz, M., Teper, D., Kucharczyk, H., & Grzybek, M. (2017). Insights into the biochemical defence and methylation of the solitary bee *Osmia rufa* L.: A foundation for examining eusociality development. *PLoS One*, 12(4), e0176539. <https://doi.org/10.1371/journal.pone.0176539>
- Szentgyörgyi, H., Moroń, D., Nawrocka, A., Tofilski, A., & Woyciechowski, M. (2017). Forewing structure of the solitary bee *Osmia bicornis* developing on heavy metal pollution gradient. *Ecotoxicology (London, England)*, 26(8), 1031–1040. <https://doi.org/10.1007/s10646-017-1831-2>
- Szentgyörgyi, H., & Woyciechowski, M. (2013). Cocoon orientation in the nests of red mason bees (*Osmia bicornis*) is affected by cocoon size and available space. *Apidologie*, 44(3), 334–341. <https://doi.org/10.1007/s13592-012-0185-2>
- Teper, D. (2007). Food plants of the red mason bee (*Osmia rufa* L.) determined based on a palynological analysis of faeces. *Journal of Apicultural Science*, 51(2), 55–62.
- Thomson, J. D. (2001). Using pollination deficits to infer pollinator declines: Can theory guide us? *Conservation Ecology*, 5(1), 1–12. <https://doi.org/10.5751/ES-00270-050106>
- Tscharntke, T., Gathmann, A., & Steffan-Dewenter, I. (1998). Bioindication using trap-nesting bees and wasps and their natural enemies: Community structure and interactions. *Journal of Applied Ecology*, 35(5), 708–719. <https://doi.org/10.1046/j.1365-2664.1998.355343.x>
- Uhl, P., Franke, L. A., Rehberg, C., Wollmann, C., Stahlschmidt, P., Jeker, L., & Brühl, C. A. (2016). Interspecific sensitivity of bees towards dimethoate and implications for environmental risk assessment. *Scientific Reports*, 6, 34439. <https://doi.org/10.1038/srep34439>
- Ungricht, S., Müller, A., & Dorn, S. (2008). *A taxonomic catalogue of the Palaearctic bees of the tribe Osmiini (Hymenoptera: Apoidea: Megachilidae)*. Magnolia Press.
- Van der Steen, J., & De Ruijter, A. (1991). The management of *Osmia rufa* L. for pollination of seed crops in greenhouses. *Proceedings of Experimental and Applied Entomology*, 2, 137–141.
- Wasielewski, O., Giejdasz, K., Wojciechowicz, T., & Skrzypski, M. (2011). Ovary growth and protein levels in ovary and fat body during adult-wintering period in the red mason bee, *Osmia rufa*. *Apidologie*, 42(6), 749–758. <https://doi.org/10.1007/s13592-011-0084-y>
- Wasielewski, O., Szczepankiewicz, D., Giejdasz, K., Wojciechowicz, T., Bednářová, A., & Krishnan, N. (2014). The potential role of adiponectin-and resistin-like peptides in the regulation of lipid levels in the hemolymph of over-wintering adult females of *Osmia bicornis*. *Apidologie*, 45(4), 491–503. <https://doi.org/10.1007/s13592-013-0264-z>
- Wasielewski, O., Wojciechowicz, T., Giejdasz, K., & Krishnan, N. (2013). Overwintering strategies in the red mason solitary bee - physiological correlates of midgut metabolic activity and turnover of nutrient reserves in females of *Osmia bicornis*. *Apidologie*, 44(6), 642–656. <https://doi.org/10.1007/s13592-013-0213-x>
- Wasielewski, O., Wojciechowicz, T., Giejdasz, K., & Krishnan, N. (2015). Enhanced UV-B radiation during pupal stage reduce body mass and fat content, while increasing deformities, mortality and cell death in female adults of solitary bee *Osmia bicornis*. *Insect Science*, 22(4), 512–520. <https://doi.org/10.1111/1744-7917.12124>
- Wilkaniac, Z., & Giejdasz, K. (2003). Suitability of nesting substrates for the cavity-nesting bee *Osmia rufa*. *Journal of Apicultural Research*, 42(3), 29–31. <https://doi.org/10.1080/00218839.2003.11101084>
- Wilkaniac, Z., Giejdasz, K., & Fliszkiewicz, M. (2004). The influence of food amount consumed during the larval development on the body weight of the imago of the red mason bee (*Osmia rufa* L., Megachilidae). *Journal of Apicultural Science*, 48(1), 29–36.
- Wilkaniac, Z., & Radajewska, B. (1996). Solitary bee *Osmia rufa* L. (Apoidea, Megachilidae) as pollinator of strawberry cultivated in an unheated plastic tunnel. *Acta Horticulturae*, 439(1), 489–494.
- Woodcock, B. A., Bullock, J. M., Shore, R. F., Heard, M. S., Pereira, M. G., Redhead, J., Ridding, L., Dean, H., Sleep, D., Henrys, P., Peyton, J., Hulmes, S., Hulmes, L., Sárosataki, M., Saure, C., Edwards, M., Genersch, E., Knäbe, S., & Pywell, R. F. (2017). Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. *Science (New York, N.Y.)*, 356(6345), 1393–1395. <https://doi.org/10.1126/science.aaa1190>
- Yoon, H., Lee, K., Kim, S., Lee, Y., Kim, N., & Jin, B. (2015). Effects of location, direction, altitude, and placement of trap nests on the rate of trap-nesting of *Osmia* solitary bees. *Journal of Asia-Pacific Entomology*, 18(4), 695–700. <https://doi.org/10.1016/j.aspen.2015.08.004>
- Zajdel, B., Kucharska, K., Kucharski, D., Fliszkiewicz, M., & Gąbka, J. (2014). Accompanying fauna of red mason bees in annual and perennial nesting sites. *Veterinary Medicine – Science and Practice*, 70(12), 745–749.
- Zaobidna, E., Dmochowska, K., Frączek, R., Dymczyk, E., & Żółtowska, K. (2014). The proteolytic activity of diapausing and newly hatched red mason bees (*Osmia rufa*: Megachilidae). *Journal of Apicultural Science*, 58(1), 85–91. <https://doi.org/10.2478/jas-2014-0008>
- Zurbuchen, A., Landert, L., Klaiber, J., Müller, A., Hein, S., & Dorn, S. (2010). Maximum foraging ranges in solitary bees: Only few individuals have the capability to cover long foraging distances. *Biological Conservation*, 143(3), 669–676. <https://doi.org/10.1016/j.biocon.2009.12.003>

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# The associations among the breeding performance of *Osmia bicornis* L. (Hymenoptera: Megachilidae), burden of pathogens and nest parasites along urbanisation gradient

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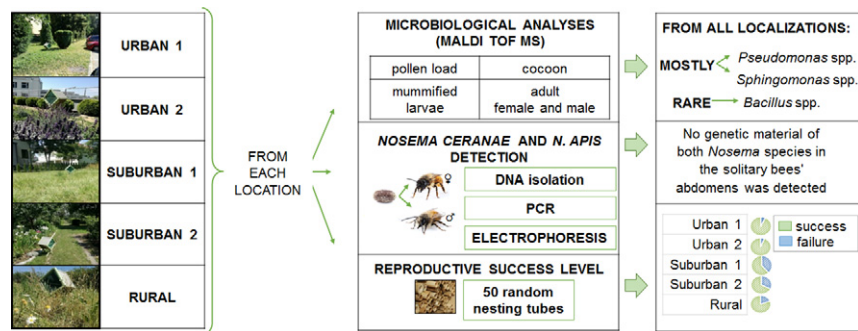
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## HIGHLIGHTS

- Reproduction of solitary bees was investigated along the urbanisation gradient.
- *O. bicornis* bees and their nests were neither a reservoir nor a vector of pathogens.
- Presence of parasites negatively correlated with the reproductive success of bees.
- *Osmia* has surprisingly high level of successful development in urban environment.

## GRAPHICAL ABSTRACT



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*Nosema* spp.

## ABSTRACT

Urban environments may negatively affect the development of organisms. In host-pathogen/parasite systems, this impact may lead to increased manifestations of pathogens that decrease the success of their hosts in urban environments compared to rural ones. We tested this hypothesis in the solitary bee *Osmia bicornis* L. We estimated the development of bees, their reproductive success and the manifestation of different pathogens and nest parasites along an urbanisation gradient. We conducted the experiment in an urbanisation gradient in sites representing three environments: urban, suburban and rural. First, we analysed the manifestation of bacterial and fungal microorganisms in pollen loads, within dried/mummified individuals, on the surface of cocoons and on the surface of diapausing adult individuals by using the MALDI-TOF MS technique. We also verified genetic samples from diapausing individuals for the presence of the parasitic *Nosema apis* (Zandler, 1909) and *N. ceranae* (Fries et al., 1996) species. Finally, we assessed the level of reproductive success and manifestations of brood parasites. Not any biological material from the nests was infected by pathogenic microorganisms. This result indicates that the nests are not a reservoir of the pathogenic bacteria and that *O. bicornis* offspring are not a source or vectors of these pathogens to the surrounding environment and indirectly to other bee species. In urban sites, there was a lower number of parasites than in suburban and rural environments. The presence of parasites

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was negatively correlated with the reproductive success and may be a limiting factor for *O. bicornis* populations. We also found that urban sites had the highest indices of reproductive success and the lowest number of breeding failures compared to suburban and rural sites. Moreover, bacterial and fungal transmission is not a serious threat in the studied region.

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## 1. Introduction

Urbanisation is increasing worldwide, as today over 50% of the human population around the world lives in cities, increasing to approximately 80% in the industrialized countries (McKinney, 2008). Urbanisation has a large impact on the development and morphology of specimens, shapes the functioning of local populations and affects the composition of species assemblages and thereby the level of biodiversity (Jones and Leather, 2013). Sustaining biodiversity via pollination and plant population functioning for environmental balance is a key task attributed mainly to bees.

The clade Anthophila includes approximately 20,000 species, which mostly lead solitary lives, as do the over 300 species of mason bees (genus *Osmia* Panzer, 1806, Megachilidae) (Michener, 2007). These bees are worldwide common in different environments and accompanying other pollinating insect species (Evertaars et al., 2011; Michener, 2007; Revilla and Křivan, 2018). One of the most ubiquitous European representatives of solitary bees is the red mason bee (*Osmia bicornis* L., syn. *O. rufa* L.), which is increasingly kept as an agriculture (especially orchard) pollinator and has recently been introduced in cities as environmental complements (Krunić and Stanisavljević, 2006; Evertaars et al., 2011; Gruber et al., 2011; Maclvor and Packer, 2016; Schindler and Peters, 2011; Sedivy and Dorn, 2014; Teper and Biliński, 2009). However, cities are highly altered, rapidly developing environments throughout the world (Pauchard et al., 2006). Urban environments impose constraints on the functioning of species populations and biodiversity (McKinney, 2002). The burden of factors such as high temperatures, high level of air, soil and light pollution, noise, traffic, and dense human populations affect urban animal populations through higher road mortality (Riley et al., 2014), altered activity (Ditchkoff et al., 2006), or impaired development compared to rural environments (Isaksson, 2015). For example, Kierat et al. (2017b) and Giejdasz and Fliszkiewicz (2016) stated that high temperature has a negative influence on *O. bicornis* body size and longevity. Biotic interactions may also be altered in urban environments. Augmented levels of environmental stressors may lower immune competence and thus favour pathogens and parasites in urban environments (Giraudeau et al., 2014). This risk raises concerns about the suitability of urban environments for the introduction of pollinating bees. A way to estimate the effects of urbanisation on species is to compare the structure and function of populations between urban and rural areas (McKinney, 2008).

*Osmia bicornis* emerges from a cocoon when the spring temperature reaches 15 °C for several days in a row. Related insects hatched from the same or adjacent nesting cavities prefer to stay at the birth place (so called breeding philopatry/breeding-site fidelity). *Osmia bicornis* females create agglomerates but do not cooperate with each other (Coudrain et al., 2016). Red mason bees ignore the presence of other individuals, competing bee species, parasitical or accompanying insects, birds and mammals, including rodents or even people (Kierat et al., 2017a; Krunić et al., 2005). In spring, when there is low abundance of blooming plants in the environment, different species of pollinating insects, especially solitary bees, honeybees and bumblebees, visit exactly the same flowers at different times of a given day (Bağ et al., 2003; Dramstad et al., 2003; Shimizu et al., 2014). Therefore, numerous individuals from various species can touch the same plants and indirectly contact each other through the environment, which aids interspecies exchange of neutral, symbiotic and/or pathogenic microorganisms

(Fürst et al., 2014; Ruiz-González and Brown, 2006). The first spring-time flight of overwintering bees is also associated with moving faeces accumulated through the winter along with pathogen spores. In this way, bee excrement contributes to the spread of pathogens, parasites and diseases throughout an environment (Hung, 2000; Ribiere et al., 2007; Ruiz-González and Brown, 2006).

A group of brood parasites, such as *Cacoxenus indagator* (Loew, 1858) (Drosophilidae), *Monodontomerus obscurus* (Westwood, 1833) (Torymidae), or *Chaetodactylus osmiae* (Dufour, 1839) (Chaetodactylidae), is frequently believed to be another serious threat for solitary bee populations (Krunić et al., 2005). These natural enemy species are recorded in bee agglomerations and parasite the immature stages, reducing the bee population up to 50% or, in extreme cases, even up to 95% (Krunić et al., 2001, 2005). Brood parasites have been noted to accompany *O. bicornis* populations, as well as other solitary bees from the genus *Osmia*, such as *Osmia cornuta* (Latreille, 1805) in southern and central Europe or *Osmia lignaria* (Say, 1837) in Canada and USA. However, these brood parasites do not pose a danger for all bee species, especially social ones, such as bumblebees or honeybees.

Repeatedly visiting the same (potentially infected) flowers in a small area makes *O. bicornis* a potential vector of microorganisms (horizontal transmission). This transmission contributes to a more serious problem, which is bringing pathogens into the solitary bee nests and transmitting them vertically to the pollen loads and offspring. As the urban environment may negatively impact the development of invertebrates, urbanisation may increase the impact of pathogens and parasites (Martin et al., 2010). We hypothesized that (1) pollen loads especially those in urban environments, can be a source of infection inside nests of *O. bicornis*, which means that the nests are a reservoir of pathogenic microorganisms collected in the previous season by females from the surrounding environment. Further, we investigated if (2) adult offspring individuals diapausing inside the cocoons may also be a source of pathogenic microorganisms or brood parasites for the surrounding environment and indirectly a threat to other bee species. Moreover (3), pathogens and brood parasites influence some population parameters and reproductive success. Finally, (4) the level of pathogen manifestation, microorganism taxa composition, number of parasites and number of breeding failures and reproductive success vary among urban, suburban and rural environments. In order to compare the performance of the populations, data on the content of nesting tubes were collected from particular environments. The aim of the research was also to examine whether and what kind of pathogens are present in various types of biological material from the nest of *O. bicornis* bees in newly initiated breedings.

## 2. Material and methods

### 2.1. Red mason bee breeding and selection of study sites

All cocoons with adult red mason bees originally came from the same population bred by employees of The Research Institute of Horticulture in Puławy, Poland (51°40'N, 21°96'E). We prepared five constructions of 45 cm × 45 cm × 45 cm dimensions on a wooden frame 0.5 m above ground level with a protective net in the front (mesh of 2 cm diameter). As nesting material, 700 dry reed tubes of 7–12 mm inner diameters and of 15 cm length obliquely cut at the end were used.

To compare results from sites differing in the level of urbanisation, we chose five locations in the Lubelskie region (Eastern Poland) to set up the constructions. We mapped the area to assign the composition of the territory within a radius of 500 m around the nest structures. We used QGIS software and satellite images freely available in Geoportal (<https://www.geoportal.gov.pl>). Moreover, we used distance to the city centre as the additional metric of urbanisation level. The chosen urban locations – U1 (51°14'N, 22°32'E) and U2 (51°14'N, 22°30'E) – consisted of a built-up infrastructure within which we classified approximately 90% as buildings, roads, sidewalks, etc., with the remaining 10% composed of greenery and they were located 1.8 and 2.5 km from the city centre. The suburban locations – suburb1 (51°14'N, 22°29'E) and suburb2 (51°17'N, 22°42'E) – comprised 55–65% of a built-up infrastructure and were located 3.3 and 4.5 km from the. The selected rural area (51°30'N, 22°55'E) of low-intensity cultured grasslands included a built-up infrastructure up to 20% and was outside the city borders. Illustrative photos from each location in which the experimental breedings of *O. bicornis* were maintained are given in Suppl. 1.

At every site, a nesting construction was set in a place protected from wind. In order to begin each new breeding, exactly 500 cocoons were randomly chosen. In March inside the nesting constructions on specially designed shelves, cocoons in random order and direction were placed in identical cardboard boxes (without lids) of dimensions 150x100x50 mm. Since then breedings were daily inspected and after three weeks when all the bees emerged boxes with empty cocoons were removed and burned to kill potential parasites (left inside the cocoons and/or boxes) before hatching. All nesting material (reed tubes) from each location was removed from the wooden constructions and placed in separate cardboard boxes with ventilation holes at the end of November and stored in an air-conditioned chamber at approx. 4 °C until the biological material was removed from tubes and sampled (according to the recommendations by Sedivy and Dorn, 2014).

## 2.2. Microbiological analyses (MALDI-TOF MS)

From each location, masoned nesting tubes were randomly selected. Each tube was carefully cut in half with a sharp knife. Into separate 2 ml Eppendorf tubes, the following materials (from each of 10 samples) were collected:

- unused pollen stored in an individual brood cell,
- undeveloped red mason bees, dried and mummified at larval stage (if present),
- randomly chosen cocoons (Suppl. 2).

Samples were taken with sterile tweezers and kept at 4 °C until the analysis of the bacterial and fungal present. Additionally, during sampling, the parameters described in Section 2.4. were noted and counted for 50 nesting tubes. Each tube was given a unique number called tube identity.

Inoculates were prepared from biological materials. Each sample was transferred into a separate test tube with 1 ml of solution for Hymenoptera (the full composition of the solution is given in Suppl. 3) (Barbosa et al., 2014). Test tubes were mixed thoroughly by vortexing for 60 s. to transfer and suspend bacteria and fungi from the samples. After the solutions were prepared from the surface of the cocoons, the cocoons were removed from the test tubes and dissected with sterile scissors, and the diapausing bees found inside were removed. *Osmia bicornis* were also suspended into 1 ml of solution and mixed thoroughly by vortexing. A 50 µl volume of each suspension was inoculated onto solid Blood Lab-Agar (5%), McConkey Lab-Agar, Chapman Lab-Agar and Sabouraud Dextrose Lab-Agar (Biocorp). The samples collected were incubated at 36 °C for 48 h. Bacteria and fungi were sifted out on subsequent media and incubated until pure cultures were isolated for each material type at every location.

The identification of microbiological strains had to be preceded by preliminary extraction of proteins with ethanol and formic acid. For this purpose, a single colony of a bacterial/fungal culture was suspended in 150 µl of sterile deionized water, after which 450 µl of pure ethanol (Merck) was added. Then, each sample was mixed thoroughly by vortexing. The resulting sample was then centrifuged for 5 min at 13000 rpm/min. After the supernatant was discarded, 40 µl of 70% aqueous formic acid and then 40 µl of acetonitrile (Merck) were added to the precipitate, and the sample was thoroughly mixed by vortexing. After another centrifugation (13,000 rpm/min for 2 min), 1 µl of the obtained supernatant was applied on a metal plate (Ancorchip™ 800/384, Bruker) and allowed to dry at room temperature. Then, 1 µl of matrix solution (cyano-4-hydroxycinnamic acid, Bruker) was applied, and the sample was also left to dry at room temperature. The metal plate with these samples was subsequently placed in a MALDI chamber for analysis. The MALDI (Matrix assisted laser desorption and ionization) TOF (time of flight) MS (Mass Spectrometry) technique identifies microorganisms by measuring unique proteins. These proteins are found in every microorganism and are its “fingerprint”. Protein spectra are compared to a constantly updated spectrum library, and on this basis, a microorganism can be identified to the species level. Automatic measurement of the spectrum and a comparative analysis with reference spectra of bacteria were performed using the Ultraflex extreme Mass Spectrometer and MALDI Biotyper 3.0 software (Bruker Daltonik, Germany).

## 2.3. *Nosema ceranae* and *N. apis* detection

We verified if the red mason bee is a transmitter of honey bee *Nosema apis* and *N. ceranae* parasites via microsatellite analysis of genetic material from the abdomen of diapausing adult *O. bicornis* individuals.

From each location, masoned nesting tubes were randomly chosen. Each tube was carefully cut in half with a sharp knife. Cocoons were pulled out from brood cells with sterile tweezers. Each cocoon was cut open with sterile scissors, and the gender of the adult was identified (on the basis of sexual dimorphism). Five males and five females were sampled from each location. The abdomen was severed from each insect into a separate 2 ml Eppendorf tube. Samples were frozen at –20 °C until genetic analysis.

DNA from *O. bicornis* abdomens was isolated using the DNeasy Blood and Tissue Kit® (Qiagen, Germany) according to the manufacturer's instruction. To verify the presence of *N. apis* and *N. ceranae*, standard Polymerase Chain Reaction (PCR) was conducted with a Qiagen Taq PCR Core Kit® (Qiagen, Germany) reaction mixture. The primers were specific for both *Nosema* species' 16S rDNA genes, and the temperature-time profile of the PCR reaction was established according to Ptaszyńska et al. (2016) and the literature within (Suppl. 4). The results were visualized on a 2% agarose gel, stained with ethidium-bromide and run at 5 V/cm for approx. 30 min. The results of electrophoresis were analysed on photographs taken under ultra-violet light with a CCD camera and the Scion Image program on a computer (Suppl. 5).

## 2.4. Evaluation of the reproductive success level

To evaluate the level of red mason bee reproductive success at each location, 50 nesting reed tubes were randomly chosen. Each tube was given a unique number called tube identity. Tubes were cut open with a knife to verify the following parameters:

I. total number of reproductive “failures” counted by unsuccessful development of offspring, such as:

- a) number of brood cells with the unused portion of pollen – where eggs did not hatch,
- b) number of brood cells with undeveloped, dried and mummified individuals at larval or pupal level (so-called “mummies”),

- c) number of brood cells with dead adult red mason bee individuals outside the cocoon,  
 d) number of brood cells with parasites – we encountered only the best-known parasites: *Cacoxenus indagator* and *Monodontomerus obscurus* eggs at every location.

II. Total number of all healthy adult red mason bee individuals developed inside the cocoons.

The level of reproductive success (RS [%]) was estimated using the following equation:

$$\frac{\text{II}}{\text{II} + [(a) + (b) + (c) + (d)]} * 100\% = \frac{\text{II}}{\text{II} + \text{I}} * 100\% = \text{RS}$$

## 2.5. Statistical analysis

We performed three sets of statistical analyses. First, we analysed differences in the bacteria/fungi species composition among environments. We used partial canonical correspondence analysis (pCCA) to formally test the differences in bacterial species composition among five sites. The type of biological material (pollen, adult bee, surface of cocoons and mummified individuals) was used as a categorical supplementary variable, the effect of which was to parse responses out from the effect of sites. The pCCAs were performed using the Canoco 5.1 software (Šmilauer and Lepš, 2014). The significance of ordination axes and the explanatory variables were tested using 1000 Monte Carlo permutations with the *P*-values adjusted for the false discovery rate. We used a generalized linear mixed model (GLMM) with Poisson error variance and log-link function to determine if the number of microorganism taxa differed among sites in urban, suburban and rural environments as well as among samples of biological material (cocoons, imago bees, “mummies”, and pollen loads). Tube identity was assigned as a random effect.

Second, using GLMM with binomial error variance and logit link function, we determined whether the probability that a cocoon survives, probability that there is a brood parasite in a dead larvae, probability that there is a dead larvae (“mummies”) and probability that there is an unused pollen differed among sites in urban, suburban and rural environments. Tube identity was assigned as a random effect.

Third, we considered differences at the level of the tubes. Using a generalized linear model (GLM) with Poisson error variance and log-link function, we analysed whether the number of all cocoons, number of alive diapausing bees inside cocoons, number of brood parasites, number of dead larvae (“mummies”), and number of unused pollen per tube differed among locations in urban, suburban and rural environments. We also used the number of cells as a covariate in these analyses to control for differences in the number of cocoons among the tubes.

For the generalized linear (mixed) model, we used the packages “glmmADMB” (Fournier et al., 2012; Skaug et al., 2016) and “car” (Fox and Weisberg, 2011). A Tukey post-hoc test was used to find significant difference among locations using the “multcomp” (Hothorn et al., 2008) package for R (R Core Team, 2018).

## 3. Results

MALDI-TOF MS analysis of bacterial and fungal cultures has shown that the biological material collected from the red mason bee nests was mostly inhabited by 29 representatives of *Pseudomonas* (69% of total number of all certainly identified species) (e.g. *P. rhodesiae*, *P. tolaasii*, *P. extremorientalis*, *P. azotoformans*) and 5 representatives of the *Sphingomonas* (12% of total number of all certainly identified species) (e.g. *S. parapaucimobilis*, *S. paucimobilis*) genera. *Pseudomonas* and *Sphingomonas* are gram-negative bacteria and some species may be pathogenic to humans and/or animals. There are several reports of

these bacteria genera being widely found on honeybees, as well as on some solitary bees (Donkersley et al., 2018; Loncaric et al., 2011). Due to their prevalence among bees and unknown influence on them, we treated these organisms as non-pathogenic. A detailed inventory of the microorganisms identified by the MALDI-Biotyper 3.0 software is available in Supplementary Material (Suppl. 6) along with the condensed summary (Suppl. 7).

Partial canonical correspondence analysis revealed that there were statistically significant differences in bacterial and fungal cultures among different environments (Fig. 1A). Two ordination axes explained 10% of the microorganism taxa composition after accounting for the nest material investigated (test of two axes,  $F = 2.9$ ,  $P = 0.001$ ). The first pCCA axis separated mostly rural and one suburban site from the other suburban site (Fig. 1A). The second pCCA axis clearly separated rural and suburban sites from urban ones (Fig. 1A). *P. marginalis* occurred the most frequently in rural and suburb site1, representatives of *Bacillus* such as *B. thuringiensis* and *B. cereus* were present in suburban2 site, whereas *P. brassicacearum* and *S. sanguinis* were bacteria species mostly associated with two urban sites (Fig. 1A; Suppl. 6 and 7). Generally, the sites that had the most significant effect on the differences in species composition were suburb2 ( $F = 2.8$ ,  $P = 0.007$ ), urban2 ( $F = 2.4$ ,  $P = 0.028$ ) and urban1 ( $F = 2.0$ ,  $P = 0.03$ ).

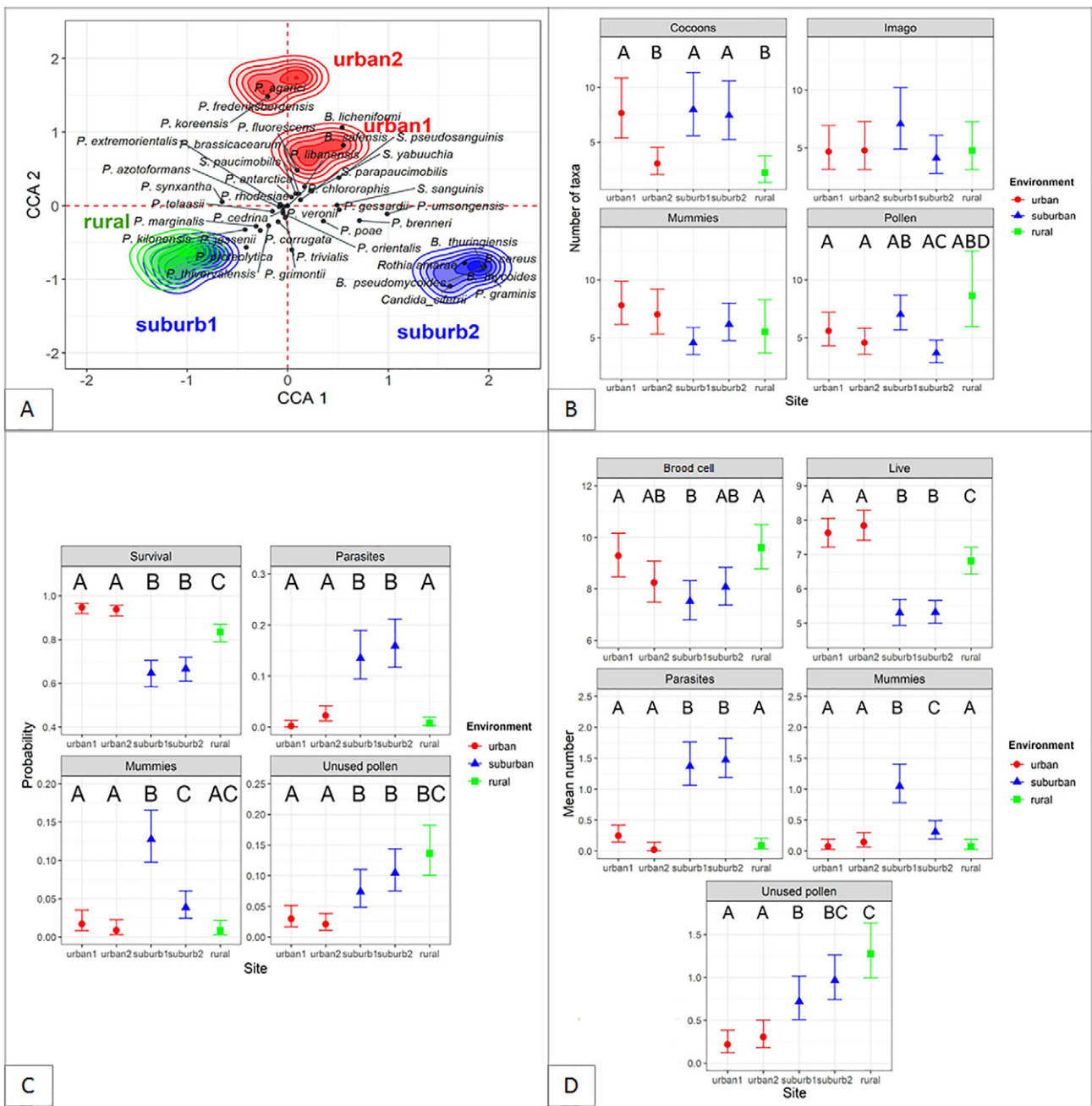
Analysis of the microorganisms in the samples revealed that the number of taxa was not related to site (GLMM, Wald  $\chi^2 = 8.401$ ,  $df = 4$ ,  $P = 0.08$ ) but was associated with the type of nest material investigated (GLMM, Wald  $\chi^2 = 8.888$ ,  $df = 4$ ,  $P = 0.031$ ) and the interaction between these two variables (GLMM, Wald  $\chi^2 = 68.492$ ,  $df = 12$ ,  $P < 0.001$ ). The interaction term indicated that there were no differences in the number of taxa among sites in samples from imago bees and “mummies”, but there were differences in the samples of cocoons and pollen, as indicated by Tukey post-hoc tests. In samples of cocoons, the number of microorganisms was the highest in urban1 and the two suburban sites. In samples of pollen, the number of microorganisms was the highest in the rural and suburb1 sites (Fig. 1B).

No genetic material of either *Nosema* species in the solitary bees' abdomens was detected. *Osmia bicornis*' offspring was not infested with *Nosema* spp., therefore, *O. bicornis* cannot be a source of these pests in the environment.

The environment type has a significant effect on the probability of reproductive success (GLMM  $F_{4, 2194} = 37.385$ ,  $P < 0.001$ ), probability of occurrence of unused pollen (GLMM  $F_{4, 2192} = 11.345$ ,  $P < 0.001$ ), occurrence of mummified larvae (GLMM  $F_{4, 2194} = 19.094$ ,  $P < 0.001$ ) and presence of *Cacoxenus indagator* and *Monodontomerus obscurus* parasites (GLMM  $F_{4, 2194} = 18.756$ ,  $P < 0.001$ ). The probability of reproductive success (survival of bees) was the highest in the sites located in urban centres (Fig. 1C). The probabilities of occurrence of “mummies”, packages of unused pollen and brood parasites were the lowest in the sites in the city centre (Fig. 1C).

Analyses performed at the tube level revealed that the studied localities differed in the number of cells per tube with the highest values for urban1 and rural sites (GLMM  $df = 4$ , Wald  $\chi^2 = 17.852$ ,  $P = 0.001$ , Fig. 1D). There was a significant effect of the site on the number of live bees in a tube (GLMM  $df = 4$ , Wald  $\chi^2 = 70.108$ ,  $P < 0.001$ ), number of brood parasites (GLMM  $df = 4$ , Wald  $\chi^2 = 185.187$ ,  $P < 0.001$ ), number of mummified larvae (GLMM  $df = 4$ , Wald  $\chi^2 = 76.797$ ,  $P < 0.001$ ) and number of unused pollen in a tube (GLMM  $df = 4$ , Wald  $\chi^2 = 65.403$ ,  $P < 0.001$ ). The number of live bees was the highest in the sites located in the urban centre, then in the rural area and the lowest in suburbs, after controlling for differences in the number of brood cells in tubes among locations (Fig. 1D). The number of brood parasites, number of “mummies” and number of packages of unused pollen per tube were the lowest in the sites in the city centre, after controlling for the differences in the number of brood cells per tube among sites (Fig. 1D) (Table 1).

There was a negative relationship between the number of brood parasites and number of alive bees (estimate =  $-0.129 \pm 0.030$ ; GLMM  $df$



**Fig. 1.** Images of statistical analysis of the results obtained with MALDI-TOF MS technique. A. The effect of environment on the composition of bacterial species in nests of red mason bees; results from the partial canonical correspondence analysis. Sample scores are shown as kernel density estimates. B. Number of microorganism taxa (bacterial species) found in different nest materials in different environments. Estimates (points) from the generalized linear mixed models are shown with 95% confidence intervals (whiskers). Levels not connected by the same capital letter indicate statistically significant differences revealed by Tukey post-hoc tests. C. The effect of environment on the probability of (a) bees survival, (b) occurrence of nest parasites, (c) occurrence of mummified larvae and (d) occurrence of unused pollen loads. Estimates (points) from the generalized linear mixed models are shown with 95% confidence intervals (whiskers). Levels not connected by the same capital letter indicate statistically significant differences revealed by Tukey post-hoc tests. D. The effect of environment on the probability of bees survival, occurrence of nest parasites, occurrence of mummified larvae and occurrence of unused pollen. Estimates (points) from the generalized linear mixed models are shown with 95% confidence intervals (whiskers). Levels not connected by the same capital letter indicate statistically significant differences revealed by Tukey post-hoc tests.

= 1, Wald  $\chi^2 = 18.943, P < 0.001$ ) after controlling for the differences in sites and number of brood cells in a tube as well as the interaction term between these factors.

#### 4. Discussion

We did not confirm our hypothesis that the urban environment has elevated levels of bee pathogens. Solitary *O. bicornis* bees do not seem to transmit or bring pathogens to the nests in any environment. Neither pollen nor any other biological material from the nests was infected.

These results indicate that the nests were not a reservoir of pathogenic microorganisms from the surrounding environment. However, the etiology of failures in the early stage of insect development (unused pollen and mummification of larvae) remains unknown. Adult offspring diapausing inside the cocoons had no signs of bacterial infection, so they were probably not transmitters and hence not a source of pathogenic microorganisms for the surrounding environment or indirectly a threat to other bee species.

We also did not confirm the hypothesis that the urban environment had a higher occurrence and abundance of nest parasites. On the

**Table 1**  
Summary statistics of the reproductive success based on the content of 50 nesting tubes. Comparison of number of brood cells with unused pollen, mummified bees, compartments with parasites and dead bees with number of healthy *Osmia bicornis* adults in the same nesting boxes localized in sites of urban (U), suburban (suburb) and rural environments.

	Total number in 50 nesting tubes				Total number of failures	Number of healthy bees inside the cocoons	Level of reproductive success
	Cell with pollen	Cell with mummified larvae	Cell with parasites outside or inside the cocoons	Dead bee outside the cocoons			
U1	12	4	14	4	34	437	92.78%
U2	14	7	1	4	26	392	93.78%
Suburb1	38	46	62	1	147	236	61.62%
Suburb2	54	17	83	1	155	324	67.64%
Rural	83	4	4	1	92	391	80.95%

contrary, the manifestation of nest parasites was the lowest in the urban environment. Additionally, the abundance of parasites was negatively correlated with the reproductive success of *O. bicornis* populations. Breeding failures were rare in the urban environment, and breeding success was the highest there.

A large diversity of pollinators is important to support environmental sustainability. It is believed that a few dominant bee species and many rare ones are needed for native flora and crops to be pollinated at a sufficient level (Kremen, 2018). Depending on the environment, the proportions of pollinating insect species composition differ (Kremen, 2018). Variation in species richness, especially in the Apoidea superfamily, occurs in all biotopes of the urbanisation gradient from rural areas to urban agglomerations (Banaszak-Cibicka and Żmihorski, 2012; Fortel et al., 2014; Hudewenz and Klein, 2015; Verboven et al., 2014). However, rich biodiversity of insects may promote the spread of interspecific diseases. Flowers act as dispersal platforms for a variety of pathogens. Graystock et al. (2015) showed that wild foraging insects transfer diseases horizontally and spread parasites in the environment, even if they are not a given parasite's host. Similar finding observed in experiments by Maccagnani et al. (2009) showed that *Osmia cornuta* and *Apis mellifera* bees can be the agent which is causing secondary colonisation of bacteria (*Bacillus subtilis* were used as a model organism) on apple flowers – so pollinators can play an important role as secondary carriers. Additionally, *Apis mellifera* L. have been implicated in the spread of the fire blight pathogen (*Erwinia amylovora*), and may transmit other bacterial plant pathogens in the process of pollinating crops (Pattemore et al., 2014). Moreover, Pattemore et al. (2014) showed that *Pseudomonas syringae* – a pathogen of kiwifruit (*Actinidia* spp.) – can survive in beehives and spread within a hive. Snyder et al. (1998) suggested that rhizobacteria can escape the rhizosphere by moving in or onto foliage, where they can then be acquired and transmitted by insects. This transmission route may be common among naturally occurring rhizobacteria and facilitate the dispersal of both beneficial and harmful soilborne microorganisms. Therefore it is clear that bees are potential diseases carriers. Ravoet et al. (2014) confirmed that in homogenates made from 10 individuals of an adult *O. bicornis* caught flying in the area near the apiaries, genetic material of *N. ceranae* and protists *Crithidia bombi* (Lipa & Triggiani, 1988) and *Apicystis bombi* (Liu et al., 1974) was present. Additionally, Fürst et al. (2014) claimed that managed honeybees drive interspecies pathogen transmission for wild pollinators. Our results broaden this knowledge with information that the studied representative of solitary bees does not bring pathogenic bacteria and fungi (including *Nosema* spp.) inside the nests, so in the studied populations would be no pathogens, and its nests are not a source of diseases. Our finding coincides with the results obtained by Hedtke et al. (2015) with genetic analyses of *Osmia cornifrons* (Radoszkowski, 1887). The solitary horned mason bee offspring collected from breedings in different biotopes was not infected with microsporidia (*N. apis*), *Wolbachia* or trypanosomes. For other hand, Graystock et al. (2017) claimed that microbiota of social and wild bee species are significantly varied and differentiated, and also may be associated with the microbiological flora composition of stored and consumed pollen. Voulgari-Kokota et al. (2018) also identified cosmopolitan bacterial

taxa that showed consistent occurrence in various wild bee species (including *O. bicornis*) larvae and stored pollen – however still with high differentiation among species. Biological material collected in this study from the *O. bicornis* nests was mostly inhabited by non-pathogenic representatives of the *Pseudomonas* and *Sphingomonas* genera. These bacteria (among others) are the typical core microorganisms of *Apis* bees and pollen flora (Donkersley et al., 2018; Graystock et al., 2017; Loncaric et al., 2011). Graystock et al. (2017) found *Pseudomonas* and *Sphingomonas* among other genera of bacteria also in small carpenter *Ceratina* bees as well as in pollen samples. It is interesting that among these non-pathogenic bacteria, there were significant differences in taxa composition among environments. The most probable reason for the differences in nest microbiota is a variable composition of the flora and pollinating insect species. While Voulgari-Kokota et al. (2018) stated that solitary bee bacterial microbiota in the nesting environment are mostly homogeneous within species, and not significantly affected by landscape, they also admitted that the microbiomes of early stage pollen provisions from the most abundant host species were influenced by site. In future studies, it might be worth comparing the microbiome of different bee species in the same environment and conduct research on a wide scale to comprehensively study this subject. Moreover, as higher trophic levels such as brood parasites are more sensitive to intensively managed habitats such as meadows (Albrecht et al., 2007), it would be interesting to investigate why they were more diverse in suburban areas, with focus on habitat fragmentation along urbanisation gradient. Lozo et al. (2015) identified only 29 species of bacteria in biological material from *Osmia cornuta* and assumed that the microbial diversity detected in the tested samples probably come from the environment. In this study, we had up to 42 (highly probable) species identifications for the closely related *O. bicornis* bee, which significantly improved the database for further microbiological analyses.

Phylogenetic reports of Branstetter et al. (2017) indicated that the social representatives of Apidae family are evolutionarily older than representatives of the solitary Megachilidae family (see also Murray et al., 2018). The social lifestyle, along with offspring nursing, is associated with problems of pathogen proliferation, and solitary living may be an evolutionary solution. In honey bee colonies, transmission of disease by one individual can cause an epidemic and might decimate the colony (Betti et al., 2014). Although *Apis mellifera* developed protective mechanisms and behaviours to eliminate the threat of disease, a high manifestation of infection may not be possible to eradicate in a human-modified environment (Natsopoulou et al., 2016; Paris et al., 2018). The use of drugs may have negative effects on bees' lives (Raymann et al., 2017). Increasing the number of individual bees in a colony (e.g., for better overwintering) facilitates the spread of diseases (Seeley and Smith, 2015). The life cycle of solitary *Osmia* bees allows keepers to conduct only a limited number of breeding operations, which have a positive effect on the bee health and limit the number of parasites (Sedivy and Dorn, 2014).

Our results showed that the population of *O. bicornis* in different environments is not threatened by pathogenic bacteria or fungi but only by brood parasites. Fliszkiewicz et al. (2012) reported that red mason bees bred in meadow and forest environments had higher ratios of



brood parasite invasion in comparison to orchard and park sites. We noticed a serious manifestation of brood parasites in suburban areas, whereas in breedings conducted in urban and meadow sites, the infestation was minor. It seems that urban environments may negatively affect brood parasites, which supposedly are the most important limiting factor for solitary bees. Although Groulx and Forrest (2018) related the invasion of brood parasites with the density of wild solitary bee individuals in a given territory, our results and those of Fliszkiewicz et al. (2012) were obtained for equinumerous initial populations and even then showed differences in parasite manifestation. Zajdel et al. (2014) noted that in a breeding conducted continuously for 10 years in the same place, the number of brood parasites was significantly higher than in one-year breeding.

There may be, of course, other factors affecting the reproductive success of solitary bee populations. *Osmia bicornis*, compared to social bees, have a significantly reduced period of annual activity (Fliszkiewicz et al., 2011; Strachecka et al., 2017). This restriction also reduces the spread of an epidemic on a wider temporal scale, which is especially important considering these bees stay close to their birth place and have short, up to 500 m flight ranges (Gathmann and Tscharntke, 2002; Zurbuchen et al., 2010). The usage of specific blooming flower's resources can also provide additional protection. Spear et al. (2016) suggested that e.g. Sapyga parasites (Sapygidae) are unable to develop on Asteraceae pollen provisions. *Osmia* bees as pollinators foraging only on protein pollen from flowers may be less attractive as pathogen/parasite hosts than bees that also collect nectar rich in carbohydrates. In our experiment, the sites chosen as breeding locations were rich in various plant species, so floral resources could not be a factor limiting the bees' reproductive success. However, an overall lack of pathogenic bacterial and fungal infection in breedings conducted in different environments can be directly connected with the low (in carbohydrates) value of resource storages inside the solitary bee nests. Palladini and Maron (2014) noticed that brood parasites cause mortality of *Osmia lignaria* offspring in environments which are less human-modified and have the advantage of native-dominated plant species. Our results corroborate those observations because the greatest manifestation of brood parasites was noticed in the most "natural" localization, while the highest reproductive success was achieved by the insects from the most urbanised nesting sites.

As Fürst et al. (2014) and Graystock et al. (2016) suggested, managed bees can have negative effects on wild bees through the spread of microorganisms. There are also data showing that solitary bees spread a variety of pathogens in the environment and horizontally transmit them to other pollinators (Ravoet et al., 2014). Our results, however, indicate the need to look more closely at the causes and characteristics of the spread of pathogens as well as the human-mediated impact on pollinators' parasites and pests.

## 5. Conclusions

Bees play a significant role in the environment and human economy. The growing popularity of breeding solitary bees and their introduction into new territories may be profitable for sustainable development of the urban landscape. We rejected the hypothesis that nests of ubiquitous *O. bicornis* are reservoirs of diseases in urban environments. The collateral prediction that some diseases are vertically transmitted and, therefore, *Osmia* bees are a spring-like pathogen vector also seems debunked. Differences among broods in terms of parasite manifestations indicate that the human-modified environment creates favourable living conditions for pollinating insects. Altogether, to date, *Osmia* bees seem to be a good and environmentally safe pollinator. Further research on the effects of urbanisation in different areas are needed to draw final robust conclusions, particularly regarding the cause-and-effect relationship between bee diseases and parasites.

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## Declaration of competing interest

All authors declare no conflict of interest.

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## References

- Albrecht, M., Duelli, P., Schmid, B., Müller, C.B., 2007. Interaction diversity within quantified insect food webs in restored and adjacent intensively managed meadows. *J. Anim. Ecol.* 76, 1015–1025. <https://doi.org/10.1111/j.1365-2656.2007.01264.x>.
- Bąk, B., Wilde, J., Bratkowski, J., 2003. The monitoring of the flight activity of the red mason bee (*Osmia rufa* L.). *Acta. Biol. Univ. Daugavp.* 3, 97–100.
- Banaszak-Cibicka, W., Zmihorski, M., 2012. Wild bees along an urban gradient: winners and losers. *J. Ins. Conserv.* 16 (3), 331–343. <https://doi.org/10.1007/s10841-011-9419-2>.
- Barbosa, P., Berry, D., Kary, C.K., 2014. Dissecting fluids and saline solutions. *Insect Histology: Practical Laboratory Techniques*. John Wiley & Sons, New York, pp. 325–331.
- Betti, M.I., Wahl, L.M., Zamir, M., 2014. Effects of infection on honey bee population dynamics: a model. *PLoS One* 9 (10), e110237. <https://doi.org/10.1371/journal.pone.0110237>.
- Branstetter, M.G., Danforth, B.N., Pitts, J.P., Faircloth, B.C., Ward, P.S., Buffington, M.L., Gates, M.W., Kula, R.R., Brady, S.G., 2017. Phylogenomic insights into the evolution of stinging wasps and the origins of ants and bees. *Curr. Biol.* 27 (7), 1019–1025. <https://doi.org/10.1016/j.cub.2017.03.027>.
- Coudrain, V., Rittiner, S., Herzog, F., Tinner, W., Entling, M.H., 2016. Landscape distribution of food and nesting sites affect larval diet and nest size, but not abundance of *Osmia bicornis*. *Insect Sci* 23 (5), 746–753. <https://doi.org/10.1111/1744-7917.12238>.
- Ditchkoff, S.S., Saalfeld, S., Gibson, C., 2006. Animal behavior in urban ecosystems: modifications due to human-induced stress. *Urban Ecosyst.* 9, 5–12.
- Donkersley, P., Rhodes, G., Pickup, R.W., Jones, K.C., Wilson, K., 2018. Bacterial communities associated with honeybee food stores are correlated with land use. *Ecol. Evol.* 8 (10), 4743–4756. <https://doi.org/10.1002/ece3.3999>.
- Dramstad, W.E., Fry, G.L., Schaffer, M.J., 2003. Bumblebee foraging – is closer really better? *Agric. Ecosyst. Environ.* 95 (1), 349–357. [https://doi.org/10.1016/S0167-8809\(02\)00043-9](https://doi.org/10.1016/S0167-8809(02)00043-9).
- Everaars, J., Strohbach, M.W., Gruber, B., Dormann, C.F., 2011. Microsite conditions dominate habitat selection of the red mason bee (*Osmia bicornis*, Hymenoptera: Megachilidae) in an urban environment: a case study from Leipzig, Germany. *Landsc. Urban Plan.* 103 (1), 15–23. <https://doi.org/10.1016/j.landurbplan.2011.05.008>.
- Fliszkiewicz, M., Giejdasz, K., Wilkaniec, Z., 2011. The effect of diapause termination time on the successful insemination of *Osmia rufa* L. females. *J. Apic. Sci.* 55 (2), 79–84.
- Fliszkiewicz, M., Kuśnierczak, A., Szymaś, B., 2012. The accompanying fauna of solitary bee *Osmia bicornis* (L.) syn. *Osmia rufa* (L.) nests settled in different biotopes. *J. Apic. Sci.* 56 (1), 51–58. <https://doi.org/10.2478/v10289-012-0006-x>.
- Fortel, L., Henry, M., Guilbaud, L., Guirao, A.L., Kuhlmann, M., Mouret, H., Rollin, O., Vaissière, B.E., 2014. Decreasing abundance, increasing diversity and changing structure of the wild bee community (Hymenoptera: Anthophila) along an urbanization gradient. *PLoS One* 9 (8), e104679. <https://doi.org/10.1371/journal.pone.0104679>.
- Fournier, D.A., Skaug, H.J., Ancheta, J., Ianelli, J., Magnusson, A., Maunder, M., Nielsen, A., Sibert, J., 2012. AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optim. Methods Softw.* 27, 233–249.
- Fox, J., Weisberg, S., 2011. An {R} Companion to Applied Regression. Second edition. Sage, Thousand Oaks CA URL <http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>.
- Fürst, M.A., McMahon, D.P., Osborne, J.L., Paxton, R.J., Brown, M.J.F., 2014. Disease associations between honeybees and bumblebees as a threat to wild pollinators. *Nature* 506 (7488), 364. <https://doi.org/10.1038/nature12977>.
- Gathmann, A., Tscharntke, T., 2002. Foraging ranges of solitary bees. *J. Anim. Ecol.* 71 (5), 757–764. <https://doi.org/10.1046/j.1365-2656.2002.00641.x>.

- Giejdasz, K., Fliszkiewicz, M., 2016. Effect of temperature treatment during development of *Osmia rufa* L., on mortality, emergence and longevity of adults. *J. Apic. Sci.* 60 (2), 221–232. <https://doi.org/10.1515/JAS-2016-0029>.
- Giraudeau, M., Mousel, M., Earl, S., McGraw, K., 2014. Parasites in the City: degree of urbanization predicts poxvirus and coccidian infections in house finches (*Haemorrhous mexicanus*). *PLoS One* 9 (2), e86747. <https://doi.org/10.1371/journal.pone.0086747>.
- Graystock, P., Goulson, D., Hughes, W.O., 2015. Parasites in bloom: flowers aid dispersal and transmission of pollinator parasites within and between bee species. *Proc. R. Soc. B* 282 (1813), 20151371. <https://doi.org/10.1098/rspb.2015.1371>.
- Graystock, P., Blane, E.J., McFrederick, Q.S., Goulson, D., Hughes, W.O., 2016. Do managed bees drive parasite spread and emergence in wild bees? *Int. J. Parasitol. Parasites. Wildl.* 5 (1), 64–75. <https://doi.org/10.1016/j.ijppaw.2015.10.001>.
- Graystock, P., Rehan, S.M., McFrederick, Q.S., 2017. Hunting for healthy microbiomes: determining the core microbiomes of *Ceratina*, *Megalopta*, and *Apis* bees and how they associate with microbes in bee collected pollen. *Conserv. Genet.* 18 (3), 701–711. <https://doi.org/10.1007/s10592-017-0937-7>.
- Groulx, A.F., Forrest, J.R., 2018. Nesting aggregation as a predictor of brood parasitism in mason bees (*Osmia* spp.). *Ecol. Entomol.* 43 (2), 182–191. <https://doi.org/10.1111/een.12484>.
- Gruber, B., Eckel, K., Everaars, J., Dormann, C.F., 2011. On managing the red mason bee (*Osmia bicornis*) in apple orchards. *Apidologie* 42 (5), 564–576. <https://doi.org/10.1007/s13592-011-0059-z>.
- Hedtke, S.M., Blitzer, E.J., Montgomery, G.A., Danforth, B.N., 2015. Introduction of non-native pollinators can lead to trans-continental movement of bee-associated fungi. *PLoS One* 10 (6), e0130560. <https://doi.org/10.1371/journal.pone.0130560>.
- Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. *Biom. J.* 50 (3), 346–363. <https://doi.org/10.1002/bimj.200810425>.
- Hudewenz, A., Klein, A.M., 2015. Red mason bees cannot compete with honey bees for floral resources in a cage experiment. *Ecol. Evol.* 5 (21), 5049–5056. <https://doi.org/10.1002/ece3.1762>.
- Hung, A.C.F., 2000. PCR detection of Kashmir bee virus in honey bee excreta. *J. Apic. Res.* 39 (3–4), 103–106. <https://doi.org/10.1080/00218839.2000.11101029>.
- Isaksson, C., 2015. Urbanisation, oxidative stress and inflammation: a question of evolving, acclimatizing or coping with urban environmental stress. *Funct. Ecol.* 29, 913–923. <https://doi.org/10.1111/1365-2435.12477>.
- Jones, E.L., Leather, S.R., 2013. Invertebrates in urban areas: a review. *Eur. J. Entomol.* 109 (4), 463–478. <https://doi.org/10.14411/eje.2012.060>.
- Kierat, J., Filipiak, M., Szentgyörgyi, H., Woyciechowski, M., 2017a. Predation cues in solitary bee nests. *J. Insect Behav.* 30 (4), 385–393. <https://doi.org/10.1007/s10905-017-9626-0>.
- Kierat, J., Szentgyörgyi, H., Czarnoleski, M., Woyciechowski, M., 2017b. The thermal environment of the nest affects body and cell size in the solitary red mason bee (*Osmia bicornis* L.). *J. Therm. Biol.* 68, 39–44. <https://doi.org/10.1016/j.jtherbio.2016.11.008>.
- Kremen, C., 2018. The value of pollinator species diversity. *Science* 359 (6377), 741–742. <https://doi.org/10.1126/science.aar7614>.
- Krunić, M., Stanisavljević, L., 2006. Augmentation of managed populations of *Osmia cornuta* and *O. rufa* (Hymenoptera: Megachilidae) in Southeastern Europe. *Eur. J. Entomol.* 103 (3), 695. <https://doi.org/10.14411/eje.2006.091>.
- Krunić, M., Stanisavljević, L., Brajković, M., Tomanović, Ž., Radović, I., 2001. Ecological studies of *Osmia cornuta* (Latr.) (Hymenoptera, Megachilidae) populations in Yugoslavia with special attention to their diapause. *Acta Hort.* 561, 297–301.
- Krunić, M., Stanisavljević, L., Pinzauti, M., Felicioli, A., 2005. The accompanying fauna of *Osmia cornuta* and *Osmia rufa* and effective measures of protection. *B Insectol* 58, 141–152.
- Loncaric, I., Ruppitsch, W., Licek, E., Moosbeckhofer, R., Busse, H.J., Rosengarten, R., 2011. Characterization of selected Gram-negative non-fermenting bacteria isolated from honey bees (*Apis mellifera carnica*). *Apidologie* 42 (3), 312–325. <https://doi.org/10.1007/s13592-011-0019-7>.
- Lozo, J., Berić, T., Terzić-Vidojević, A., Stanković, S., Fira, D., Stanisavljević, L., 2015. Microbiota associated with pollen, bee bread, larvae and adults of solitary bee *Osmia cornuta* (Hymenoptera: Megachilidae). *Bull. Entomol. Res.* 105 (4), 470–476. <https://doi.org/10.1017/S0007485315000292>.
- Maccagnani, B., Giacomello, F., Fanti, M., Gobbin, D., Maini, S., Angeli, G., 2009. *Apis mellifera* and *Osmia cornuta* as carriers for the secondary spread of *Bacillus subtilis* on apple flowers. *Biocontrol* 54 (1), 123–133. <https://doi.org/10.1007/s10526-008-9163-z>.
- MacIvor, J.S., Packer, L., 2016. The bees among us: modelling occupancy of solitary bees. *PLoS One* 11 (12), e0164764. <https://doi.org/10.1371/journal.pone.0164764>.
- Martin, L.B., Hopkins, W.A., Mydlarz, L.D., Rohr, J.R., 2010. The effects of anthropogenic global changes on immune functions and disease resistance. *Ann. N. Y. Acad. Sci.* 1195 (1), 129–148. <https://doi.org/10.1111/j.1749-6632.2010.05454.x>.
- McKinney, M.L., 2002. Urbanization, biodiversity, and conservation. *BioSci* 52 (10), 883–890. [https://doi.org/10.1641/0006-3568\(2002\)052\[0883:UBAC\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2002)052[0883:UBAC]2.0.CO;2).
- McKinney, M.L., 2008. Effects of urbanization on species richness: a review of plants and animals. *Urban Ecosyst.* 11 (2), 161–176. <https://doi.org/10.1007/s11252-007-0045-4>.
- Michener, C.D., 2007. *The Bees of the World*, 2nd ed. Johns Hopkins, Baltimore.
- Murray, E.A., Bossert, S., Danforth, B.N., 2018. Pollinivory and the diversification dynamics of bees. *Biol. Lett.* 14 (11), 20180530. <https://doi.org/10.1098/rsbl.2018.0530>.
- Natsopoulos, M.E., McMahon, D.P., Paxton, R.J., 2016. Parasites modulate within-colony activity and accelerate the temporal polyethism schedule of a social insect, the honey bee. *Behav. Ecol. Sociobiol.* 70 (7), 1019–1031. <https://doi.org/10.1007/s00265-015-2019-5>.
- Palladini, J.D., Maron, J.L., 2014. Reproduction and survival of a solitary bee along native and exotic floral resource gradients. *Oecologia* 176 (3), 789–798. <https://doi.org/10.1007/s00442-014-3028-3031>.
- Paris, L., El Alaoui, H., Delbac, F., Diogon, M., 2018. Effects of the gut parasite *Nosema ceranae* on honey bee physiology and behavior. *Curr. Opin. Insect. Sci.* 26, 149–154. <https://doi.org/10.1016/j.cois.2018.02.017>.
- Pattemore, D.E., Goodwin, R.M., McBrydie, H.M., Hoyte, S.M., Vanneste, J.L., 2014. Evidence of the role of honey bees (*Apis mellifera*) as vectors of the bacterial plant pathogen *Pseudomonas syringae*. *Australas Plant Path* 43 (5), 571–575. <https://doi.org/10.1007/s13313-014-0306-7>.
- Pauchard, A., Aguayo, M., Pena, E., Urrutia, R., 2006. Multiple effects of urbanization on the biodiversity of developing countries: the case of a fast-growing metropolitan area (Concepcion, Chile). *Biol. Conserv.* 127, 272–281. <https://doi.org/10.1016/j.biocon.2005.05.015>.
- Ptaszyńska, A.A., Borsuk, G., Zdybicka-Barabas, A., Cytryńska, M., Małek, W., 2016. Are commercial probiotics and prebiotics effective in the treatment and prevention of honeybee nosemosis? *Parasitol. Res.* 115 (1), 397–406. <https://doi.org/10.1007/s00436-015-4761-z>.
- R Core Team, 2018. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria URL <https://www.R-project.org/>.
- Ravoet, J., De Smet, L., Meeus, I., Smaghe, G., Wenseleers, T., de Graaf, D.C., 2014. Widespread occurrence of honey bee pathogens in solitary bees. *J. Invertebr. Pathol.* 122, 55–58. <https://doi.org/10.1016/j.jip.2014.08.007>.
- Raymann, K., Shaffer, Z., Moran, N.A., 2017. Antibiotic exposure perturbs the gut microbiota and elevates mortality in honeybees. *PLoS Biol.* 15 (3), e2001861. <https://doi.org/10.1371/journal.pbio.2001861>.
- Revilla, T.A., Krivan, V., 2018. Competition, trait-mediated facilitation, and the structure of plant–pollinator communities. *J. Theor. Biol.* 440, 42–57. <https://doi.org/10.1016/j.jtbi.2017.12.019>.
- Ribiere, M., Lallemand, P., Iscache, A.L., Schurr, F., Celle, O., Blanchard, P., Olivier, V., Faucon, J.P., 2007. Spread of infectious chronic bee paralysis virus by honeybee (*Apis mellifera* L.) feces. *Appl. Environ. Microbiol.* 73 (23), 711–716. <https://doi.org/10.1128/AEM.01053-07>.
- Riley, S.P.D., Brown, J.L., Sikkich, J.A., Schoonmaker, C.M., Boydston, E.E., 2014. Wildlife friendly roads: The impacts of roads on wildlife in urban areas and potential remedies. In: McCleery, R.A., Moorman, C.E., Peterson, M.N. (Eds.), *Urban Wildlife Conservation: Theory and Practice*. Springer US, Boston, MA, pp. 323–360.
- Ruiz-González, M.X., Brown, M.J., 2006. Honey bee and bumblebee trypanosomatids: specificity and potential for transmission. *Ecol. Entomol.* 31 (6), 616–622. <https://doi.org/10.1111/j.1365-2311.2006.00823.x>.
- Schindler, M., Peters, B., 2011. Mason bees *Osmia bicornis* and *Osmia cornuta* as suitable orchard pollinators. *Erwerbs-Obstbau* 52 (3–4), 111–116. <https://doi.org/10.1007/s10341-010-0118-z>.
- Sedivy, C., Dorn, S., 2014. Towards a sustainable management of bees of the subgenus *Osmia* (Megachilidae: *Osmia*) as fruit tree pollinators. *Apidologie* 45 (1), 88–105. <https://doi.org/10.1007/s13592-013-0231-8>.
- Seeley, T.D., Smith, M.L., 2015. Crowding honeybee colonies in apiaries can increase their vulnerability to the deadly ectoparasite *Varroa destructor*. *Apidologie* 46 (6), 716–727. <https://doi.org/10.1007/s13592-015-0361-2>.
- Shimizu, A., Dohzono, I., Nakaji, M., Roff, D.A., Miller III, D.G., Osato, S., Yajima, T., Niitsu, S., Utsugi, N., Sugawara, T., Yoshimura, J., 2014. Fine-tuned bee-flower coevolutionary state hidden within multiple pollination interactions. *Sci. Rep.* 4, 3988. <https://doi.org/10.1038/srep03988>.
- Skaug, H., Fournier, D., Bolker, B., Magnusson, A., Nielsen, A., 2016. *Generalized Linear Mixed Models Using 'AD Model Builder'*. R Package Version 0.8.3.3.
- Šmilauer, P., Lepš, J., 2014. *Multivariate analysis of ecological data using CANOCO 5*. Cambridge University Press, Cambridge.
- Snyder, W.E., Tonkyn, D.W., Kluepfel, D.A., 1998. Insect-mediated dispersal of the rhizobacterium *Pseudomonas chlororaphis*. *Phytopathology* 88 (12), 1248–1254. <https://doi.org/10.1094/PHYTO.1998.88.12.1248>.
- Spears, D.M., Silverman, S., Forrest, J.R., 2016. Asteraceae pollen provisions protect *Osmia* mason bees (Hymenoptera: Megachilidae) from brood parasitism. *Amer. Nat.* 187 (6), 797–803. <https://doi.org/10.1086/686241>.
- Strachecka, A., Chobotow, J., Paleolog, J., Łoś, A., Schulz, M., Teper, D., Kucharczyk, H., Grzybek, M., 2017. Insights into the biochemical defence and methylation of the solitary bee *Osmia rufa* L.: a foundation for examining eusociality development. *PLoS One* 12 (4), e0176539. <https://doi.org/10.1371/journal.pone.0176539>.
- Teper, D., Biliński, M., 2009. Red mason bee (*Osmia rufa* L.) as a pollinator of rape plantations. *J. Apic. Sci.* 53 (2), 115–120.
- Verboven, H.A., Uyttenbroeck, R., Brys, R., Hermy, M., 2014. Different responses of bees and hoverflies to land use in an urban–rural gradient show the importance of the nature of the rural land use. *Landsc. Urban Plan.* 126, 31–41. <https://doi.org/10.1016/j.landurbplan.2014.02.017>.
- Voulgari-Kokota, A., Grimmer, G., Steffan-Dewenter, I., Keller, A., 2018. Bacterial community structure and succession in nests of two megachilid bee genera. *FEMS Microbiol. Ecol.* 95 (1), fiy218.
- Zajdel, B., Kucharska, K., Kucharski, D., Fliszkiewicz, M., Gąbka, J., 2014. Accompanying fauna of red mason bees in annual and perennial nesting sites. *Med. Weter.* 70 (12), 745–749. <https://doi.org/10.2478/v10289-012-0006-x>.
- Zurbuchen, A., Landert, L., Klaiber, J., Müller, A., Hein, S., Dorn, S., 2010. Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. *Biol. Conserv.* 143 (3), 669–676. <https://doi.org/10.1016/j.biocon.2009.12.003>.

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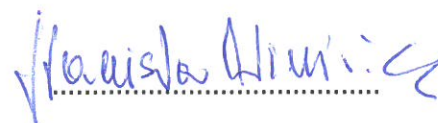
Łoś, A., Skórka, P., Strachecka, A., Winiarczyk, S., Adaszek, Ł., Winiarczyk, M., & Wolski, D. (2020). The associations among the breeding performance of *Osmia bicornis* L. (Hymenoptera: Megachilidae), burden of pathogens and nest parasites along urbanisation gradient. *Science of the Total Environment*, 710, 135520. DOI: 10.1016/j.scitotenv.2019.135520

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Mój udział był następujący:

Merytoryczny udział w publikacji:

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06.05.2022  
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### **PUBLIKACJA 3**

Splitt A., Pustkowiak S., Borański M., Strachecka A., Skórka P. Sex and the city: *Osmia bicornis* have more numerous and bigger female offspring in urban environment. Landscape and Urban Planning [*w trakcie procesu redakcyjnego w czasopiśmie Landscape and Urban Planning*]

1 **Sex and the city: *Osmia bicornis* has more numerous and bigger female offspring in urban**  
 2 **environment**

3 Aleksandra Splitt<sup>1,2\*</sup>, Sylwia Pustkowiak<sup>1</sup>, Mikołaj Borański<sup>2</sup>, Aneta Strachecka<sup>3</sup>, Piotr Skórka<sup>1</sup>

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 5 Poland

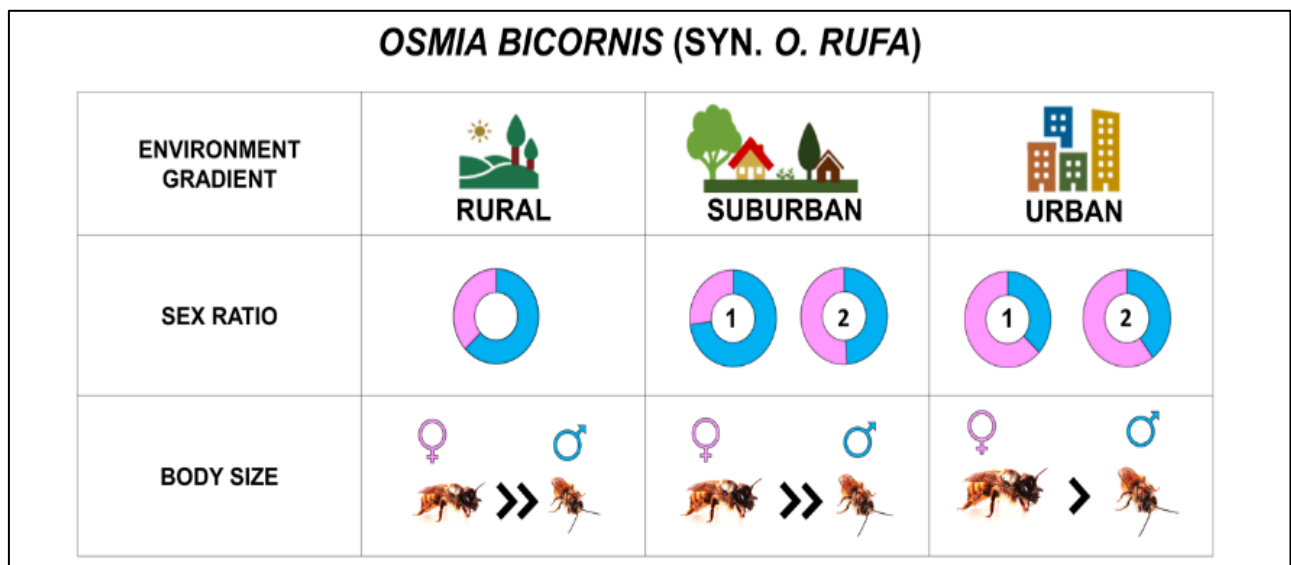
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11 **HIGHLIGHTS**

- 12 ● The sex ratio of *Osmia bicornis* changes along the urban-rural gradient
- 13 ● The sex ratio shifted towards females in an urban environment
- 14 ● Both female and male offspring were the largest in urban sites
- 15 ● Environment, location in tube and size of cocoon are best predictors of bee sex
- 16 ● Urban areas may be a suitable environment for the species



17 **ABSTRACT**

18 Urbanization has a high impact on wild bee populations. Shifts of the bee sex ratio along an  
19 urbanization gradient may affect population dynamics. The aims of this research were to compare  
20 influence of urbanization gradient (urban, suburban and rural environments) on the sex ratio of  
21 the first generation of *Osmia bicornis* (syn. *O. rufa*) offspring and its size. Results revealed that  
22 the sex ratio of the *O. bicornis* offspring is shifted towards females in urban sites (in comparison  
23 to rural one). The wider the tubes the higher probability that females were laid. Sex of the  
24 offspring was highly correlated with the total cocoon mass and volume, as well as the offspring  
25 mass: males were significantly smaller and lighter than females. The brood chamber position was  
26 correlated with the sex of the bee - the chambers located from the posterior end had higher  
27 probability to include a female offspring. As *O. bicornis* is a manageable bee species, and  
28 females are believed to be more effective pollinators than males, their prevalence in the  
29 population is often desirable. When the combinations of features that potentially allow sex  
30 identification were compared, the model that included total cocoon weight and the position in the  
31 tube showed a better fit than the model with cocoon volume and the position in the tube. Based  
32 on the shift in the sex ratio towards females in the offspring, it can be concluded that the urban  
33 environment creates favorable conditions for the *O. bicornis* development.

34 **Key words:** *Osmia rufa*, red mason bee, solitary bee, urbanization gradient, sex ratio, sex  
35 allocation

36 **INTRODUCTION**

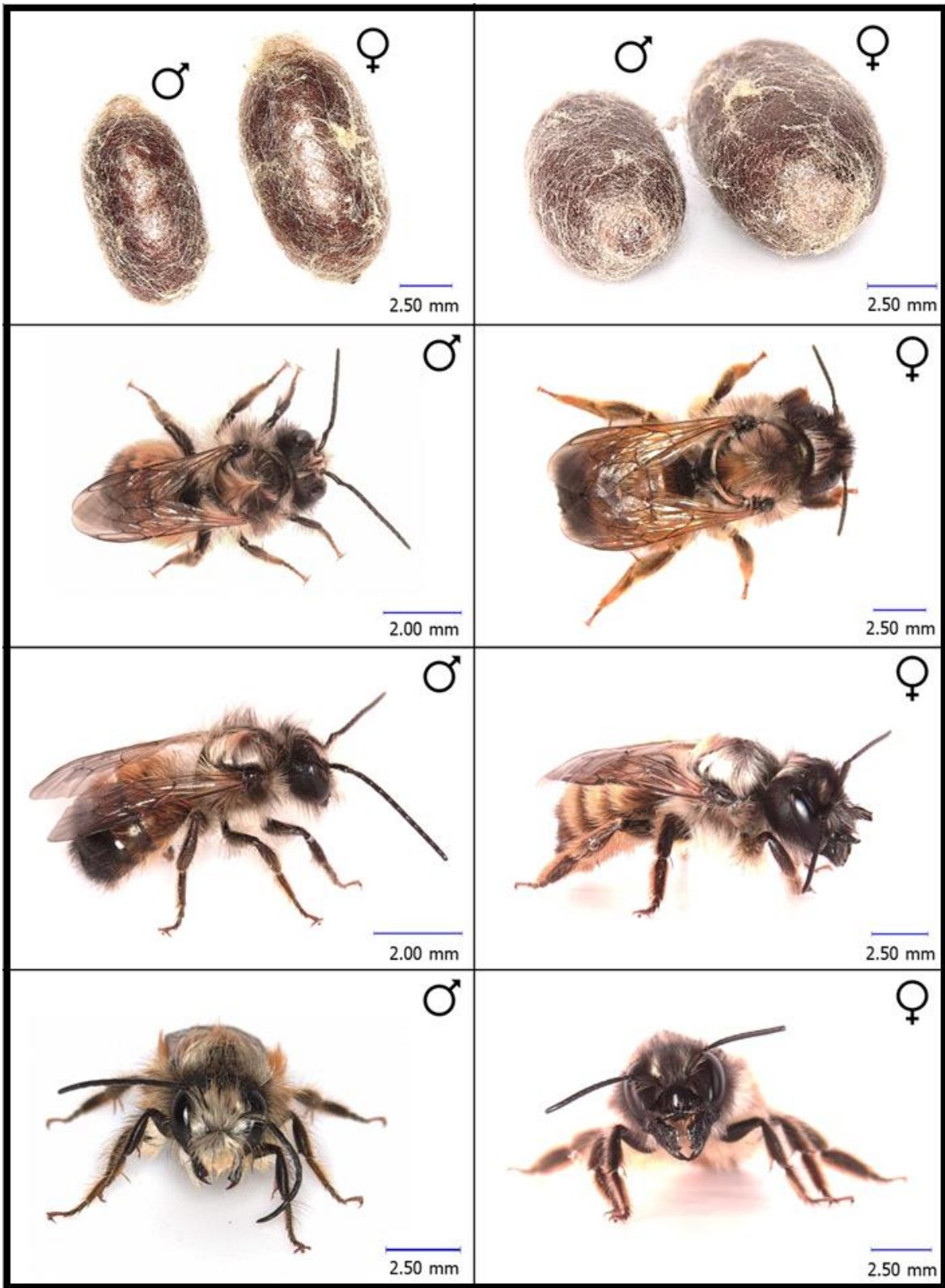
37 The sex ratio in local populations of pollinating insect may affect plant communities and  
38 crop production because females are credited with greater pollination efficiency (Ne'eman et al.,  
39 2006). Changes in sex ratios may also affect population dynamics and lead to incorrect

40 assessment of the population trends (Bessa-Gomes et al., 2004). As cost of production males and  
41 females differs the estimation of factors affecting sex-ratio may indicate population performance  
42 of pollinating insects. The red mason bee (*Osmia bicornis*, syn. *O. rufa*) is a major pollinator of  
43 wild plants and crops in Europe, Northern Africa and adjacent parts of Asia (Banaszak &  
44 Romasenko, 1998; Müller, 2019; Praz et al., 2008). Wild *O. bicornis* predominantly tend to  
45 allocate in favor of males (Sedivy and Dorn, 2014). Male predominance may be induced by the  
46 polyandry in this species. It can also be increased by the fact that even over 85% of the males do  
47 not mate at all but these males that copulate may do so repeatedly (Siedlmann, 2015). Single  
48 male can participate in as many as 10 matings (no more than four daily) (Fliszkiewicz &  
49 Wilkaniec, 2009; Seidelmann, 2015; Boomsma et al., 2005; Paxton, 2005; Seidelmann, 2014).  
50 During the mating season univoltine females of *O. bicornis* may collect 1400 to 5650  
51 spermatozoa in the spermatheca (Fliszkiewicz & Wilkaniec, 2009). The gathered spermatozoa  
52 supply serves the female throughout her foraging period (46 - 70 days; (Raw, 1972; Radmacher,  
53 2011) to produce diploid daughters whereas material from the father is not necessary to produce  
54 haploid sons.

55       Offspring sex in *O. bicornis* is determined mostly by available nesting conditions and  
56 availability of food (Ivanov, 2006). Sons are usually deposited in hollow plant stems of 5–6 mm  
57 inner diameter, whereas daughters mostly in larger tubes with a diameter of 7–8 mm (even up to  
58 10 mm) (Ivanov, 2006; Radmacher & Strohm, 2010; Seidelmann et al., 2016). Length of nesting  
59 material matters and the percentage of daughters within offspring increases with the length of the  
60 nesting tube (Gruber et al., 2011). Typically, daughters require bigger pollen provision mass than  
61 sons (Rust et al., 1989; Radmacher & Strohm, 2010; Seidelmann, 2006). Therefore, the offspring  
62 sex ratio is also correlated with the efficiency of pollen collection by adult females. The  
63 decreased efficiency is associated with a higher proportion of males, and also with an increased

64 rate of male eggs laying (Radmacher & Strohm, 2010; Seidelmann, 2006). Consequently, male  
65 eggs are usually laid closer to the opening of the tube.

66         Inside separated brood compartments, each larva consumes available pollen provision,  
67 afterwards it spins waterproof and completely closes the cocoon in order to pupate into dimorphic  
68 female or male imago (Murawska et al., 2021). Whereas sex of cocoons are visually  
69 indistinguishable, adult females have black heads with strongly developed mandibles and their  
70 lower lateral parts of the clypeus protrude forward as strong angles or horns. On the other hand,  
71 males have black heads with white hair in front and long antennae which are bent backwards and  
72 reach the trunk, among other typical features (Splitt et al., 2021) (Figure 1). Although most  
73 reports state that females are larger than males in the same population their size range varies  
74 among populations. Therefore, it is possible that small females from one population may have  
75 similar size as big males from other populations and environmental gradients (e.g. urbanization)  
76 may be responsible for this phenomenon (Splitt et al., 2022).



77 **Figure 1.** There is a lack of visual difference between *Osmia bicornis* (syn. *O. rufa*) male (on the  
78 left) and female (on the right) cocoons, whereas sexual dimorphism of adults is clearly visible.  
79 The photos were taken with KEYENCE VHX-970F microscope under 10x magnification.

80 Food resources for solitary *O. bicornis* vary depending on the environment and are  
81 generally more abundant and diverse in rural environment than in urban one (Splitt et al., 2021).  
82 *O. bicornis* is commonly kept for commercial or recreational purposes in various environments  
83 with increasing introductions to urban areas (Gruber et al., 2011; Hansted et al., 2014; Holzschuh  
84 et al., 2012; Schindler & Peters, 2011; Sedivy & Dorn, 2014). Thus, as food resources vary along  
85 urbanization gradient it may affect sex ratio in local populations, and thus pollination efficiency.

86 Generally, urbanization has a high impact on wild bee populations (Cane et al., 2006,  
87 Banaszak-Cibicka & Żmihorski, 2011, Fortel, L. et al., 2014, Fitch et al., 2019). According to  
88 Fitch et al. (2019) shifts of bee sex ratio along an urbanization gradient may be caused by  
89 systematic changes in the ability of foragers to provision their brood, resulting from either the  
90 abundance or distribution of suitable floral resources or brood parasitism rates. Also, as bee  
91 emergence is related to temperature, the urban heat island effect is a possible explanation for  
92 differences in sex distribution across an urban-to-rural gradient. Development of bees may be  
93 accelerated in more urbanized areas, where temperatures are higher than in the surrounding  
94 landscape (Bartomeus et al., 2011, Kim, 1992). Fitch et al. (2019) claimed that sex allocation or  
95 dispersal-based theories may explain male predominance due to the influence of urbanization for  
96 medium and large bees. These results proclaimed that previously documented negative effects of  
97 urbanization on bees may underestimate the full impact of urbanization, and highlight the need  
98 for improved understanding of bee population functioning in urban environments.

99 The aims of this research were to compare influence of three types of the environments  
100 (urban, suburban and rural) on (1) the sex ratio of the first generation of *O. bicornis* offspring and  
101 (2) its size (considering the sex of the offspring). We also analyzed reliability of the size  
102 (volume) and weight of the cocoon and location of its brood chamber in the nesting tube in  
103 predicting the sex of *O. bicornis*.



## 104 MATERIALS AND METHODS

### 105 *Osmia bicornis* sites

106 The cocoons with *O. bicornis* originally came from the population of the National  
107 Institute of Horticultural Research, Puławy, Poland (51°40'N 21°96'E). Five wooden nesting  
108 constructions of 45 x 45 x 45 cm dimensions on the wooden frame 0.5 m above ground level with  
109 protective net in the front (meshes of 2 cm each) were settled. As nesting material 700 dry reed  
110 tubes of randomly chosen inner diameters from 5 to 9 mm, obliquely cut, with nodes at one end  
111 were used. In order to compare results from different types of environments, five study sites  
112 within the Lublin region were chosen to set up nesting constructions. The chosen study sites were  
113 assigned to categories of an urbanization gradient as follows: urban\_1 (51°14'N, 22°32'E) and  
114 urban\_2 (51°14'N, 22°30'E) [90% of built up infrastructure like buildings, roads, sidewalks, etc.],  
115 suburban\_1 (51°14'N, 22°29'E) and suburban\_2 (51°17'N, 22°42'E) [55-65% of built up  
116 infrastructure], and rural [up to 20% of built up infrastructure]. Exactly 500 randomly chosen  
117 cocoons of mixed sex were placed in carton boxes inside wooden constructions in March, and  
118 three weeks later after emergence of bees, empty cocoons were removed in order to eradicate  
119 potential parasites before they hatch.

### 120 *Collecting data*

121 When the foraging and nest establishing season of *O. bicornis* was over, in order not to  
122 disturb offspring development, the nesting material was left intact in all of the study sites. At the  
123 end of October, the nesting material from each construction was removed, placed in separate  
124 carton boxes with ventilation holes and stored in 4°C. Later from each site 50 reed tubes sealed  
125 by *O. bicornis* were randomly chosen to cut open. The contents of each brood chamber were  
126 verified and counted (see also Łoś et al., 2020), the cocoons were taken out, their width and

127 length was measured with an electronic digital caliper in millimeters to two decimal places (150  
128 mm / 0.01 mm accuracy 0.02 mm, Limit®, Poland), and their mass (in grams to four decimal  
129 places) was checked with an electronic analytical scale (Radwag AS 110.R2 [110 g / 0.1 mg],  
130 Poland, with fully automatic calibration). Afterward each cocoon was opened with entomological  
131 scissors (Paradox co., SCI 015, Poland) and offspring bees were removed from the inside of the  
132 cocoons. The sexual dimorphism characteristics of this species (see Figure 1 and Splitt et al.,  
133 2022) were used to identify the sex of the offspring and then each bee was weighed (Radwag AS  
134 110.R2 (110g / 0.1 mg), Poland).

### 135 *Statistical analysis*

136 The brood chambers of *O. bicornis* were numbered starting from the posterior end  
137 towards vestibulum (Fig. 5B) and the number of brood chambers per tube was counted. For  
138 further statistics only, data on cocoons with determined sex were used (parasited, dead larvae or  
139 empty cocoons were excluded). The volume of cocoons was calculated using the volume formula  
140 for an elongated rotating ellipsoid  $V = (4/3)\pi ab^2$  (see raw data in Supplementary Material 1).  
141 Finally, data from 215 tubes were analyzed (rural - 42; suburban\_1 - 40; suburban\_2 - 48;  
142 urban\_1 - 42; urban\_2 - 43; Supplementary Material 2) and the sex ratio of the offspring in each  
143 tube was expressed as a proportion of the females. Because the inner tube diameter may impact  
144 the sex ratio of the offspring (Ivanov, 2006; Radmacher & Strohm, 2010; Seidelmann et al.,  
145 2016), the differences of this feature among the study sites were tested with a simple linear  
146 model. The tubes had significantly lower inner diameter in suburban\_1 ( $\beta = -0.67$ , SE = 0.179, p  
147 < 0.001) and higher in suburban\_2 ( $\beta = 0.37$ , SE = 0.171, p = 0.03) comparing to the rural site,  
148 so this variable was included as a covariate in further models.

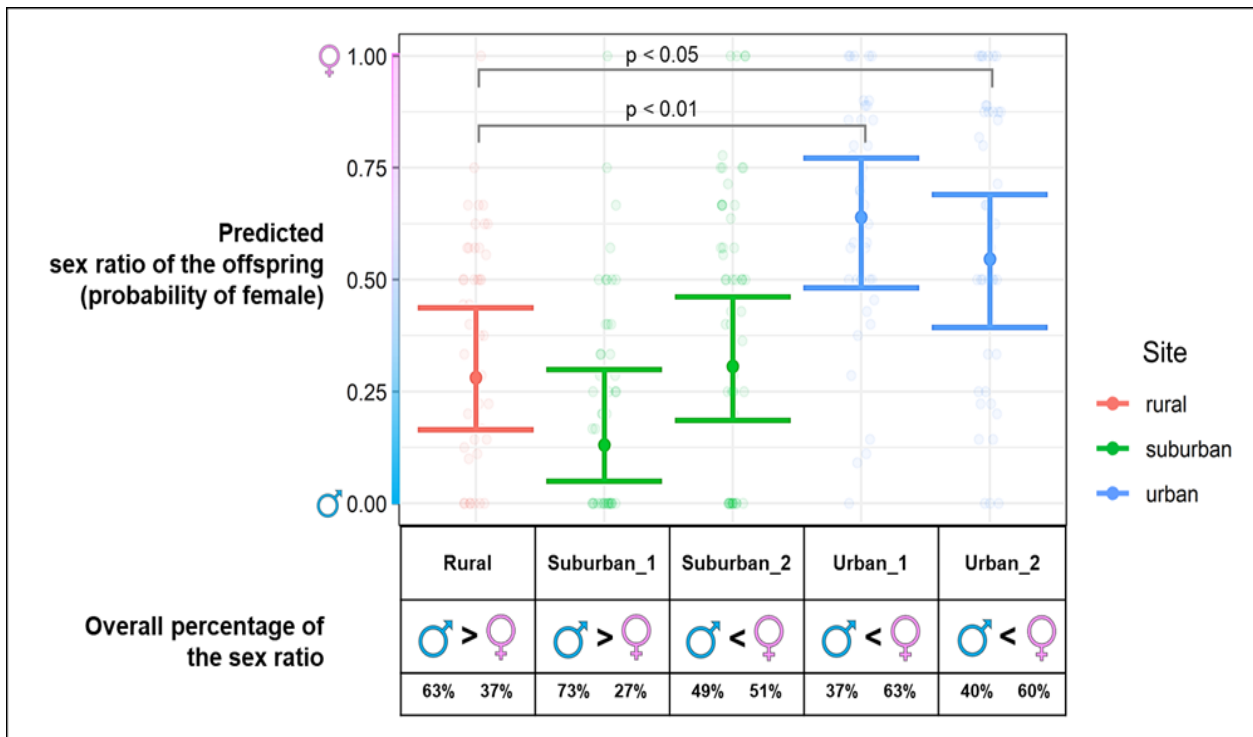
149 Three linear mixed models (LMM) with normal distribution and four generalized linear  
150 mixed models (GLMM) with binomial distribution were fitted, with either a cocoon and offspring  
151 measurements (mass, volume) or sex ratio of offspring and chamber content (male/female) as a  
152 response variable, respectively. Each model included tube inner diameter as a covariate and tube  
153 ID kept as a random factor. The models were built using “lme4” package (Bates et al., 2015) in R  
154 (R Core Team, 2021). GLMM1 explained the sex ratio of offspring in each tube using  
155 environment (site) as a predictor (categorical, with five levels: rural, suburban\_1, suburban\_2,  
156 urban\_1, urban\_2). LMM1, LMM2 and LMM3 explained total cocoon mass (mass of a cocoon  
157 with a bee inside), cocoon volume and offspring mass (respectively) using interaction of  
158 offspring sex and site as a predictor. To check the relationship between the position in the tube,  
159 site and the sex of the offspring the GLMM 2 was built with the interaction of subsequent brood  
160 chamber number and the site as a predictor. To compare the performance of the studied features  
161 in predicting *O. bicornis* offspring sex the next two models were built. GLMM3 was built to  
162 predict sex based on cocoon volume, position in the tube and the interaction of these traits with a  
163 site. GLMM4 was built to predict the sex based on total cocoon mass, the position in the tube and  
164 the interaction of these variables with the site. The GLMM3 and GLMM4 were compared using  
165 The Akaike information criterion (AIC).

## 166 **RESULTS**

167 The sex ratio of the *O. bicornis* offspring differed among rural and both urban sites, the  
168 latter having significantly more females (Table 1, Figure 2, Supplementary Material 2).

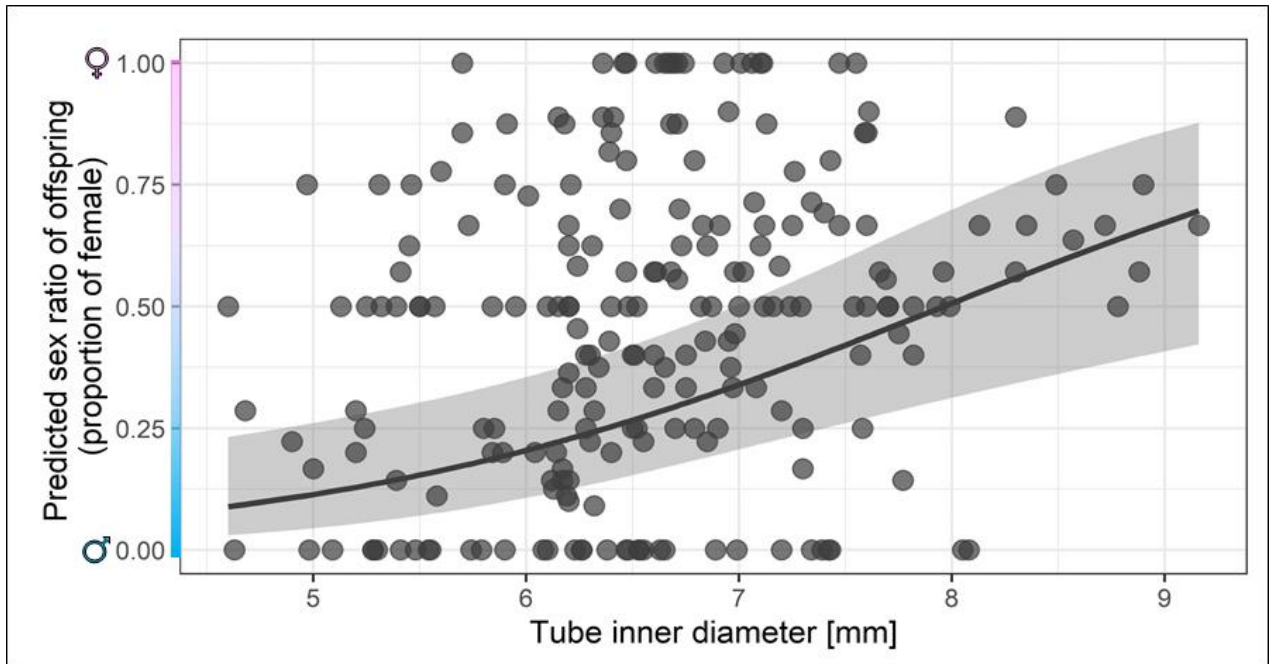
169 **Table 1.** The effects of the environment (study site) and inner tube diameter on the sex ratio of the  
 170 *Osmia bicornis* (syn. *O. rufa*) offspring. Tube ID was set as a random factor. The rural site is a  
 171 reference level for other term estimates. Statistically significant effects are emboldened and marked  
 172 with asterisks: \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ .

Explanatory variables	Response variable
	Sex ratio of offspring (proportion of females)
<i>GLMM estimates of slopes with standard errors (in brackets) for explanatory variables and assumed binomial response</i>	
Site: rural (intercept)	<b>-5.514 (1.445)***</b>
Site: suburban_1	-0.960 (0.645)
Site: suburban_2	0.123 (0.472)
Site: urban_1	<b>1.513 (0.481)**</b>
Site: urban_2	<b>1.123 (0.475)*</b>
Tube inner diameter [mm]	<b>0.693 (0.204)***</b>



173 **Figure 2.** Predicted sex ratio the *Osmia bicornis* (syn. *O. rufa*) offspring along the urbanization  
 174 gradient environments. The whiskers represent confidence intervals of estimated mean (bar  
 175 points) and transparent points represent original data.

176 The tube inner diameter also had an impact on sex ratio - the wider the tubes the higher  
177 probability that female eggs were laid (Table 1, Figure 3).

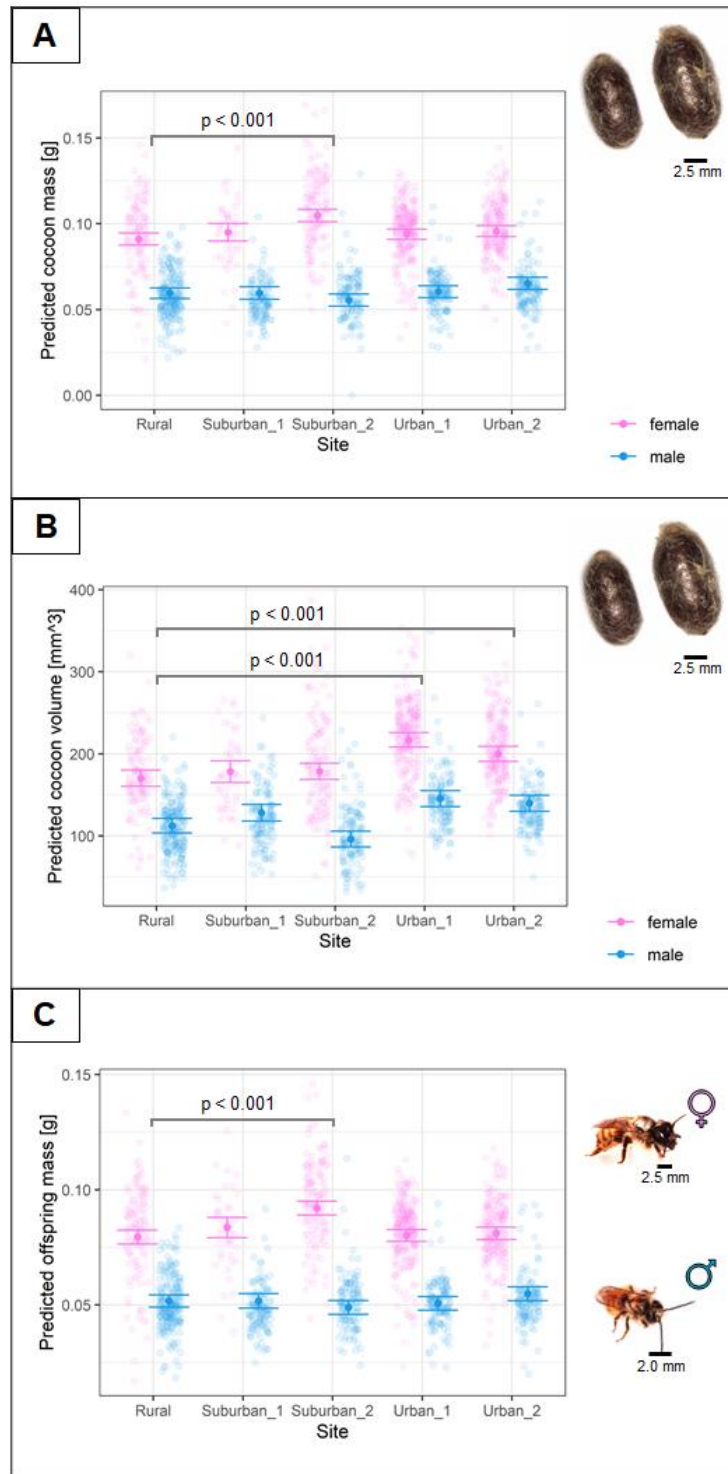


178 **Figure 3.** The association between the inner tube diameter on the *Osmia bicornis* (syn. *O. rufa*)  
179 and the offspring sex ratio. The predicted relationship is represented by a line, confidence  
180 intervals are represented by the shaded region, while points represent original data.

181 Sex of the offspring was highly correlated with the total cocoon mass and volume, as well  
182 as the offspring mass: offspring males were significantly smaller and lighter than females (Table  
183 2, Figure 4A-C). There were also differences among the sites: in suburban\_2 cocoons and  
184 offspring were heavier than in the rural site, and in both urban sites the cocoons had a greater  
185 volume in comparison to the rural site (Table 2). There was an interaction between offspring sex  
186 and site: in both suburban sites the differences between sexes in the total cocoon mass, volume  
187 and offspring mass were higher than in rural site (Table 2, Figure 4A-C). Bigger differences  
188 between males and females in cocoon volume as compared to the rural site were also noted in  
189 urban\_1 site (Table 2, Figure 4B). Tube inner diameter also had a positive effect on all the  
190 dependent variables (Table 2).

191 **Table 2.** The effects of *Osmia bicornis* (syn. *O. rufa*) offspring sex (brood chamber content), study  
 192 environment (site), inner tube diameter, and the interaction between offspring sex and study site  
 193 on the total cocoon mass (sum mass of cocoon and a bee), cocoon volume and offspring mass.  
 194 Tube ID was set as a random factor. The female sex and rural site are a reference level for other  
 195 terms estimates. Statistically significant effects are emboldened and marked with asterisks: \*\*\*p <  
 196 0.001, \*\*p < 0.01, \*p < 0.05.

Explanatory variables	Response variables		
	Total cocoon mass [g]	Cocoon volume [mm <sup>3</sup> ]	Offspring mass [g]
<i>LMM estimates of slopes with standard errors (in brackets) for explanatory variables and assumed linear response</i>			
(Intercept)	<b>0.063 (0.006)***</b>	<b>122.475 (17.216)***</b>	<b>0.055 (0.005)***</b>
Chamber content: male	<b>-0.032 (0.002)***</b>	<b>-57.658 (4.292)***</b>	<b>-0.028 (0.002)***</b>
Site: suburban_1	0.004 (0.003)	8.254 (8.481)	0.004 (0.003)
Site: suburban_2	<b>0.014 (0.003)***</b>	8.571 (7.084)	<b>0.013 (0.002)***</b>
Site: urban_1	0.003 (0.002)	<b>46.911 (6.742)***</b>	0.001 (0.002)
Site: urban_2	0.005 (0.002)	<b>29.819 (6.860)***</b>	0.002 (0.002)
Tube inner diameter [mm]	<b>0.004 (0.001)***</b>	<b>7.188 (2.441)**</b>	<b>0.004 (0.001)***</b>
Male*suburban_1	-0.004 (0.003)	7.559 (7.606)	-0.004 (0.003)
Male*suburban_2	<b>-0.018 (0.003)***</b>	<b>-25.090 (6.275)***</b>	<b>-0.015 (0.002)***</b>
Male*urban_1	-0.002 (0.002)	<b>-13.936 (5.939)*</b>	-0.002 (0.002)
Male*urban_2	0.001 (0.003)	-2.553 (6.208)	0.001 (0.002)



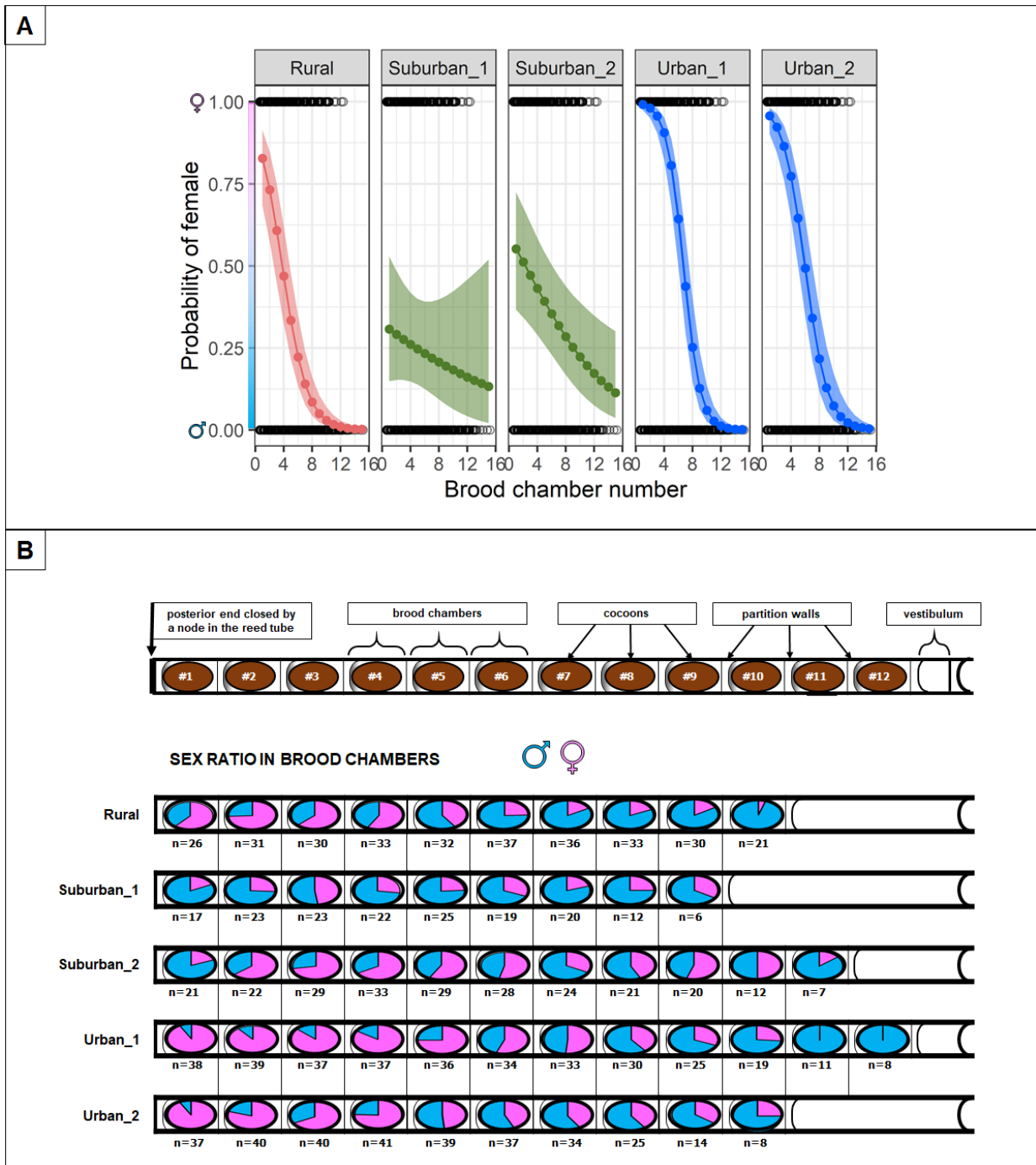
197 **Figure 4.** Size (volume), mass of the cocoons and mass of *Osmia bicornis* (syn. *O. rufa*)  
 198 offspring along the urbanization gradient: A - Predicted male and female cocoon mass; B -  
 199 Predicted male and female cocoon volume; C - Predicted male and female offspring mass. The  
 200 whiskers represent confidence intervals of the estimated mean (bar points) and transparent points  
 201 represent original data.

202 The last analyzed variable, brood chamber position, turned out to be highly correlated  
 203 with the sex of the offspring - the chambers located from the posterior end had higher probability  
 204 of the offspring being female (Table 3, Figure 5A-B). There was also significant interaction  
 205 between brood chamber position and site (Table 3). The probability of finding females at the  
 206 posterior end in suburban \_1 and suburban\_2 was much lower than in other sites and decreased  
 207 towards the vestibulum less rapidly (Figure 5A). In contrast, in urban\_1 the probability of female  
 208 offspring decreases towards the vestibulum faster than in rural site (Table 3, Figure 5A).  
 209 Moreover, all of the suburban and urban sites differ significantly from the rural in the term of sex  
 210 distribution: in suburban sites probability of female offspring occurrence was lower, whereas in  
 211 urban sites was higher than in rural site (Table 3). Tube inner diameter included in the model was  
 212 again positively associated with the probability of the offspring being female.

213 **Table 3.** The effects of the study environment (site), inner tube diameter, brood chamber position  
 214 (number) and its interaction with the study site on the sex ratio of the *Osmia bicornis* (syn. *O. rufa*)  
 215 offspring expressed as the probability of being a female. Tube ID was set as a random factor. The  
 216 rural site is a reference level for other term estimates. Statistically significant effects are  
 217 emboldened and marked with asterisks: \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ .

Explanatory variables	Response variables
	Chamber content (probability of female)
<i>GLMM estimates of slopes with standard errors (in brackets) for explanatory variables and assumed binomial response</i>	
Site: rural (intercept)	-2.280 (1.249)
Site: suburban_1	<b>-2.870 (0.707)***</b>
Site: suburban_2	<b>-1.762 (0.621)**</b>
Site: urban_1	<b>3.478 (0.781)***</b>
Site: urban_2	<b>1.604 (0.683)*</b>
Tube inner diameter [mm]	<b>0.666 (0.176)***</b>
Brood chamber number	<b>-0.564 (0.069)***</b>
Brood chamber number*suburban_1	<b>0.488 (0.112)***</b>
Brood chamber number*suburban_2	<b>0.402 (0.088)***</b>
Brood chamber number*urban_1	<b>-0.273 (0.109)*</b>
Brood chamber number*urban_2	-0.063 (0.102)



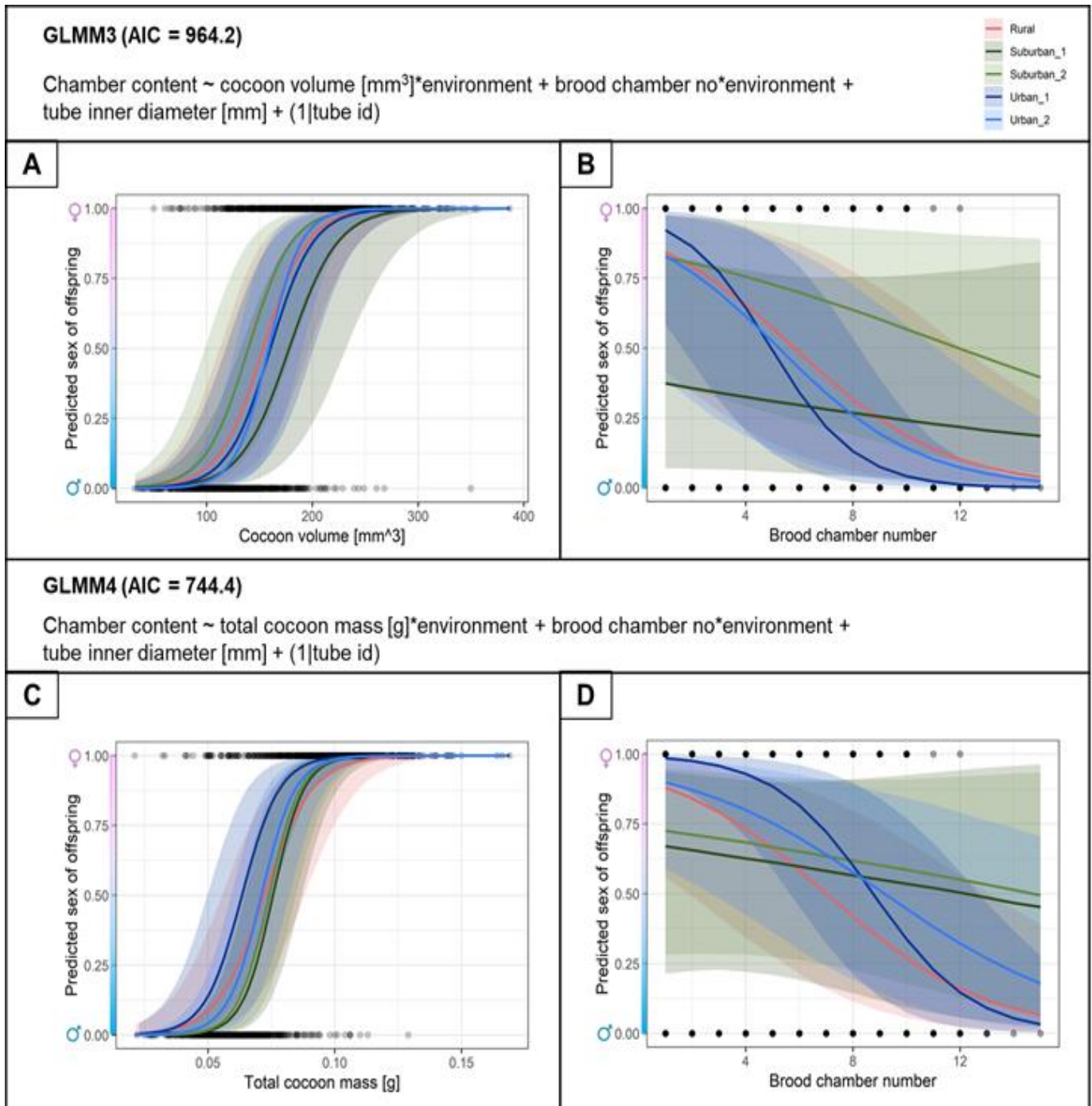


218 **Figure 5.** The sex distribution of the *Osmia bicornis* (syn. *O. rufa*) offspring in the subsequent  
 219 brood chambers of nesting tubes: A - Predicted probability of female occurrence in subsequent  
 220 brood chambers in each site (the predicted relationship is represented by line, confidence  
 221 intervals are represented by the shaded region, black points represent original data); B -  
 222 Percentage distribution of offspring sex in tubes in each site based on raw data.

223           When the combinations of features that potentially allow sex identification were  
224 compared, the model that included total cocoon weight, the position in the tube (brood chamber  
225 number) and site showed a better fit than the model with cocoon volume and the position in the  
226 tube (GLMM3 AIC = 964.2, GLMM4 AIC = 744.4). Brood chamber position was a significant  
227 predictor of offspring sex in both models, but in GLMM3 it had significant interactions with sites  
228 suburban\_1, suburban\_2 and urban\_1 (Table 4, Fig. 6BD). Cocoon volume in GLMM3 and total  
229 cocoon mass in GLMM4 also showed significant association with offspring sex, but only cocoon  
230 mass showed a significant interaction with a site (Table 4). For smaller cocoon mass in  
231 suburban\_1, suburban\_2 and urban\_2 the probability of offspring being female was lower than in  
232 rural site but for higher cocoon mass the probability was higher (Fig. 6C). There was also  
233 significant association between site and sex ratio: model with cocoon volume (GLMM3)  
234 predicted lower probability of female offspring in suburban\_1, whereas model with cocoon mass  
235 (GLMM4) predicted higher probability of female offspring in urban\_1 than in other sites (Table  
236 4).

237 **Table 4.** The comparison of the two GLMM models predicting the brood chamber content  
 238 (*Osmia bicornis*, syn. *O. rufa* offspring sex expressed as the probability of female occurrence)  
 239 using either brood chamber position (number) and cocoon volume or brood chamber position  
 240 (number) and total cocoon mass depending on the breeding environment (site). Inner tube  
 241 diameter was included as a covariate and tube ID was set as a random factor. The rural site is a  
 242 reference level for other terms estimates. Statistically significant effects are emboldened and  
 243 marked with asterisks: \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ .

Explanatory variables	Response variable: Chamber content (probability of female)	
	Model 1:	Model 2:
	Chamber content ~ cocoon volume [mm <sup>3</sup> ]*environment + brood chamber position*environment + tube inner diameter [mm] + (1 tube id)	Chamber content ~ total cocoon mass [g]*environment + brood chamber position*environment + tube inner diameter [mm] + (1 tube id)
<i>GLMM estimates of slopes with standard errors (in brackets) for explanatory variables with assumed binomial response</i>		
(Intercept)	-0.628 (1.193)	0.703 (1.287)
Site: suburban_1	<b>-1.012 (0.481)*</b>	-0.161 (0.527)
Site: suburban_2	0.752 (0.457)	0.130 (0.454)
Site: urban_1	-0.388 (0.448)	<b>1.325 (0.438)**</b>
Site: urban_2	-0.211 (0.432)	0.496 (0.417)
Tube inner diameter [mm]	0.144 (0.169)	-0.126 (0.186)
Brood chamber number	<b>-1.019 (0.213)***</b>	<b>-0.953 (0.225)***</b>
Brood chamber number*suburban_1	<b>0.815 (0.369)*</b>	0.720 (0.470)
Brood chamber number*suburban_2	<b>0.606 (0.303)*</b>	0.733 (0.380)
Brood chamber number*urban_1	<b>-0.793 (0.339)*</b>	-0.620 (0.354)
Brood chamber number*urban_2	-0.081 (0.329)	0.202 (0.343)
Cocoon volume [mm <sup>3</sup> ]	<b>2.821 (0.390)***</b>	-
Cocoon volume [mm <sup>3</sup> ]*suburban_1	-0.241 (0.564)	-
Cocoon volume [mm <sup>3</sup> ]*suburban_2	0.063 (0.513)	-
Cocoon volume [mm <sup>3</sup> ]*urban_1	0.088 (0.533)	-
Cocoon volume [mm <sup>3</sup> ]*urban_2	0.945 (0.607)	-
Total cocoon mass [g]	-	<b>2.683 (0.345)***</b>
Total cocoon mass [g]*suburban_1	-	<b>1.483 (0.706)*</b>
Total cocoon mass [g]*suburban_2	-	<b>1.485 (0.625)*</b>
Total cocoon mass [g]*urban_1	-	0.758 (0.537)
Total cocoon mass [g]*urban_2	-	<b>1.132 (0.549)*</b>
AIC	964.2	744.4



244 **Figure 6.** The relationships between predictor variables - cocoon volume, total cocoon mass and  
 245 brood chamber number (position in tube) and the probability of the *Osmia bicornis* (syn. *O. rufa*)  
 246 offspring sex (0 - 100% male, 1 - 100% female) in different experimental sites compared in two  
 247 models. Lower AIC of GLMM4 model indicates better fit. The formula of each model is  
 248 provided on the chart. The predicted relationship is represented by line, confidence intervals are  
 249 represented by the shaded region, black points represent original data.

250 **DISCUSSION**

251 For the red mason bee having daughters may be a bigger investment than having sons.  
252 The high proportion of female offspring in the urban environment suggests there are favorable  
253 living conditions for this species. The sex ratio of *Osmia* is associated with bee size, so it is worth  
254 considering these two aspects alongside (Bosch & Vicens, 2006; Greenleaf, et al., 2007;  
255 Seidelmann, 2006; Seidelmann et al., 2010).

256 Predominance of sons can be expected when the bee nest is threatened by predators or  
257 parasites. Firstly, at the beginning of the season, as the bees are haplodiploid, the females need to  
258 collect sperm to produce daughters throughout their whole lives, whereas the possibility of  
259 producing sons is not dependent on mating (Sedivy & Dorn, 2014; Seidelmann, 2015). If females  
260 are threatened by predators or parasites or other bee species occupying the same environment  
261 during nuptials they may not receive satisfactory post-copulatory display and may try to mate  
262 again before laying eggs (Seidelmann, 2014). Secondly, later on the season when a nest is at  
263 higher risk of parasite invasion the female bee may prefer to stay close to the nest, distribute  
264 brood cells to higher number of tubes, collect smaller portions of pollen and hence shift the sex  
265 ratio of offspring towards haploid sons (Seidelmann, 2006, Seidelmann et al., 2010). Indeed *O.*  
266 *bicornis* populations placed in the city are rarely attacked by parasites, whereas this was  
267 predominantly the case in suburban and rural areas (Łoś et al., 2020). Also, Zajdel et al. (2021)  
268 found parasites and kleptoparasites occupying almost three times more brood chambers in nests  
269 located in suburbs, than in other habitats. Thus, urban areas may be an environmental filter  
270 releasing bee populations from various nest parasites. This may be the cause of shifting the sex  
271 ratio of offspring in urbanized areas towards females. Also, the presence of parasites may  
272 indirectly affect the size of bees in the next generation. In that case females may make fewer  
273 foraging flights or / and collect smaller pollen loads, and larvae get less food and both the

274 daughters and the sons may be smaller than in populations with low prevalence of parasites. This  
275 is also in line with our results as both females and males from urban sites were bigger than the  
276 ones from suburban and rural sites.

277         A second important factor influencing the offspring sex ratio, is the food abundance in the  
278 environment. In order to feed daughter larvae a female has to collect a bigger pollen load and  
279 make more foraging flights than for male larvae (Radmacher & Strohm, 2010; Seidelmann,  
280 2006). Therefore, plant species richness and pollen abundance may be a limiting factor for  
281 solitary bees. Polylectic bees, such as *O. bicornis*, opportunistically collect pollen from plants  
282 present in the environment. However, there were no shortages in resources in studied  
283 environments (Splitt et al., 2021b). If competition for food is very high females will be unable to  
284 collect enough pollen for their offspring even in the environment with abundant food resources.  
285 As there is lower diversity of bee species in urban environments than in more natural habitats  
286 (Hernandez et al., 2009) it is reasonable to suspect that this limited inter-specific competition has  
287 enabled *O. bicornis* unrestricted pollen foraging in the city. This abundance of supply may have  
288 been a factor in shifting the offspring sex ratio towards daughters and better provisioning of all  
289 larvae, so that later the whole generation was larger than in other environments.

290         When considering the sex ratio of mason bees, the availability, type and diameter of the  
291 nesting material should be considered. Bees from all urban-to-rural sites had access to the same  
292 variety of substrates with randomized range of diameters (see subsection *Osmia bicornis sites* in  
293 ‘Materials and methods’) in the extent that they may use (Gruber et al., 2011; Ivanov, 2006;  
294 Radmacher & Strohm, 2010; Seidelmann et al., 2016). Based on the results, it can be concluded  
295 that when bees have access to a large number of different diameters of nesting substrate, they  
296 decide on a basis of the intended sex of the offspring, which tube they use. It was found that  
297 female offspring was laid in tubes of higher inner diameter than male offspring, which is in line

298 with other reports (Ivanov, 2006; Radmacher & Strohm, 2010; Seidelmann et al., 2016).  
299 Interestingly, *O. bicornis* chose tubes of bigger diameter in urban areas where it had a higher  
300 percentage of daughters and tubes of smaller diameter where it preferred to have sons. While  
301 various studies examine the choice of nesting material and the mason bees' preferred diameter,  
302 the sex ratio of the offspring is often omitted (e.g. Michořap et al., 2020; Wilkaniec & Giejdasz,  
303 2003).

304 Both the size of *O. bicornis* females and males fit in an extended size range (Splitt et al.,  
305 2022). Therefore, some populations can be expected to contain very large males or very small  
306 females. This study showed that when a female produces more daughters, they have also bigger  
307 mass than in other cases. At the same time, the sons were probably also supplied with more  
308 generous pollen loads, which enabled them to reach larger sizes and reduce the gender gap. This  
309 in turn may affect the sex ratio in subsequent generations of bees. According to Bosch & Vicens  
310 (2006) small females biased their investment toward males. Moreover, it may also affect the  
311 dispersion of bees in the environment, as large females are more likely to establish their nest at  
312 the release site, probably in relation to size-dependent vigor at emergence. On the other hand,  
313 movement and dispersal distance in bees is strongly correlated with body size; larger specimens  
314 are more likely to disperse sufficiently far from their natal nest to reach resource patches in urban  
315 landscapes (Greenleaf et al., 2007; Fitch et al., 2009). Zuburchen et al. (2009) also confirmed that  
316 maximum foraging distance is smaller for smaller bee species. These findings suggest that a close  
317 neighborhood of nesting and foraging habitat is crucial to maintain populations of small  
318 pollinators.

319 As *O. bicornis* is a manageable bee species, and females are believed to be more effective  
320 pollinators than males, their prevalence in the population is considered desirable (Kronic &  
321 Stanisavljevic, 2006). There are algorithms that allow breeders to estimate how many individuals

322 - specifically how many *O. bicornis* females - they need to provide for the pollination of different  
323 species of mass flowering plants (especially orchards of different fruit species, like apples, pears,  
324 sweet and sour cherries, etc.) (Biodar). Furthermore, scientists also specifically select and include  
325 females for research (Sgolastra et al., 2017; Sgolastra et al., 2018; Azpiazu et al., 2019). This has  
326 forced the need to identify the sex of an individual by some easily measurable parameters. Many  
327 sources (e.g. Bosch & Kemp, 2001; Mader et al., 2010) suggest considering the size of the  
328 cocoon and its placement in the tube as reliable measures to indicate the sex of the individual. It  
329 is believed that as the eggs of females are laid early in the season their placement is deep in the  
330 tube (closer to the node), whereas male eggs due to their protandry are placed closer to the nest  
331 entrance. Also, cocoon size is considered a reliable sex indicator, as females are usually larger  
332 than males. We tested different combinations of traits and indeed the placement in the tube  
333 together with the weight of the cocoons can be a good determinant of the bee sex. However, our  
334 research shows that it is also desirable to consider the impact of the environment and to set  
335 typical nesting parameters for a particular site.

## 336 **CONCLUSIONS**

337       Based on the shift in the sex ratio towards having more daughters in the offspring, it can  
338 be concluded that the urban environment creates favorable conditions for the development of *O.*  
339 *bicornis*. Yet, it is worth considering further studies on a larger scale to better understand this  
340 phenomenon. The model that includes total cocoon weight, the position in the tube (brood  
341 chamber number) and site shows the best offspring sex prediction. This is an important hint for  
342 traders and researchers who try to identify sex without opening the cocoon, although further  
343 analysis on bigger data is needed to develop clear guidelines.



344 **DATA STATEMENT**

345 The raw source data are included as an online supplement to this paper (Supplementary Material  
346 1).

347 **REFERENCES**

- 348 1. Azpiazu, C., Bosch, J., Viñuela, E., Medrzycki, P., Teper, D., & Sgolastra, F. (2019).  
349 Chronic oral exposure to field-realistic pesticide combinations via pollen and nectar: effects  
350 on feeding and thermal performance in a solitary bee. *Scientific Reports*, 9(1), 1-11.  
351 <https://doi.org/10.1038/s41598-019-50255-4>
- 352 2. Banaszak, J., & Romasenko, L. (1998). Megachilid bees of Europe (Hymenoptera, Apoidea,  
353 Megachilidae). Wydawnictwo Bydgoszcz University.
- 354 3. Banaszak-Cibicka, W., & Żmihorski, M. (2012). Wild bees along an urban gradient: winners  
355 and losers. *Journal of Insect Conservation*, 16(3), 331-343. [https://doi.org/10.1007/s10841-](https://doi.org/10.1007/s10841-011-9419-2)  
356 [011-9419-2](https://doi.org/10.1007/s10841-011-9419-2)
- 357 4. Bartomeus, I., Ascher, J. S., Wagner, D., Danforth, B. N., Colla, S., Kornbluth, S., &  
358 Winfree, R. (2011). Climate-associated phenological advances in bee pollinators and bee-  
359 pollinated plants. *Proceedings of the National Academy of Sciences*, 108(51), 20645-20649.  
360 <https://doi.org/10.1073/pnas.1115559108>
- 361 5. Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models  
362 using lme4. *Journal of Statistical Software*, 67(1), 1-48. <https://doi.org/10.18637/jss.v067.i01>
- 363 6. Bessa-Gomes, C., Legendre, S., & Clobert, J. (2004). Allee effects, mating systems and the  
364 extinction risk in populations with two sexes. *Ecology Letters*, 7(9), 802-812.  
365 <https://doi.org/10.1111/j.1461-0248.2004.00632.x>
- 366 7. BIODAR (accessed 8th June 2022)  
367 [https://www.biodar.com.pl/EN/oferta\\_murarka\\_ogrodowa.php?page=murarka\\_ilosci](https://www.biodar.com.pl/EN/oferta_murarka_ogrodowa.php?page=murarka_ilosci)
- 368 8. Boomsma, J. J., Baer, B., & Heinze, J. (2005). The evolution of male traits in social insects.  
369 *Annual Review of Entomology*, 50, 395-420.  
370 <https://doi.org/10.1146/annurev.ento.50.071803.130416>
- 371 9. Bosch, J., & Kemp, W. P. (2001). How to manage the blue orchard bee. In *Systematics*.

- 372 10. Bosch, J., & Vicens, N. (2006). Relationship between body size, provisioning rate, longevity  
373 and reproductive success in females of the solitary bee *Osmia cornuta*. *Behavioral Ecology*  
374 *and Sociobiology*, 60(1), 26-33. <https://doi.org/10.1007/s00265-005-0134-4>
- 375 11. Cane, J. H., Minckley, R. L., Kervin, L. J., Roulston, T. A. H., & Williams, N. M. (2006).  
376 Complex responses within a desert bee guild (Hymenoptera: Apiformes) to urban habitat  
377 fragmentation. *Ecological Applications*, 16(2), 632-644. [https://doi.org/10.1890/1051-  
378 0761\(2006\)016\[0632:CRWADB\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2006)016[0632:CRWADB]2.0.CO;2)
- 379 12. Fitch, G., Glaum, P., Simao, M. C., Vaidya, C., Matthijs, J., Iuliano, B., & Perfecto, I.  
380 (2019). Changes in adult sex ratio in wild bee communities are linked to urbanization.  
381 *Scientific Reports*, 9(1), 1-10. <https://doi.org/10.1038/s41598-019-39601-8>
- 382 13. Fliszkiewicz, M., & Wilkaniec, Z. (2009). Potential possibilities of insemination of mason  
383 bee (*Osmia rufa* L.) females by a single male in laboratory conditions. *Annals of Warsaw*  
384 *University of Life Sciences—SGGW: Animal Science*, 46, 51-58.
- 385 14. Fortel, L., Henry, M., Guilbaud, L., Guirao, A. L., Kuhlmann, M., Mouret, H., ... &  
386 Vaissière, B. E. (2014). Decreasing abundance, increasing diversity and changing structure  
387 of the wild bee community (Hymenoptera: Anthophila) along an urbanization gradient. *PloS*  
388 *one*, 9(8), e104679. <https://doi.org/10.1371/journal.pone.0104679>
- 389 15. Greenleaf, S. S., Williams, N. M., Winfree, R., & Kremen, C. (2007). Bee foraging ranges  
390 and their relationship to body size. *Oecologia*, 153(3), 589-596.  
391 <https://doi.org/10.1007/s00442-007-0752-9>
- 392 16. Gruber, B., Eckel, K., Everaars, J., & Dormann, C. F. (2011). On managing the red mason  
393 bee (*Osmia bicornis*) in apple orchards. *Apidologie*, 42(5), 564-576.  
394 <https://doi.org/10.1007/s13592-011-0059-z>
- 395 17. Hansted, L., Grout, B. W., Toldam-Andersen, T. B., & Eilenberg, J. (2014). An assessment  
396 of *Osmia rufa* (syn. *bicornis*) as a pollinator of the sour cherry (*Prunus cerasus*) cv.  
397 Stevnsbaer in eastern Denmark. *Journal of Apicultural Research*, 53(1), 177-182.  
398 <https://doi.org/10.3896/IBRA.1.53.1.20>
- 399 18. Hernandez, J. L., Frankie, G. W., & Thorp, R. W. (2009). Ecology of urban bees: a review of  
400 current knowledge and directions for future study. *Cities and the Environment (CATE)*, 2(1),  
401 3.

- 402 19. Holzschuh, A., Dudenhöffer, J. H., & Tschardtke, T. (2012). Landscapes with wild bee  
403 habitats enhance pollination, fruit set and yield of sweet cherry. *Biological Conservation*,  
404 153, 101-107. <https://doi.org/10.1016/j.biocon.2012.04.032>
- 405 20. Kim, H. H. (1992). Urban heat island. *International Journal of Remote Sensing*, 13(12),  
406 2319-2336.
- 407 21. Kronic, M., & Stanisavljevic, L. (2006). Population management in the mason bee species  
408 *Osmia cornuta* and *O. rufa* for orchard pollination in Serbia (Hymenoptera: Megachilidae).  
409 *Entomologia Generalis*, 29(1), 27-38.
- 410 22. Łoś, A., Skórka, P., Strachecka, A., Winiarczyk, S., Adaszek, Ł., Winiarczyk, M., & Wolski,  
411 D. (2020). The associations among the breeding performance of *Osmia bicornis* L.  
412 (Hymenoptera: Megachilidae), burden of pathogens and nest parasites along urbanisation  
413 gradient. *Science of the Total Environment*, 710, 135520.  
414 <https://doi.org/10.1016/j.scitotenv.2019.135520>
- 415 23. Mader, E., Spivak, M., & Evans, E. (2010). Managing alternative pollinators (1th ed.).  
416 NRAES.
- 417 24. Michoła, P., Cierpisz, M., Sikora, A., & Sikora, M. (2020). Nesting effectiveness of red  
418 mason bee *Osmia rufa* (L.) in reed stalk *Phragmites australis* (Cav.). *Journal of Apicultural*  
419 *Science*, 64(2), 345-354. <https://doi.org/10.2478/jas-2020-0031>
- 420 25. Müller, A. (2022), Palearctic Osmiine Bees, ETH Zürich, <http://blogs.ethz.ch/osmiini>
- 421 26. Murawska, A., Migdał, P., Zajdel, B., Popiela, E., & Roman, A. (2021). The composition of  
422 red mason bee cocoons. *Journal of Apicultural Research*, 61(2), 227-232.  
423 <https://doi.org/10.1080/00218839.2021.1988278>
- 424 27. Ne'eman, G., Shavit, O., Shaltiel, L., & Shmida, A. (2006). Foraging by male and female  
425 solitary bees with implications for pollination. *Journal of Insect Behavior*, 19(3), 383-401.  
426 <https://doi.org/10.1007/s10905-006-9030-7>
- 427 28. Paxton, R. (2005). Male mating behaviour and mating systems of bees: An overview.  
428 *Apidologie*, 36(2), 145-156. <https://doi.org/10.1051/apido:2005007>
- 429 29. Praz, C. J., Müller, A., Danforth, B. N., Griswold, T. L., Widmer, A., & Dorn, S. (2008).  
430 Phylogeny and biogeography of bees of the tribe Osmini (Hymenoptera: Megachilidae).  
431 *Molecular Phylogenetics and Evolution*, 49(1), 185-197.  
432 <https://doi.org/10.1016/j.ympev.2008.07.005>

- 433 30. R Core Team (2021) R: A language and environment for statistical computing. Vienna,  
434 Austria. URL <https://www.R-project.org/>
- 435 31. Radmacher, S. (2011). Temperature effects on the development in the solitary bee *Osmia*  
436 *bicornis* (Hymenoptera, Megachilidae) - Development time, brain microstructure and  
437 cognitive abilities [Doctoral dissertation]. University of Regensburg. [https://epub.uni-](https://epub.uni-regensburg.de/)  
438 [regensburg.de/](https://epub.uni-regensburg.de/)
- 439 32. Radmacher, S., & Strohm, E. (2010). Factors affecting offspring body size in the solitary bee  
440 *Osmia bicornis* (Hymenoptera, Megachilidae). *Apidologie*, 41(2), 169-177.  
441 <https://doi.org/10.1051/apido/2009064>
- 442 33. Raw, A. (1972). The biology of the solitary bee *Osmia rufa* (L.) (Megachilidae).  
443 *Transactions of the Royal Entomological Society of London*, 124(3), 213-229.
- 444 34. Rust, R., Torchio, P., & Trostle, G. (1989). Late embryogenesis and immature development  
445 of *Osmia rufa cornigera* (Rossi) (Hymenoptera: Megachilidae). *Apidologie*, 20(4), 359-367.  
446 <https://doi.org/10.1051/apido:19890408>
- 447 35. Schindler, M., & Peters, B. (2011). Mason bees *Osmia bicornis* and *Osmia cornuta* as  
448 suitable orchard pollinators. *Erwerbs-Obstbau*, 52(3-4), 111-116.  
449 <https://doi.org/10.1007/s10341-010-0118-z>
- 450 36. Sedivy, C., & Dorn, S. (2014). Towards a sustainable management of bees of the subgenus  
451 *Osmia* (Megachilidae; *Osmia*) as fruit tree pollinators. *Apidologie*, 45(1), 88-105.  
452 <https://doi.org/10.1007/s13592-013-0231-8>
- 453 37. Seidelmann, K. (1999a). The function of the vestibulum in nests of a solitary stem-nesting  
454 bee, *Osmia rufa* (L.). *Apidologie*, 30(1), 19-29. <https://doi.org/10.1051/apido:19990103>
- 455 38. Seidelmann, K. (1999b). The race for females: The mating system of the red mason bee,  
456 *Osmia rufa* (L.) (Hymenoptera: Megachilidae). *Journal of Insect Behavior*, 12(1), 13-25.  
457 <https://doi.org/10.1023/A:1020920929613>
- 458 39. Seidelmann, K. (2006). Open-cell parasitism shapes maternal investment patterns in the red  
459 mason bee *Osmia rufa*. *Behavioral Ecology*, 17(5), 839-848.  
460 <https://doi.org/10.1093/beheco/arl017>
- 461 40. Seidelmann, K. (2014). Optimal progeny body size in a solitary bee, *Osmia bicornis*  
462 (Apoidea: Megachilidae). *Ecological Entomology*, 39(5), 656-663.  
463 <https://doi.org/10.1111/een.12145>

- 464 41. Seidelmann, K. (2015). Double insurance of paternity by a novel type of mating plug in a  
465 monandrous solitary mason bee *Osmia bicornis* (Hymenoptera: Megachilidae). *Biological*  
466 *Journal of the Linnean Society*, 115(1), 28-37. <https://doi.org/10.1111/bij.12472>
- 467 42. Seidelmann, K. (2018). Optimal resource allocation, maternal investment, and body size in a  
468 solitary bee, *Osmia bicornis*. *Entomologia Experimentalis et Applicata*, 166(9), 790-799.  
469 <https://doi.org/10.1111/eea.12711>
- 470 43. Seidelmann, K., Bienenach, A., & Pröhl, F. (2016). The impact of nest tube dimensions on  
471 reproduction parameters in a cavity nesting solitary bee, *Osmia bicornis* (Hymenoptera:  
472 Megachilidae). *Apidologie*, 47(1), 114-122. <https://doi.org/10.1007/s13592-015-0380-z>
- 473 44. Seidelmann, K., Ulbrich, K., & Mielenz, N. (2010). Conditional sex allocation in the red  
474 mason bee, *Osmia rufa*. *Behavioral Ecology and Sociobiology*, 64(3), 337-347.  
475 <https://doi.org/10.1007/s00265-009-0850-2>
- 476 45. Sgolastra, F., Medrzycki, P., Bortolotti, L., Renzi, M. T., Tosi, S., Bogo, G., ... & Bosch, J.  
477 (2017). Synergistic mortality between a neonicotinoid insecticide and an ergosterol-  
478 biosynthesis-inhibiting fungicide in three bee species. *Pest Management Science*, 73(6),  
479 1236-1243. <https://doi.org/10.1002/ps.4449>
- 480 46. Sgolastra, F., Arnan, X., Cabbri, R., Isani, G., Medrzycki, P., Teper, D., & Bosch, J. (2018).  
481 Combined exposure to sublethal concentrations of an insecticide and a fungicide affect  
482 feeding, ovary development and longevity in a solitary bee. *Proceedings of the Royal Society*  
483 *B*, 285(1885), 20180887. <https://doi.org/10.1098/rspb.2018.0887>
- 484 47. Splitt, A., Schulz, M., & Skórka, P. (2022). Current state of knowledge on the biology and  
485 breeding of the solitary bee – *Osmia bicornis*. *Journal of Apicultural Research*, 1-17.  
486 <https://doi.org/10.1080/00218839.2021.1957610>
- 487 48. Splitt, A., Skórka, P., Strachecka, A., Borański, M., & Teper, D. (2021). Keep trees for bees:  
488 Pollen collection by *Osmia bicornis* along the urbanization gradient. *Urban Forestry &*  
489 *Urban Greening*, 64, 127250. <https://doi.org/10.1016/j.ufug.2021.127250>
- 490 49. Wilkaniec, Z., & Giejdasz, K. (2003). Suitability of nesting substrates for the cavity-nesting  
491 bee *Osmia rufa*. *Journal of Apicultural Research*, 42(3), 29-31.  
492 <https://doi.org/10.1080/00218839.2003.11101084>
- 493 50. Zajdel, B., Borański, M., Kucharska, K., & Teper, D. (2021). Reproduction and  
494 accompanying fauna of red mason bee *Osmia rufa* L. (syn. *Osmia bicornis* L.) in areas with

495 different levels of urbanization. *Journal of Apicultural Science*, 65(1), 123-137.

496 <https://doi.org/10.2478/JAS-2021-0009>

497 51. Zurbuchen, A., Landert, L., Klaiber, J., Müller, A., Hein, S., & Dorn, S. (2010). Maximum

498 foraging ranges in solitary bees: only few individuals have the capability to cover long

499 foraging distances. *Biological Conservation*, 143(3), 669-676.

500 <https://doi.org/10.1016/j.biocon.2009.12.003>

501 **Supplementary Material 1.** Raw data on the sex distribution of *Osmia bicornis* (syn. *O. rufa*)

502 [csv].

503 **Supplementary Material 2.** Descriptive statistics [docx].

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## Keep trees for bees: Pollen collection by *Osmia bicornis* along the urbanization gradient

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### ABSTRACT

Development of urban agglomerations and the intensification of agriculture profoundly affect bees' food resources, hence ecosystem services such as pollination. A solitary bee, *Osmia bicornis* (syn. *O. rufa*), is an effective springtime pollinator of crops, decorative and wild plants. However, it is largely unknown if this species is conservative or plastic in pollen collection in different environments. New breedings of *O. bicornis* were established in localizations qualified as urban (90 % of built-up infrastructure), suburban (55–65 %), and rural (up to 20 %). From each nest randomly chosen samples of unused pollen provisions were collected and analysed. Moreover, literature databases of food composition of *O. bicornis* was compiled to show overall tendencies in the choice of plant type, habitat, as well as pollen coating and size. Our field study showed that in the less human-modified environment *O. bicornis* collected higher diversity of pollen types to build its provision, compared to more urban areas (Simpson diversity index was 3.7 in rural, 2.8 in suburban and 2.2 in urban sites). Literature review showed that bees repeatedly collected pollen from commonly available trees like oaks, maples, horse chestnut and elms. Field data also revealed that the use of tree pollen was especially common in urban sites while bees from suburban and rural sites included pollen of herbaceous plants and shrubs. Neither the shape nor the size of the pollen mattered to bee foraging choices. However, bees frequently used pollen dispersed by wind in urban sites. The main conclusion is that polylectic bees opportunistically collect pollen of plants present in the environment and number of plant taxa may be limiting factor for studied bees. The welfare of *O. bicornis* requires planting trees such as oaks, willows, maples, and representatives from Rosaceae family, and it is especially advisable in urban sites where herbaceous flowering plants are less common than in urban and suburban areas. Hence, keeping even singular trees may complement the bee food base in urbanized areas.

### 1. Introduction

Many research, e.g. Belsky and Joshi (2019), report that there has been multitiered large-scale declines in bee abundance and species richness over the last decade. As one reason climatic alterations are mentioned, because they have disrupted synchronous bee emergence with flower blooming and reduced the availability of diverse floral resources. Also, urbanization, namely urban sprawl, often is pointed as having negative influence on insects' populations (Newman, 2016). All these phenomena may lead to impoverishing the diet of pollinating insects. Initially honeybees, because of their economic importance, appear to be of the greatest concerns, while it is wild bees that are more likely to

be adversely affected by shortages of food sources. Honeybees use a wide range of resources (pollen, nectar, resin) and also themselves produce e.g. royal jelly and store food in large quantities for the entire colony, whereas adult solitary bees use the flower resources on an ongoing basis and provide the offspring with food mainly in the form of pollen loads on which the larvae must feed themselves. While honeybees, as a managed species, might be fed by beekeepers when food is scarce, wild bees, which lead mostly solitary life and inhabit hollow plant stems or nest in the ground, are vulnerable to periods of starvation. It is particularly important as many solitary bees, such as *Osmia* in Europe are early-spring pollinators and their emerging period is just at the time when flower resources are scarce and only a limited number of

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plants produce pollen. This takes on added significance if we consider that in temperate regions solitary bees are considered among the best pollinators for most plants and significantly outperform honeybees in pollination effectiveness (Willmer et al., 2017). However, filling gaps in knowledge regarding solitary bees' use of resources may help to indicate most effective plants to enrich environment, especially an anthropogenized one. Following Ayers and Rehan (2021) it can be stated that green spaces like parks and urban gardens may be potential refuges for pollinators. Also, Majewska and Altizer (2020) indicate that pollinators responded positively to high plant species diversity and woody vegetation in gardens. These may be supported also by Donkersley (2019) statement that woody habitat features like trees and hedgerows provide more efficient resources for pollinators in a number of ways. Tree and hedgerow are more efficient forage targets due to absolute resource density and also could provide more optimised foraging landscapes for pollinators. These measures might augment population of pollinators, especially solitary bees from the genus *Osmia* (Bosch and Kemp, 2002; Haider et al., 2013).

Currently, *Osmia bicornis* L. (Hymenoptera: Megachilidae) (syn. *O. rufa*) (species description is in Material and Methods) is known as being polylectic / polylege bee, which means that they are pollen generalists collecting food resources from the flowers of a variety of plants (e.g. Cripps and Rust, 1989; Haider et al., 2013; Krunic and Stanisavljević, 2006). However, it has been noted that *O. bicornis* might show a local specialization for either a genus or family of flowering plants. *Osmia bicornis* may prefer trees especially *Quercus* spp., *Salix* spp. and *Rosaceae*, and among herbaceous plant *Ranunculus* spp. (Raw, 1974; Radmacher and Strohm, 2010; Sedivy et al., 2011; Hansted et al., 2014; Coudrain et al., 2016). It is not known what affects these local preferences. One may suspect that difference in habitat composition e.g. along the urbanization gradient may be a major factor affecting pollen composition collected by these bees.

In this paper we tested if composition of pollen in nests of *O. bicornis* differ among sites varying with urbanization level. We predicted that number of collected pollen types is lower in urban sites than in suburban and rural because the latter have higher cover of green areas. We also checked pollen properties basing on the literature. Moreover, we compared our findings from field with the systematically searched literature. We implied that there are recurring patterns of bee' choices in the literature. Our goal was to search for all available, to our knowledge, pollen analyses of materials obtained from *O. bicornis* and to compare them in search of schemes, reoccurring information or environment influence on preferences.

## 2. Materials and methods

In our study we choose *O. bicornis* as research object. This species is good study object to address stated research questions. This bee gained interest among researchers due to practical usage as a pollinator of crops that resulted in numerous studies on its biology allowing effective literature search. Moreover, it has specific traits that make this species a good model. *Osmia bicornis* creates small collectives composed of non-cooperating individuals which shows breeding-site fidelity (Steffan-Dewenter and Schiele, 2004) with flight range up to 200–500 m from the nesting place (Gathmann and Tschardtke, 2002; Yoon et al., 2015; Zurbuchen et al., 2010). Adult individuals consume pollen and nectar resources (Howell and Alarcón, 2007). Females perform pollen-harvesting flights during which they use modified hairs on the abdomen (so called scopa) in order to provide pollinivory larvae with food supply (Haider et al., 2013; Seidelmann, 2018). Each fertile female builds separate compartment for a single egg with individual provision of pollen, wherein offspring is left unattended and has to feed and develop on the resources left by the mother (Seidelmann, 2018). According to Sedivy and Dorn (2014), one female produces approximately 30 descendants on average, and as Giejdasz et al. (2016) claimed, they are deposited into 5 nesting tubes. Moreover, according to Konrad et al.

(2008) and the publications cited therein, some results obtained for *O. bicornis* may be relevant as a model for approximately 700 other polylectic European solitary bees, especially foraging in an overlapping period.

Original population of *O. bicornis* came from The National Institute of Horticultural Research, Apiculture Division in Puławy (51°40'N, 21°96'E). The cocoons from this population were divided randomly into 500 pieces for each experimental breeding. To establish new breedings, composition of the chosen area was mapped to assign a built-up infrastructure (buildings, roads, sidewalks, etc.) and qualified as urban (90 % of built-up infrastructure), suburban (55–65 %), and rural (up to 20 %) category of an urbanization gradient. Categorization of the urbanization gradient followed Fortel et al. (2014), however, the range has been narrowed to the local conditions. We used QGIS software and satellite images freely available in Geoportal (<https://www.geoportal.gov.pl>). Moreover, we used distance to the city centre as the additional metric of urbanisation level. Nesting constructions of 45cm × 45cm × 45 cm dimensions on a wooden frame 0.5 m above ground level with 700 reed tubes of ±15 cm length and 7–12 mm inner diameter as nesting material and protected by a net in the front (mesh of 2 cm diameter) were settled in chosen locations: urban1, urban2, suburban1, suburban2, and rural (low-intensity cultured grasslands) in the Lubelskie region (Eastern Poland). Each localization was attended one month after bees' emergence to identify the presence of the 10 flowering plants with the highest cover level on designated round plots of 50 m radius from centralized nesting constructions (Table 1).

Beil et al. (2008) indicated pollen analysis as the method to provide valuable information about foraging on different spatial scales. We used the microscopic palynological analysis of the species composition of pollen samples to determine constitution of brood provisions. At the end of the season of bee activity in October reed tubes with *O. bicornis* nests were taken from the experimental localizations. Randomly chosen 50 sealed reeds with nesting chambers inside were cut open in order to inspect their contents. From these randomly chosen chambers found in different tubes in the mixed position in the tube (at least one from the end, one from the beginning and one from the middle of the tube) five samples of unused (not eaten by larvae) pollen provisions were collected from each localization into 2 mL Eppendorf type tubes. To state the relative frequency of pollen grains into each pollen package sample distilled water was added (5:1 by volume) and stirred for 1 h (until complete dispersion) in order to transfer 15 µl of homogeneous pollen solution to a microscope slide glass with further 30 µl of distilled water. The slide was dried and then protected with a cover slip and glycerol gelatine as a mounting medium. For each microscopic slide to achieve consistent results over 300 consecutive pollen grains were determined, when possible, into genus, species, family or type of build with the usage of pollen atlases, on-line databases (among others PalDat – a palynological database [2000 onwards, [www.paldat.org](http://www.paldat.org)]) and own collection of over 300 reference preparations using the classification by Zander (1935, 1937, 1941, 1949, 1951) in The National Institute of Horticultural Research, Apiculture Division in Puławy. The obtained numerical results (Suppl. 1) were summed up for particular types of environments in the urbanization gradient and a percentage table was made (Table 2). Additionally, originated plant traits, such as plant habit and vector of plant pollination along with the pollen coating and range of pollen sizes have been determined (Fig. 4A-D). It is supplemented with the traits of individual types of pollen (and origin plants) on a basis of PalDat – a palynological database (2000 onwards, [www.paldat.org](http://www.paldat.org)). Furthermore, an extensive literature search was performed using the Web of Science Core Collection™ bibliographical database section (<http://apps.webofknowledge.com/>). The TS function was used to define the search strings (i.e. the criteria key-words) in order to find articles in the topic of interest. All literature from 1900 until 2021 were searched. The complete search string was “[TS = *Osmia* AND TS = *rufa* OR TS = *bicornis* AND TS = pollen] AND [TS = *Osmia* AND TS = pollen]”. This revealed 131 studies. Abstracts and full-texts of these 131 studies were

**Table 1**  
Plant of high cover level on designated round plots of 50 m radius from centralized nesting constructions.

	Urban1	Urban2	Suburban1	Suburban2	Rural
<b>Geographical coordinates</b>	51°14'N, 22°32'E	51°14'N, 22°30'E	51°14'N, 22°29'E	51°17'N, 22°42'E	51°30'N, 22°55'E
<b>Description</b>	highly human-modified environment with local planting of decorative flowers and the presence of old trees		human-modified environment, without high buildings, plentiful lawns with decorative flowers and the presence of young trees		low-intensity cultured grasslands
<b>Plant list</b>	<i>Acer platanoides</i> <i>A. pseudoplatanus</i> <i>Aesculus hippocastanum</i> <i>Frangula alnus</i> <i>Juglans regia</i> <i>Magnolia loebneri</i> <i>Quercus petraea</i> <i>Q. robur</i> <i>Rhamnus cathartica</i> <i>Salix alba</i> <i>S. caprea</i>	<i>Acer platanoides</i> <i>A. pseudoplatanus</i> <i>Aesculus hippocastanum</i> <i>Euphorbia</i> spp. <i>Frangula alnus</i> <i>Iris reticulata</i> <i>Juglans regia</i> <i>Quercus petraea</i> <i>Q. robur</i>	<i>Brassica napus</i> <i>Aubrieta deltoidea</i> <i>Euphorbia</i> spp. <i>Juglans regia</i> <i>Malus</i> spp. <i>Ranunculus</i> spp. <i>Ranunculus acris</i> <i>Rosa</i> spp. <i>Ulmus laevis</i> <i>U. minor</i>	<i>Brassica napus</i> <i>Euphorbia</i> spp. <i>Juglans regia</i> <i>Ranunculoideae</i> spp. <i>Ranunculus acris</i> <i>Rhamnus</i> spp. <i>Rosa canina</i> <i>Rosa</i> spp. <i>Ulmus laevis</i> <i>U. minor</i>	<i>Acer platanoides</i> <i>A. pseudoplatanus</i> <i>Euphorbia</i> spp. <i>Juglans regia</i> <i>Papaver rhoeas</i> <i>Quercus petraea</i> <i>Q. robur</i> <i>Ranunculus</i> spp. <i>Rhamnus cathartica</i> <i>Rubus idaeus</i>

**Table 2**  
Proportions [%] of the identified pollens from pollen provisions sampled from the red mason bees (*Osmia bicornis*, syn. *O. rufa*) breedings conducted in environments of the urbanization gradient.

	Rural	Suburban 1	Suburban 2	Urban 1	Urban 2
<i>Acer</i>	25.73%		2.89%	20.73%	73.29%
<i>Aesculus</i>	1.98%	1.78%		6.32%	4.99%
<i>Betula</i>	0.21%				
Brassicaceae	0.78%	7.94%	3.49%		
Caryophyllaceae			1.68%		
Euphorbiaceae	4.03%	16.19%	3.13%	3.08%	4.63%
<i>Frangula</i>	1.89%		0.30%		
<i>Iris</i>					10.21%
<i>Juglans regia</i>	11.82%	28.32%	3.31%	5.17%	4.93%
Papaveraceae	9.47%			2.09%	
<i>Pinus</i>		0.70%	0.24%	0.22%	0.47%
<i>Plantago</i>		0.63%			
Poaceae			4.69%	0.33%	
<i>Quercus</i>	11.94%			33.86%	0.47%
Ranunculaceae	4.36%	0.89%	64.52%	6.93%	
Rhamnaceae	5.23%	3.68%		9.40%	
<i>Robinia</i>					1.01%
Rosaceae	1.61%	7.17%	7.04%		
Rosaceae - <i>Fragaria</i>	0.33%		0.66%		
Rosaceae - <i>Prunus</i> type	0.91%	0.57%		2.58%	
Rosaceae - <i>Rubus</i> type	14.66%		3.13%	0.44%	
<i>Salix</i>	1.07%	2.67%	1.14%	8.41%	
<i>Taraxacum</i> type	0.29%	0.25%	0.24%		
<i>Trifolium</i> type	0.62%			0.44%	
<i>Ulmus</i>		27.94%			
<i>Viscum</i>		1.27%			
other	3.09%	5.02%	3.55%	1.87%	1.42%

Pollen determination was made into genus, species, family or type of build – whichever possible, table cells with pollen find as main types (exceeding 5% of the package's volume) are shaded grey.

read for relevance, in order to select empirical studies that used original data and to exclude simulation-based studies. Also, additional studies, not found in the described systematic search, were included basing on expert knowledge of the authors. Analyses were complicated because, as it turned out, there are many not standardized methods in research especially as far as sampling method is concerned (pollen sampled from pollen provisions, faeces samples, pollen obtained from scopa hair or other parts of the body etc.). Finally, a total of 53 studies were selected for the review to show overall tendencies for choosing plant type, habit, way of pollination, as well as pollen coating and size by *O. bicornis* (Suppl. 2, 3). Simplified compilation of information from these studies can be found in Table 3.

All analyses were done in R environment (R Core Team 2019). We used canonical analysis of correspondence to find out if there are differences in plant taxa composition among sites along the urbanization

gradient. The analysis was performed in R package “vegan” (Oksanen et al., 2007). Also, co-correspondence analysis (Co-CA) (ter Braak and Schaffers, 2004) was used to reveal whether or not plant taxa abundance in pollen provisions can be explained by plant community composition in studied sites. In this analysis, performed in R package “cocorresp” (Simpson, 2009) data were averaged among samples per taxon for pollen provisions (dependent data) and the presence of plant taxa in each site (explanatory data).

We used linear model to test if diversity (Simpson reciprocal index) and number of plant taxa in pollen provision differ among sites along the urbanization gradient. We used robust regression, implemented in packages “MASS” (Venables and Ripley, 2002) and “sfsmisc” (Maechler et al., 2020) to correlate the number of plant taxa and number of pollen grains in the provision with the number of plant taxa present in a site.

**Table 3**  
*Osmia bicornis* main (exceeding 5% of the sample) pollen choices based on the literature data.

No.	Characteristics of breeding localization	Sample type	Main pollens		Refs.	
			Plant	Average amount [%]		
1	Breedings next to winter oilseed rape	pollen provisions	<i>Brassica napus</i> <i>Quercus robur</i> <i>Salix</i> sp.	15.75 % 64.75 % 41.4 %	Ruddle et al. (2018)	
2	<i>Prunus cerasus</i> orchards with <i>Salix</i> , <i>Acer</i> , <i>Betula</i> and <i>Quercus</i> trees within 100 m of the orchard, surrounded by cereal crops and pasture grassland [Taastrup, eastern Denmark]	pollen provisions	<i>Acer</i> spp. <i>Betula</i> <i>Prunus</i> -type <i>Quercus robur</i> <i>Acer</i> spp.	33.24 % 8.16 % 8.16 % ? ?	Hansted et al. (2014)	
3	set-aside orchards (mainly apple trees and grassland), landscaped grounds (several tree species and lawn), and farmland (rape and cereal crops) [near the Biocenter of the University of Würzburg, Germany]	pollen provisions	<i>Papaver rhoeas</i> , <i>P. dubium</i> <i>Ranunculus</i> spp. <b>Ranunculus</b> and <b>Quercus</b>	? ? 38.8 %	Radmacher and Strohm (2010)	
4	<b>NON-APPLICABLE</b> [museum specimens across Europe]	scopal pollen			Haider et al. (2013)	
5	low-intensity grasslands of Swiss Plateau between the cities of Bern, Solothurn and Fribourg	pollen provisions	<b>Ranunculus</b> <i>Quercus</i> sp.	58.576 % 23.402 %	Coudrain et al. (2016)	
6	8 locations in orchards [the Wachtberger Ländchen south of Bonn]	pollen provisions	<b>Rosaceae</b> <i>Salix</i> sp.	32.18 % 27.59 %	Schindler and Peters (2011)	
				2003 2004		
			<i>Juglans</i>	10.87 % 17.57 %		
			<b>Brassicaceae</b>	10.33 % 16.22 %		
7	area adjacent to home gardens and rapeseed plantations	faeces	<i>Aesculus</i> <i>Rubus</i> -type <i>Ranunculus</i> <i>Pinus</i> <i>Lonicera</i>	9.24 % 5.43 % 5.43 % 6.52 % 8.15 %	10.81 % 6.76 % 7.43 % – –	Teper (2007)
8	Breedings next to oilseed rape	pollen provisions	<i>Brassica napus</i> <b>Rosaceae</b> Ranunculaceae <i>Prunus</i> -type	16.0 % 32.6 % 26.6 % 10.33 %	Peters et al. (2016)	
9	Puławy, Poland	pollen provisions	<b>Juglans</b> <i>Populus</i> <i>Salix</i> sp.	31.67 % 15.67 % 16.67 %	Biliński and Teper (2004)	
10	Plantation consisting of several hundred hectares of winter rape	pollen provisions	<b>Brassica napus</b>	29.8 %	Teper and Biliński (2009)	
11	Scania, south Sweden	pollen provisions	<b>Asteraceae</b> <i>Taraxacum</i> -type <i>Brassica napus</i> <i>Quercus</i> sp.	47.1 % 42.9 % 10 % 45 %	Söderman et al. (2018)	
12	Nidda catchment in Central Germany	pollen provisions	<i>Aesculus</i> <b>hippocastanum</b> Ranunculaceae <b>Rosaceae</b>	45 % 45 % 45 %	Jauker et al. (2012)	
13	1) grasslands adjacent to oilseed rape 2) grasslands isolated from oilseed rape 3) oilseed rape fields adjacent to grasslands 4) oilseed rape fields isolated from grassland 5) Gottingen, Lower Saxony, Germany	pollen provisions	<i>Brassica napus</i>	to 20 %	Holzschuh et al. (2013)	

### 3. Results

The experimental breeding locations were covered with typical vegetation for this region in a temperate climate. The list of plants with high cover level among locations largely overlapped (regardless of their urbanization level) (Table 1).

*Osmia bicornis* bees used limited number of main pollen types (exceeding 5% of volume) for storage as pollen provisions (Table 2). Diversity ( $F = 2.571$ ,  $df = 4, 22$ ,  $P = 0.06$ ) and number of plant taxa ( $F = 4.252$ ,  $df = 4, 22$ ,  $P = 0.011$ ) in pollen provisions increased from urban, through suburban to rural site (Fig. 1). Bees made use of commonly available trees like oaks, maples, horse chestnut, walnuts and elms. The composition of collected pollen differed among sites as indicated by CCA analysis (Fig. 2). This CCA was statistically significant ( $F = 2.54$ ,  $df = 4, 22$ ,  $P < 0.001$ ). First axis explained 38 % of variation and separated suburban habitats from other habitats. Second axis explained 31 % of

variation and separated suburban2 from other sites (Fig. 2). Nevertheless, the composition of plant species present in a site well explained taxa composition in pollen provisions as indicated by Co-CA (Fig. 3). In this analysis axis 1 and 4 were statistically significant as indicated by the permutation tests ( $P < 0.01$ ). Moreover, we found positive relation between number of plant taxa present in a site and number of pollen grains in a provision (robust regression:  $r = 0.864$ ,  $F = 33.984$ ,  $df = 2, 3$ ,  $P = 0.01$ ) but not with number of taxa in pollen provision (robust regression:  $r = -0.307$ ,  $F = 0.024$ ,  $df = 2, 3$ ,  $P = 0.884$ ). Breedings from suburban localizations had pollen of decoratively planted Ranunculaceae, Euphorbiaceae and Papaveraceae plants families in prominent quantities (Table 2). When Rosaceae and Brassicaceae plants are available nearby the nests, the solitary bees collect a small amount of pollen from their flowers as well (Table 2, Suppl. 1, 2, 3). Literature data analysis showed repeatability of some bee' choices as oak, willow and Rosaceae pollen in provisions (Suppl. 2, 3). Results from our experiment as well as



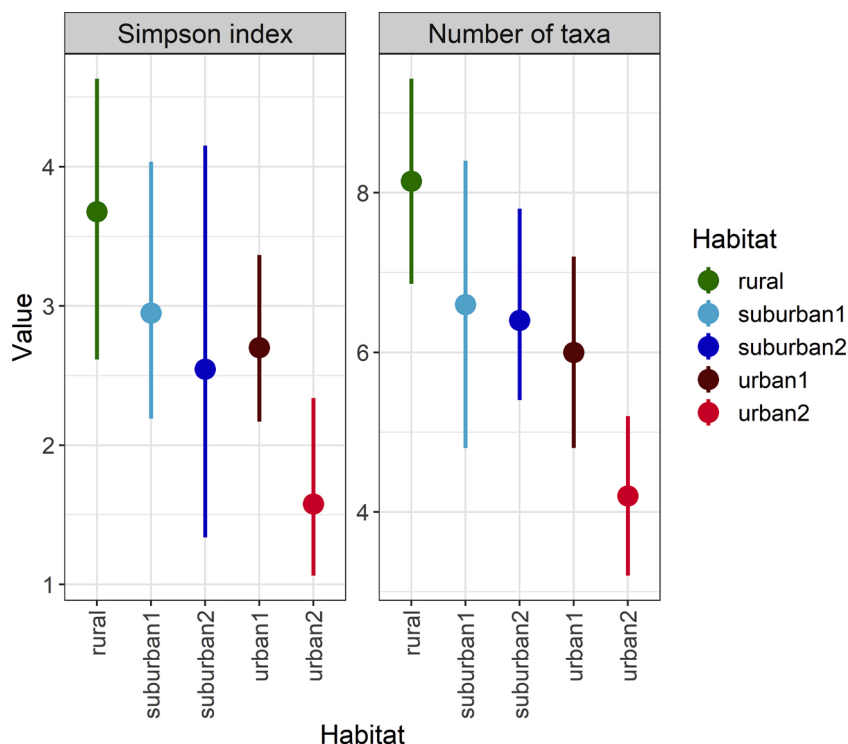


Fig. 1. Simpson diversity of plants (left) and number of plant taxa (species/families/types) in pollen collected by the red mason bees (*Osmia bicornis*, syn. *O. rufa*) in different sites and habitats along the urbanization gradient. Means with 95 % confidence intervals are presented (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

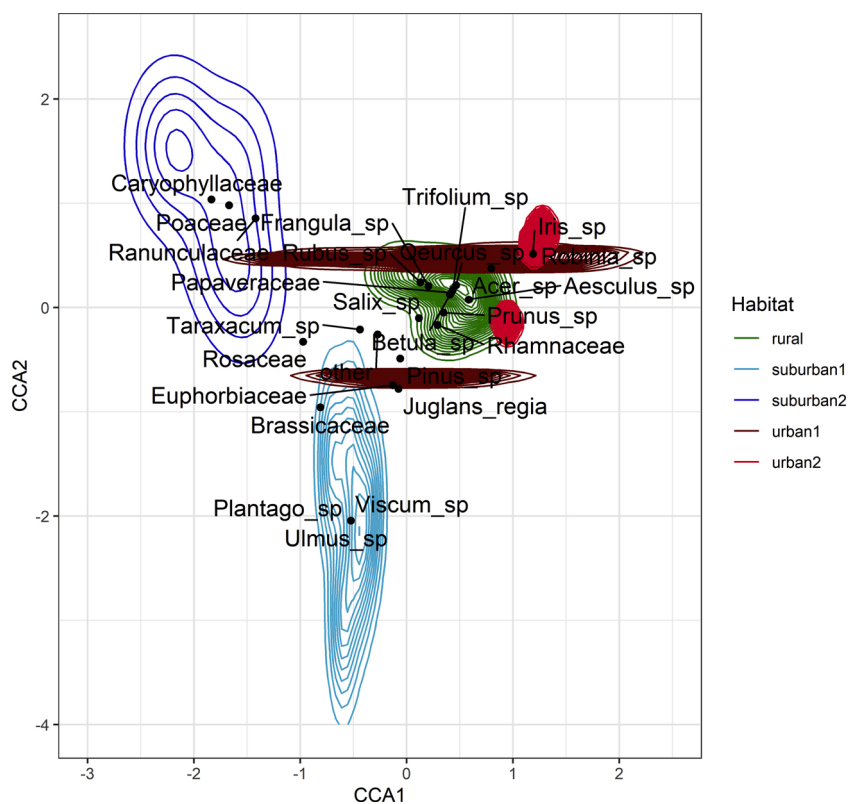


Fig. 2. The composition of plant species/families/types collected by the red mason bees (*Osmia bicornis*, syn. *O. rufa*) in different sites and habitats along the urbanization gradient. Results from the canonical correspondence analysis. For details see Table 1 (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

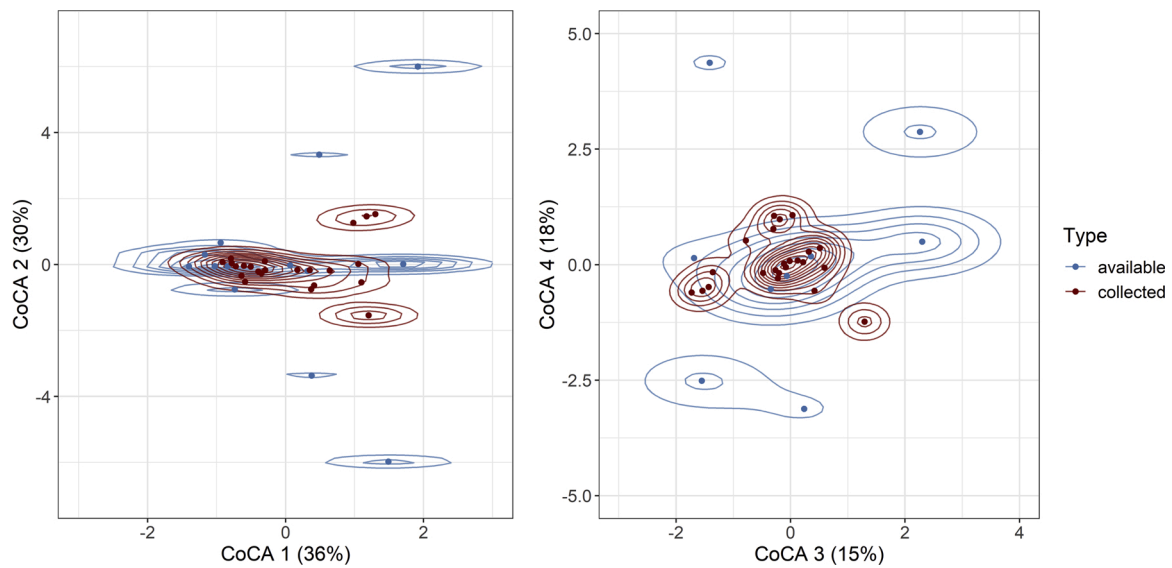


Fig. 3. Co-correspondence ordination of plant taxa found in pollen and plant taxa present in study sites. Kernel density estimator was used to show over.

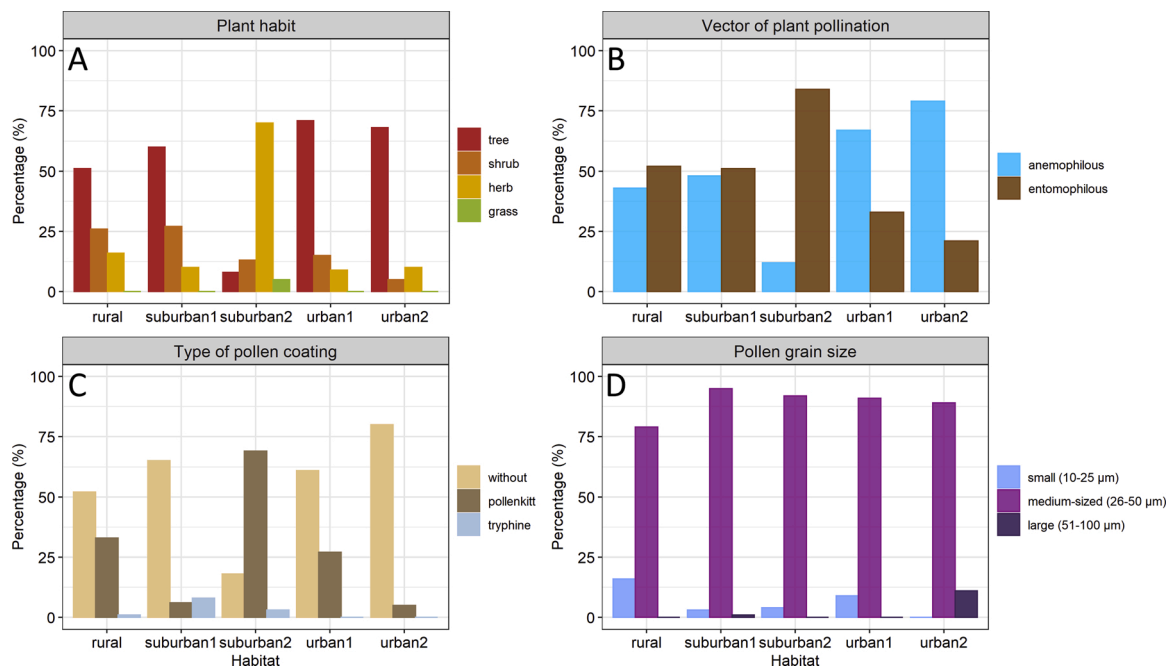


Fig. 4. Traits of pollens (and their origin plants) harvested by the red mason bees (*Osmia bicornis*, syn. *O. rufa*) to create pollen provisions in different environments of urbanization gradient: A – plant habit; B – vector of plant pollination; C – type of pollen coating; D – pollen size (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

from literature data analyses showed that *O. bicornis* tended to use pollen mostly from trees (Fig. 4A). In urban areas bees collected pollen from anemophilous plants, whereas in other areas (suburban allotments, rural meadows) *O. bicornis* seemed to visit mostly entomophilous flowers (Fig. 4B). Literature data suggested over 60 % share of pollen from entomophilous plants in provisions stored by *O. bicornis* (Suppl. 2, 3). Pollenkitt on the surface of pollen was (most often) present when insects were the pollination vector of plants – and our data allowed to link these results (Fig. 4C). Most pollen used by *O. bicornis* for storage were of a medium range of size (26–50 μm), and to a lesser extent, small grains (10–25 μm) regardless of the environment (Fig. 4D).

#### 4. Discussion

Adult bees use flower resources to obtain required nutrients i.e., pollen and nectar, in order to maintain all metabolic functions (Ollerton, 2017, 2021). According to Vaudo et al. (2020) bees obtain their protein and lipid nutrient intake from pollen, which is essential for larval growth and development as well as adult health and reproduction. As Roswell et al. (2019) confirmed the diets of male and female bees of the same species are often dissimilar as the diets of different species of bees. Additionally, Filipiak et al. (2021) stated sex-specific requirements and further development differences connected with sexual dimorphism of *O. bicornis*. The sexes differed fundamentally in the assimilation and allocation of acquired atoms, elemental phenotypes, and stoichiometric niches. Therefore, it is important to highlight that studies of *Osmia* –

including our – are largely focused on females which are believed to be more efficient pollinators and are easier to trace thanks to nest-side fidelity. Howell and Alarcón (2007) observed that in laboratory conditions adult *Osmia* females preferred flowers filled with nectar. However, analyses of free flying adult *O. bicornis* females' faeces made by Teper (2007), clearly indicated that they consume also pollen from plant which do not produce nectar at all. Moreover, Cane (2016) experimentally verified that in order to develop the basal oocytes to full size adult females of *O. californica* require access to pollen – only this enables them to lay eggs in provided nesting tubes. Ahrenfeldt et al. (2019) claimed on the example of strawberry flowers, that *O. bicornis* – commonly called red mason bee – is capable of assessing nectar and pollen quality and prioritize accordingly. The observed pattern indicated foraging changes depending on whether sugary-nectar or proteinaceous-pollen was collected. Giejdasz et al. (2005) mentioned that red mason bees with lower body mass often re-visit the same flowers and repeat scheme of foraging which entails lower energy consumption. In the same time heavier bees select flowers in more random mode and are thus able to visit higher number of flowers. However, it is important to keep in mind that bee fitness is shaped by chemical element availability in larval food (Filipiak and Filipiak, 2020). During larval development phosphorus which was firstly supporting growth is allocated mainly (55–75 %) to the cocoon. Moreover, also other elements acquired from pollen load by larvae such as Mn, Ca, Mg and Zn are later allocated to the cocoon probably influencing bee fitness by conferring protection. Additionally, an importance of pollen loads quality is emphasized by the fact that pollen nutritional value through direct influence on larval and adults' development, has high effect on bee populations (Filipiak, 2019). Dobson et al. (2012) studied chemical imprinting of *O. bicornis* larvae and suggested that rearing bees on a single plant can both directly and indirectly affect flower selection made by adults. Our results indicated that along the urbanization gradient, bees adjusted their choices and collected pollen from plants available in the surrounding environment. This showed that it is purposeful to maintain and moderate plant species composition of greeneries – especially urban ones – to influence the composition of pollen provisions and pollinators' diet.

It is worth noting, however, that despite local differences in pollen composition, some bee' choices are often repeated and even in small quantities (regardless of the environment) pollen of oak or willow can be found in the brood provisions. In our opinion pollen of *Salix* and *Quercus* trees always seem to be chosen by spring emerging bees if they are within their flight range. Some authors (e.g. Coudrain et al., 2016) indicated that high proportion of non-nectar producing anemophilous pollen *Quercus* may be connected with its high protein content (about 40 %) completed by high amount of nectar from *Salix*. Hansted et al. (2014) stated that if in the proximity of orchards competitor plant such as *Salix* is present – breeding of *O. bicornis* bees may be even insufficient to provide pollination services for crops. Persson et al. (2018) noticed that presence of oaks in 100 m proximity of nests led to higher proportions of their pollen in brood provisions, as well caused increased pace of nest construction in the beginning of nesting season and lengthened foraging flights. Meanwhile, Kratschmer et al. (2019) observed that willow and oak enhance floral resources and are frequently collected as a material for pollen provision also by *O. cornuta* (closely related to *O. bicornis*), specifically in intensively farmed agricultural areas. Interestingly, as can be followed in the Table 3, *O. bicornis* found in the vicinity of crops often also uses other food sources for pollen loads. Therefore, our and other authors' results confirmed that there are recurring patterns of bees' choices observed regardless of the environment character. Such convergent observations may indicate that the selection of food sources by bees is not completely random – they seem to prefer high-protein pollen over the less valuable ones.

Pinilla-Gallego and Isaacs (2018) found out that a relative of the red mason bee – blue orchard bee (*O. lignaria*), employed as a pollinator of commercial blueberries (*Vaccinium corymbosum*), in fact is reaching for

food-resources of: black cherries (*Prunus serotina*), white clovers (*Trifolium repens*), and red clovers (*Trifolium pratense*). Such results might suggest low usefulness of solitary bees in pollination of crop plants. In turn, in our analysis *Brassica napus* seemed to be one of the important provision sources in rural population of *O. bicornis* (Tables 2 and 3). Yet, we believe that proportion of oilseed rape pollen in brood provisions is overstated due to the fact that many experiments were deliberately conducted in the proximity of those mass-flowering spring crops (see first column of Suppl. 3). Bees consumed *B. napus* pollen without having other alternative protein source available nearby. Nevertheless, bees from oilseed studies conducted in proximity of such trees as oaks, willows or walnuts collected and profited from their pollen (e.g. Ruddle et al., 2018; Teper, 2007).

Schenk et al. (2018) concluded that short temporal mismatches (among bee emerging / foraging and plant pollinating) can cause clear fitness losses in solitary bees. Although their results suggested that bees have evolved species-specific strategies to mitigate fitness losses after temporal mismatches. What is more, according to Persson et al. (2018) pattern of resources choice is changed during the season and later *O. bicornis* forage is dominated by buttercup (*Ranunculus* spp.). Also, Coudrain et al. (2016) verified that *O. bicornis* can nest in a variety of conditions by compensating scarcity of its main larval food by exploiting alternative food sources such as *Ranunculus* flowers. Our results confirmed that Ranunculaceae pollen in brood provisions, particularly in breedings located in the suburban sites. Sedivy et al. (2011) found that *O. bicornis* developed well on *Ranunculus* pollen, whereas provision made from *Ranunculus* pollen cause dwarfism of *O. cornuta*. Eckhardt et al. (2014) results also showed that pure *Ranunculus* pollen diet have a lethal effect on developing larvae of *O. cornuta*. However, they found that larval survival and development time as well as adults' body mass remained nearly unaffected by the admixture of up to 50 % of *Ranunculus* pollen diet to the larval food. Therefore, the ability of *O. bicornis* to use *Ranunculus* pollen resources can therefore be seen as its adaptation to exploit available resources or maybe to control other potential kleptoparasites for which this pollen may be lethal. Also pollen of the Asteraceae, according to Spear et al. (2016), is known to be a poor-quality food source that significantly reduces survival of parasites' larvae. As these authors claimed – the compensatory benefits of a pollinators' poor diet may include protection from natural enemies. Therefore, it seems reasonable to say that larvae of pollen generalists can benefit from the nutrient content of unfavourable pollen without being negatively affected by its adverse chemical properties and even use them as an advantage. Additionally, against the randomness of the bees' choices is suggestion of Bukovinsky et al. (2017) that resources collected from one plant species may influence the usefulness of pollen from another plant species. In the context of bees' choice, it is established that *Osmia* bees used limited number of main pollen types (exceeding 5% of volume) for storage as pollen provisions. Therefore, Eckhardt et al. (2014) claimed that pollen mixing is a common behaviour of these solitary bees. Our results showed that from 2 to 4 main pollen resources can be expected in one pollen provision of *O. bicornis* at the same time with scarce (less than 5% of volume) addition of other pollens. We observed that the number of major pollen types appears to be higher in more rural environments. Other authors also received similar results (see Suppl. 3), what is more solitary bees from the *Osmia* genus (even from other continents) also use about 3 major food resources to create storage for offspring (e.g. MacIvor et al., 2014). Widespread pollen mixing by females of pollen generalist should be considered as a possible strategy to exploit flowers with unfavourable pollen and to optimize larval food quality (Eckhardt et al., 2014).

The problem of insects vanishing, in particular those contributing as pollinators, is widely recognized and effective remedies have been sought for some time. As one of the conservation measures wildflower seed mixtures are popularly used in variety of environments (e.g. Gresty et al., 2018; Hicks et al., 2016). However, the extent to which key pollinator groups such as solitary bees exploit and benefit from these

resources remains unclear. Rollings and Goulson (2019) stated that we do not yet fully understand what factors drive insect pollen preferences. Leonhardt et al. (2020) on a basis of literature research claimed the need for more studies on the comparative sensory ecology, underlying nutritional quality assessment, cue perception and decision making to fully understand how insects adjust resource selection and exploitation in response to environmental heterogeneity and variability. Many authors i.a. Wood et al. (2016) or Gresty et al. (2018) suggested that plant species promoted currently by agri-environment schemes (AES) are not optimal for solitary bees' foraging and whether a diverse community of pollinators is to be supported additional flora species ought to be included in these mixtures. Moreover, AES are designed for agricultural landscape while more and more research indicates role of solitary bees in urban environment (Banaszak-Cibicka and Źmihorski, 2020; Buchholz and Egerer, 2020). Pamminer et al. (2019) pointed out that in future greater emphasis in the selection of plants should be placed on their quality and nutrient content of pollen and nectar. This is supported by the newest reports of Filipiak and Filipiak (2020) and Filipiak et al. (2021) who indicated that not all pollens are nutritionally balanced for bees and the information on the fitness effects of nutritional mismatches between bee demand and the supply of specific elements in food is still to be verified. Even though some solutions such as Belgian pollinator strips of flower-rich hay meadows seems to be effective resources, they still support pollination services mainly during summer. Therefore Ouvrard et al. (2018) stated that spring and autumn food-sources remain poor and could reduce the strips' effectiveness for supporting long-term insect diversity. Klaus et al. (2021) confirmed that bee reproduction increased due to plant diversity and additionally availability of complementary flower resources can offset negative effects of neonicotinoid-treated oilseed rape on wild bee reproduction. Compared to reduced bee larval to adult development by 69 % when exposed to monocultures. Thus, Gresty et al. (2018) stated *Rosa canina* as worth adding to the plant mixtures. Nichols et al. (2019) have come to similar conclusions indicating that including a range of Apiaceae, Asteraceae, and Geraniaceae in seed mixes would cater for a wide diversity of bee species. On the other hand, Jachula et al. (2018) suggested consideration of Lamiaceae species because of their abundant flowering and good pollen nutritional value which might improve food resources, especially for bees. An interesting argument for discussion was provided by Hicks et al. (2016) who verified that perennial meadows produced up to 20x more nectar and up to 6x more pollen than annual ones and earlier in season. Ergo, the design of seed mixes should be directed at perennial plants to ensure continuity in floral resources availability throughout the year, and to identify suitable species to fill food-supply gaps in established mixes (Hicks et al., 2016). To confirm that this is a global problem it can be pointed out that this is in line with the findings of Wilson et al. (2021) that bees (in their case Australian social bee *Tetragonula carbonaria*, Meliponini) use "many small" rather than a "few large" pollen sources. Therefore, pollinators should have access to a variety of floral resources year-round which may be achieved through targeted planting of key families of plants and / or maintaining weeds while they are flowering in the orchard. Land managers may consider planting in unproductive areas such as riparian zones, edges or between crop rows if space is limited.

Apart from promoting flowers being food resources and suitable early- and late-flowering forbs as basic measure to sustain food supply for wide range of pollinators some authors (Persson et al., 2018; Filipiak, 2019; Jachula et al., 2021) implicated that increasing habitat heterogeneity with trees and shrubs, flower strips and hedgerows or man-made non-cropped areas is more profitable for in wild bee conservation and may help to mitigate the largest pollinators' food gaps. By extension human-induced environmental heterogeneity interacts to shape plant-solitary bee networks. In the urban areas Hülsmann et al. (2015) emphasized the importance of flower-rich parks and gardens even as isolated patches in the city centre and Kratschmer et al. (2018) showed that green roofs as potentially valuable habitats in urban areas, because

wild bees' diversity and abundance is strongly positively affected by increasing foraging availability. However, as Kaluza et al. (2017) stated the decreasing diversity of available resources may impact consumers primarily by reduced resource abundance and secondly by reduced resource functionality. Thus, plant species richness should support pollinators by providing not only a continuous resource supply, but also floral resources of high nutritional quality (Trinkl et al., 2020). Eventually, according to Kaluza et al. (2018) available resource diversity and abundance are related to resource (quality and quantity) intake and bees' reproduction. This indicate that plant diversity is a key driver of bee fitness as they used more resources, increased food stores and their populations grew faster in more florally diverse environments.

Not only palynological studies of the composition of *O. bicornis* pollen provisions indicated that it is worth paying attention to trees in the conservation of insects. According to Ostaff et al. (2015) willows could be used to support the early vernal pollinators' community before the flowering period of commercially valuable crops. Moquet et al. (2015) verified that willows offered pollen with higher polypeptide and essential amino acid contents than blueberry (*Vaccinium myrtillus*, Ericaceae). They observed also that during the overlapping flowering period of these two-plant species, pollinators seemed to favour high quality and easily accessible pollen of *Salix*. Although, it is necessary to pay attention to the invasive nature of some tree species, such as walnuts (e.g. Lenda et al., 2018) or *Robinia* spp. (e.g. Poblador et al., 2019) because even though they are a good food-source for bees, they still pose a threat to the environment. Therefore, as advisable measure of insect conservation in European temperate climate planting such trees as native oaks, willows, maples, elms and even various species of Rosaceae should be considered as measure to complement environment, especially in cities and towns. Nevertheless, planting individual trees could adjunct the bees' forage base, because according to Kasprzyk et al. (2019) a solitary tree produces more pollen than a tree growing near other trees of the same species. This data encourages consideration of additional plantings of singular trees in an urban environment where space for plant maintenance is limited. Moreover, as Bastin et al. (2019) suggested the restoration of trees is the most effective strategy for climate change mitigation. Global tree restoration is even mentioned as one of the most effective carbon drawdown solutions to date and also has many other positive environmental effects. Additionally, according to Bodden et al. (2019) using anemophilous plants as source of food may be bees' defence mechanism, because they are most likely not platforms for transferring diseases among insects. Our analyses revealed that solitary, spring-occurring bees tend to harvest significant amount of pollen to create provisions for offspring from trees. We believe that supplementing the landscape (especially urban one) with trees is advisable, particularly willows and oaks planting might provide a sufficient food base.

#### Author statement

AS<sup>1,2</sup>, PS, AS<sup>3</sup>: Conceptualization.  
 AS<sup>1,2</sup>, PS: Data Curation.  
 AS<sup>1,2</sup>, PS: Formal Analysis.  
 AS<sup>1,2</sup>: Funding Acquisition.  
 AS<sup>1,2</sup>, PS, DT, MB, AS<sup>3</sup>: Investigation.  
 AS<sup>1,2</sup>, PS: Methodology.  
 AS<sup>1,2</sup>: Project Administration.  
 AS<sup>1,2</sup>: Resources.  
 AS<sup>1,2</sup>, PS: Supervision.  
 AS<sup>1,2</sup>, PS: Visualization.  
 AS<sup>1,2</sup>: Writing – Original Draft Preparation.  
 PS, AS<sup>1,2</sup>: Writing – Review & Editing.

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## Declaration of Competing Interest

The authors report no declarations of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ufug.2021.127250>.

## References

- Ahrenfeldt, E.J., Sigsgaard, L., Hansted, L., Jensen, A.C., Toldam-Andersen, T.B., 2019. Forage quality and quantity affect red mason bees and honeybees differently in flowers of strawberry varieties. *Entomol. Exp. Appl.* 167 (8), 763–773. <https://doi.org/10.1111/eea.12820>.
- Ayers, A.C., Rehan, S.M., 2021. Supporting bees in cities: how bees are influenced by local and landscape features. *Insects* 12 (2), 128. <https://doi.org/10.3390/insects12020128>.
- Banaszak-Cibicka, W., Żmihorski, M., 2020. Are cities hotspots for bees? Local and regional diversity patterns lead to different conclusions. *Urban Ecosyst.* 23 (4), 1–10. <https://doi.org/10.1007/s11252-020-00972-w>.
- Bastin, J.F., Finegold, Y., Garcia, C., Mollicone, D., Rezende, M., Routh, D., Zohner, C.M., Crowther, T.W., 2019. The global tree restoration potential. *Science* 365 (6448), 76–79. <https://doi.org/10.1126/science.aax0848>.
- Beil, M., Horn, H., Schwabe, A., 2008. Analysis of pollen loads in a wild bee community (Hymenoptera: apidae) – a method for elucidating habitat use and foraging distances. *Apidologie* 39 (4), 456–467. <https://doi.org/10.1051/apido:2008021>.
- Belsky, J., Joshi, N.K., 2019. Impact of biotic and abiotic stressors on managed and feral bees. *Insects* 10 (8), 233. <https://doi.org/10.3390/insects10080233>.
- Biliński, M., Teper, D., 2004. Rearing and utilization of the red mason bee—*Osmia rufa* L. (Hymenoptera, Megachilidae) for orchard pollination. *J. Apic. Sci.* 48, 69–74.
- Bodden, J.M., Hazlehurst, J.A., Wilson Rankin, E.E., 2019. Floral traits predict frequency of defecation on flowers by foraging bumble bees. *J. Insect Sci.* 19 (5), 2. <https://doi.org/10.1093/jisesa/iez091>.
- Bosch, J., Kemp, W.P., 2002. Developing and establishing bee species as crop pollinators: the example of *Osmia* spp. (Hymenoptera: megachilidae) and fruit trees. *Bull. Entomol. Res.* 92 (1), 3–16. <https://doi.org/10.1079/BER2001139>.
- Buchholz, S., Egerer, M.H., 2020. Functional ecology of wild bees in cities: towards a better understanding of trait-urbanization relationships. *Biodivers. Conserv.* 29, 2779–2801. <https://doi.org/10.1007/s10531-020-02003-8>.
- Bukovinsky, T., Rikken, I., Evers, S., Wäckers, F.L., Biesmeijer, J.C., Prins, H.H., Kleijn, D., 2017. Effects of pollen species composition on the foraging behaviour and offspring performance of the mason bee *Osmia bicornis* (L.). *Basic Appl. Ecol.* 18, 21–30. <https://doi.org/10.1371/journal.pone.0158117>.
- Cane, J.H., 2016. Adult pollen diet essential for egg maturation by a solitary *Osmia* bee. *J. Insect Physiol.* 95, 105–109. <https://doi.org/10.1016/j.jinsphys.2016.09.011>.
- Coudrain, V., Rittiner, S., Herzog, F., Tinner, W., Entling, M.H., 2016. Landscape distribution of food and nesting sites affect larval diet and nest size, but not abundance of *Osmia bicornis*. *Insect Sci.* 23 (5), 746–753. <https://doi.org/10.1111/1744-7917.12238>.
- Cripps, C., Rust, R.W., 1989. Pollen foraging in a community of *Osmia* bees (Hymenoptera: megachilidae). *Environ. Entomol.* 18 (4), 582–589. <https://doi.org/10.1093/ee/18.4.582>.
- Dobson, H.E., Ayasse, M., O'Neal, K.A., Jacka, J.A., 2012. Is flower selection influenced by chemical imprinting to larval food provisions in the generalist bee *Osmia bicornis* (Megachilidae)? *Apidologie* 43 (6), 698–714. <https://doi.org/10.1007/s13592-012-0144-y>.
- Donkersley, P., 2019. Trees for bees. *Agric. Ecosyst. Environ.* 270, 79–83. <https://doi.org/10.1016/j.agee.2018.10.024>.
- Eckhardt, M., Haider, M., Dorn, S., Müller, A., 2014. Pollen mixing in pollen generalist solitary bees: a possible strategy to complement or mitigate unfavourable pollen properties? *J. Anim. Ecol.* 83 (3), 588–597. <https://doi.org/10.1111/1365-2656.12168>.
- Filipiak, M., 2019. Key pollen host plants provide balanced diets for wild bee larvae: a lesson for planting flower strips and hedgerows. *J. Appl. Ecol.* 56 (6), 1410–1418. <https://doi.org/10.1111/1365-2664.13383>.
- Filipiak, Z.M., Filipiak, M., 2020. The scarcity of specific nutrients in wild bee larval food negatively influences certain life history traits. *Biology (Basel)* 9 (12), 462. <https://doi.org/10.3390/biology9120462>.
- Filipiak, M., Woyciechowski, M., Czarnoleski, M., 2021. Stoichiometric niche, nutrient partitioning and resource allocation in a solitary bee are sex-specific and phosphorous is allocated mainly to the cocoon. *Sci. Rep.* 11 (1), 1–12. <https://doi.org/10.1038/s41598-020-79647-7>.
- Fortel, L., Henry, M., Guilbaud, L., Guirao, A.L., Kuhlmann, M., Mouret, H., Rollin, O., Vaissière, B.E., 2014. Decreasing abundance, increasing diversity and changing structure of the wild bee community (Hymenoptera: anthophila) along an urbanization gradient. *PLoS One* 9 (8), e104679. <https://doi.org/10.1371/journal.pone.0104679>.
- Gathmann, A., Tscharnkte, T., 2002. Foraging ranges of solitary bees. *J. Anim. Ecol.* 71 (5), 757–764. <https://doi.org/10.1046/j.1365-2656.2002.00641.x>.
- Giejdasz, K., Wilkaniec, Z., Piech, K., 2005. Effects of seed onion pollination by red mason bee females *Osmia rufa* L. (Apoidea; Megachilidae) with different body weights. *J. Apic. Sci.* 49, 21–27.
- Giejdasz, K., Fliszkiewicz, M., Bednárová, A., Krishnan, N., 2016. Reproductive potential and nesting effects of *Osmia rufa* (syn. *bicornis*) female (Hymenoptera: megachilidae). *J. Apic. Sci.* 60 (1), 75–86. <https://doi.org/10.1515/JAS-2016-0003>.
- Gresty, C.E., Clare, E., Devey, D.S., Cowan, R.S., Csiba, L., Malakasi, P., Lewis, O.T., Willis, K.J., 2018. Flower preferences and pollen transport networks for cavity-nesting solitary bees: implications for the design of agri-environment schemes. *Ecol. Evol.* 8 (15), 7574–7587. <https://doi.org/10.1002/ece3.4234>.
- Haider, M., Dorn, S., Sedivy, C., Müller, A., 2013. Phylogeny and floral hosts of a predominantly pollen generalist group of mason bees (Megachilidae: osmiini). *Biol. J. Linn. Soc.* 111 (1), 78–91. <https://doi.org/10.1111/bij.12186>.
- Hansted, L., Grout, B.W., Toldam-Andersen, T.B., Eilenberg, J., 2014. An assessment of *Osmia rufa* (syn. *bicornis*) as a pollinator of the sour cherry (*Prunus cerasus*) cv. Stevnsbaer in eastern Denmark. *J. Apic. Res.* 53 (1), 177–182. <https://doi.org/10.3896/IBRA.1.53.1.20>.
- Hicks, D.M., Ouvrard, P., Baldock, K.C., Baude, M., Goddard, M.A., Kunin, W.E., Mitschunas, N., Memmott, J., Morse, H., Nikolitsi, M., Osgathorpe, L.M., Potts, S.G., Robertson, K.M., Scott, A.V., Sinclair, F., Westbury, D.B., Stone, G.N., 2016. Food for pollinators: quantifying the nectar and pollen resources of urban flower meadows. *PLoS One* 11 (6), e0158117. <https://doi.org/10.1371/journal.pone.0158117>.
- Holzschuh, A., Dormann, C.F., Tscharnkte, T., Steffan-Dewenter, I., 2013. Mass-flowering crops enhance wild bee abundance. *Oecologia* 172 (2), 477–484. <https://doi.org/10.1007/s00442-012-2515-5>.
- Howell, A.D., Alarcón, R., 2007. *Osmia* bees (Hymenoptera: megachilidae) can detect nectar-rewarding flowers using olfactory cues. *Anim. Behav.* 74 (2), 199–205. <https://doi.org/10.1016/j.anbehav.2006.11.012>.
- Jachula, J., Wrzesień, M., Strzałkowska-Abramek, M., Denisow, B., 2018. The impact of spatio-temporal changes in flora attributes and pollen availability on insect visitors in Lamiaceae species. *Acta Bot. Croat.* 77 (2), 161–171. <https://doi.org/10.2478/botcro-2018-0018>.
- Hülsmann, Marietta, von Wehrden, Henrik, Klein, Alexandra-Maria, Leonhardt, Sara Diana, 2015. Plant diversity and composition compensate for negative effects of urbanization on foraging bumble bees. *Apidologie* 46 (6), 760–770. <https://doi.org/10.1007/s13592-015-0366-x>.
- Jachula, J., Denisow, B., Wrzesień, M., 2021. Habitat heterogeneity helps to mitigate pollinator nectar sugar deficit and discontinuity in an agricultural landscape. *Sci. Total Environ.* 782, 146909. <https://doi.org/10.1016/j.scitotenv.2021.146909>.
- Jauker, F., Peter, F., Wolters, V., Diekötter, T., 2012. Early reproductive benefits of mass-flowering crops to the solitary bee *Osmia rufa* outbalance post-flowering disadvantages. *Basic Appl. Ecol.* 13 (3), 268–276. <https://doi.org/10.1016/j.baee.2012.03.010>.
- Kaluza, B.F., Wallace, H., Keller, A., Heard, T.A., Jeffers, B., Drescher, N., Blüthgen, N., Leonhardt, S.D., 2017. Generalist social bees maximize diversity intake in plant species-rich and resource-abundant environments. *Ecosphere* 8 (3), e01758. <https://doi.org/10.1002/ecs2.1758>.
- Kaluza, B.F., Wallace, H.M., Heard, T.A., Minden, V., Klein, A., Leonhardt, S.D., 2018. Social bees are fitter in more biodiverse environments. *Sci. Rep.* 8, 12353. <https://doi.org/10.1038/s41598-018-30126-0>.
- Kasprzyk, I., Ćwik, A., Kluska, K., Wójcik, T., Cariñanos, P., 2019. Allergenic pollen concentrations in the air of urban parks in relation to their vegetation. *Urban For. Urban Green.* 46, 126486. <https://doi.org/10.1016/j.ufug.2019.126486>.
- Klaus, F., Tscharnkte, T., Bischoff, G., Grass, I., 2021. Floral resource diversification promotes solitary bee reproduction and may offset insecticide effects—evidence from a semi-field experiment. *Ecol. Lett.* 24 (4), 668–675. <https://doi.org/10.1111/ele.13683>.
- Konrad, R., Ferry, N., Gatehouse, A., Babendreier, D., 2008. Potential effects of oilseed rape expressing oryzacystatin-1 (OC-1) and of purified insecticidal proteins on larvae of the solitary bee *Osmia bicornis*. *PLoS One* 3 (7), e2664. <https://doi.org/10.1371/journal.pone.0002664>.
- Kratschmer, S., Kriechbaum, M., Pachinger, B., 2018. Buzzing on top: linking wild bee diversity, abundance and traits with green roof qualities. *Urban Ecosyst.* 21 (3), 429–446. <https://doi.org/10.1007/s11252-017-0726-6>.
- Kratschmer, S., Petrović, B., Curto, M., Meimberg, H., Pachinger, B., 2019. Pollen availability for the Horned mason bee (*Osmia cornuta*) in regions of different land use and landscape structures. *Ecol. Entomol.* 45 (3), 525–537. <https://doi.org/10.1111/een.12823>.
- Krunić, M.D., Stanisavljević, L., 2006. Population management in the mason bee species *Osmia cornuta* and *O. rufa* for orchard pollination in Serbia (Hymenoptera: megachilidae). *Entomol. Gen.* 1, 27–38.
- Lenda, M., Knops, J.H., Skórka, P., Morón, D., Woyciechowski, M., 2018. Cascading effects of changes in land use on the invasion of the walnut *Juglans regia* in forest ecosystems. *J. Ecol.* 106 (2), 671–686. <https://doi.org/10.1111/1365-2745.12827>.

- Leonhardt, S.D., Lihoreau, M., Spaethe, J., 2020. Mechanisms of nutritional resource exploitation by insects. *Insects* 11 (9), 570. <https://doi.org/10.3390/insects11090570>.
- MacIvor, J.S., Cabral, J.M., Packer, L., 2014. Pollen specialization by solitary bees in an urban landscape. *Urban Ecosyst.* 17 (1), 139–147. <https://doi.org/10.1007/s11252-013-0321-4>.
- Maechler, M., Stahel, W., Ruckstuhl, A., Keller, C., Halvorsen, K., Hauser, A., Buser, C., 2020. Package 'sfsmisc'. <https://CRAN.R-project.org/package=sfsmisc>.
- Majewska, A.A., Altizer, S., 2020. Planting gardens to support insect pollinators. *Conserv. Biol.* 34 (1), 15–25. <https://doi.org/10.1111/cobi.13271>.
- Moquet, L., Mayer, C., Michez, D., Wathelet, B., Jacquemart, A.L., 2015. Early spring floral foraging resources for pollinators in wet heathlands in Belgium. *J. Insect Conserv.* 19 (5), 837–848. <https://doi.org/10.1007/s10841-015-9802-5>.
- Newman, P., 2016. Sustainable urbanization: four stages of infrastructure planning and progress. *Adv. Sust. Urban.* 1 (1), 3–10.
- Nichols, R.N., Goulson, D., Holland, J.M., 2019. The best wildflowers for wild bees. *J. Insect Conserv.* 23 (5–6), 819–830. <https://doi.org/10.1007/s10841-019-00180-8>.
- Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M.H., Oksanen, M.J., Suggests, M.A., 2007. The Vegan Package: Community Ecology Package, 5-4(10). R package version 2, pp. 631–637. <https://CRAN.R-project.org/package=vegan>.
- Ollerton, J., 2017. Pollinator diversity: distribution, ecological function, and conservation. *Annu. Rev. Ecol. Evol. Syst.* 48, 353–376. <https://doi.org/10.1146/annurev-ecolsys-110316-022919>.
- Ollerton, J., 2021. *Pollinators and Pollination: Nature and Society*. Pelagic Publishing, Exeter.
- Ostaf, D.P., Mosseler, A., Johns, R.C., Javorek, S., Klymko, J., Ascher, J.S., 2015. Willows (*Salix* spp.) as pollen and nectar sources for sustaining fruit and berry pollinating insects. *Can. J. Plant Sci.* 95 (3), 505–516. <https://doi.org/10.4141/CJPS-2014-339>.
- Ouvrard, P., Transon, J., Jacquemart, A.L., 2018. Flower-strip agri-environment schemes provide diverse and valuable summer flower resources for pollinating insects. *Biodivers. Conserv.* 27 (9), 2193–2216. <https://doi.org/10.1007/s10531-018-1531-0>.
- Pamminger, T., Becker, R., Himmelreich, S., Schneider, C.W., Bergtold, M., 2019. Pollen report: quantitative review of pollen crude protein concentrations offered by bee pollinated flowers in agricultural and non-agricultural landscapes. *PeerJ* 7, e7394. <https://doi.org/10.7717/peerj.7394>.
- Persson, A.S., Mazier, F., Smith, H.G., 2018. When beggars are choosers – how nesting of a solitary bee is affected by temporal dynamics of pollen plants in the landscape. *Ecol. Evol.* 8 (11), 5777–5791. <https://doi.org/10.1002/ece3.4116>.
- Peters, B., Gao, Z., Zumkier, U., 2016. Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: effects on red mason bees (*Osmia bicornis*). *Ecotoxicology* 25 (9), 1679–1690. <https://doi.org/10.1007/s10646-016-1729-4>.
- Pinilla-Gallego, M.S., Isaacs, R., 2018. Pollen use by *Osmia lignaria* (Hymenoptera: megachilidae) in highbush blueberry fields. *Ann. Entomol. Soc. Am.* 111 (6), 335–340. <https://doi.org/10.1093/aesa/say028>.
- Poblador, S., Lupon, A., Martí, E., Sabater, F., Sabatè, S., Bernal, S., 2019. The influence of the invasive alien nitrogen-fixing *Robinia pseudoacacia* L. On soil nitrogen availability in a mixed Mediterranean riparian forest. *Eur. J. For. Res.* 138 (6), 1083–1093. <https://doi.org/10.1007/s10342-019-01226-x>.
- Radmacher, S., Strohm, E., 2010. Factors affecting offspring body size in the solitary bee *Osmia bicornis* (Hymenoptera, Megachilidae). *Apidologie* 41, 169–177. <https://doi.org/10.1051/apido/2009064>.
- Raw, A., 1974. Pollen preferences of three *Osmia* species (Hymenoptera). *Oikos* 25 (1), 54–60.
- Rollings, R., Goulson, D., 2019. Quantifying the attractiveness of garden flowers for pollinators. *J. Insect Conserv.* 23 (5–6), 803–817. <https://doi.org/10.1007/s10841-019-00177-3>.
- Roswell, M., Dushoff, J., Winfree, R., 2019. Male and female bees show large differences in floral preference. *PLoS One* 14, e0214909. <https://doi.org/10.1371/journal.pone.0214909>.
- Ruddle, N., Elston, C., Klein, O., Hamberger, A., Thompson, H., 2018. Effects of exposure to winter oilseed rape grown from thiamethoxam-treated seed on the red mason bee *Osmia bicornis*. *Environ. Toxicol. Chem.* 37 (4), 1071–1083. <https://doi.org/10.1002/etc.4034>.
- Schenk, M., Krauss, J., Holzschuh, A., 2018. Desynchronizations in bee – plant interactions cause severe fitness losses in solitary bees. *J. Anim. Ecol.* 87 (1), 139–149. <https://doi.org/10.1111/1365-2656.12694>.
- Schindler, M., Peters, B., 2011. Mason bees *Osmia bicornis* and *Osmia cornuta* as suitable orchard pollinators? *Erwerbsobstbau* 52 (3/4), 111–116. <https://doi.org/10.1007/s10341-010-0118-z>.
- Sedivy, C., Dorn, S., 2014. Towards a sustainable management of bees of the subgenus *Osmia* (Megachilidae; *Osmia*) as fruit tree pollinators. *Apidologie* 45 (1), 88–105. <https://doi.org/10.1007/s13592-013-0231-8>.
- Sedivy, C., Müller, A., Dorn, S., 2011. Closely related pollen generalist bees differ in their ability to develop on the same pollen diet: evidence for physiological adaptations to digest pollen. *Funct. Ecol.* 25 (3), 718–725. <https://doi.org/10.1111/j.1365-2435.2010.01828.x>.
- Seidelmann, K., 2018. Optimal resource allocation, maternal investment, and body size in a solitary bee, *Osmia bicornis*. *Entomol. Exp. Appl.* 166 (9), 790–799. <https://doi.org/10.1111/eea.12711>.
- Simpson, G.L., 2009. Co-correspondence Analysis Ordination Methods. R package version 0.4-1. <http://cran.r-project.org/package=analogue>.
- Söderman, A.M., Street, T.I., Hall, K., Olsson, O., Prentice, H.C., Smith, H.G., 2018. The value of small arable habitats in the agricultural landscape: importance for vascular plants and the provisioning of floral resources for bees. *Ecol. Indic.* 84, 553–563. <https://doi.org/10.1016/j.ecolind.2017.09.024>.
- Spear, D.M., Silverman, S., Forrest, J.R., 2016. Asteraceae pollen provisions protect *Osmia* mason bees (Hymenoptera: megachilidae) from brood parasitism. *Am. Nat.* 187 (6), 797–803. <https://doi.org/10.1086/686241>.
- Steffan-Dewenter, I.N., Schiele, S., 2004. Nest-site fidelity, body weight and population size of the red mason bee, *Osmia rufa* (Hymenoptera: megachilidae), evaluated by mark-recapture experiments. *Entomol. Gen.* 27 (2), 123–131.
- Team, R.C., 2019. R: a Language and Environment for Statistical Computing, Version 3.0. 2. R Foundation for Statistical Computing, Vienna, Austria, p. 2018. <https://www.R-project.org/>.
- Teper, D., 2007. Food plants of the red mason bee (*Osmia rufa* L.) determined based on a palynological analysis of faeces. *J. Apic. Sci.* 51 (2), 55–62.
- Teper, D., Biliński, M., 2009. Red mason bee (*Osmia rufa* L.) as a pollinator of rape plantations. *J. Apic. Sci.* 53 (2), 115–120.
- ter Braak, C.J., Schaffers, A.P., 2004. Co-correspondence analysis: a new ordination method to relate two community compositions. *Ecology* 85 (3), 834–846. <https://doi.org/10.1890/03-0021>.
- Trinkl, Moritz, Kaluza, Benjamin F., Wallace, Helen, Heard, Tim A., Keller, Alexander, Leonhardt, Sara D., 2020. Floral species richness correlates with changes in the nutritional quality of larval diets in a stingless bee. *Insects* 11 (2), 125. <https://doi.org/10.3390/insects11020125>.
- Vaudo, Anthony D., Tooker, John F., Patch, Harland M., Biddinger, David J., Coccia, Michael, Crone, Makaylee K., Fiely, Mark, Francis, Jacob S., Heather M, Hines, Hodges, Mackenzie, Jackson, Stephanie W., Michez, Denis, Mu, Junpeng, Russo, Laura, Safari, Maliheh, Treanore, Erin D., Vanderplanck, Maryse, Yip, Eric, Leonard, Anne S., Grozinger, Christina M., 2020. Pollen protein: Lipid macronutrient ratios may guide broad patterns of bee species floral preferences. *Insects* 11 (2), 132. <https://doi.org/10.3390/insects11020132>.
- Venables, W.N., Ripley, B.D., 2002. *Modern Applied Statistics With S*, fourth edition. Springer-Verlag, New York. ISBN 0-387-95457-0.
- Willmer, P.G., Cunnold, H., Ballantyne, G., 2017. Insights from measuring pollen deposition: quantifying the pre-eminence of bees as flower visitors and effective pollinators. *Arthropod. Interact.* 11 (3), 411–425. <https://doi.org/10.1007/s11829-017-9528-2>.
- Wilson, R.S., Keller, A., Shapcott, A., Leonhardt, S.D., Sichel, W., Hardwick, J.L., Heard, T.A., Kaluza, B.F., Wallace, H.M., 2021. Many small rather than few large sources identified in long-term bee pollen diets in agroecosystems. *Agric. Ecosyst. Environ.* 310, 107296. <https://doi.org/10.1016/j.agee.2020.107296>.
- Wood, T.J., Holland, J.M., Goulson, D., 2016. Diet characterisation of solitary bees on farmland: dietary specialisation predicts rarity. *Biodivers. Conserv.* 25 (13), 2655–2671. <https://doi.org/10.1007/s10531-016-1191-x>.
- Yoon, H.J., Lee, K.Y., Kim, S.Y., Lee, Y.B., Kim, N., Jin, B.R., 2015. Effects of location, direction, altitude, and placement of trap nests on the rate of trap-nesting of *Osmia* solitary bees. *J. Asia Pac. Entomol.* 18 (4), 695–700. <https://doi.org/10.1016/j.aspen.2015.08.004>.
- Zurbuchen, A., Landert, L., Klaiher, J., Müller, A., Hein, S., Dorn, S., 2010. Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. *Biol. Conserv.* 143 (3), 669–676. <https://doi.org/10.1016/j.biocon.2009.12.003>.

**Oświadczenie Kandydata o zakresie wkładu merytorycznego  
w publikacjach, z których fragment stanowi  
samodzielną i wyodrębnioną część rozprawy doktorskiej**

Stopień naukowy, imię i nazwisko Kandydata  
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Kraków**

Oświadczam, że w pracy:

**Splitt, A.**, Skórka, P., Strachecka, A., Borański, M., & Teper, D. (2021). Keep trees for bees: Pollen collection by *Osmia bicornis* along the urbanization gradient. *Urban Forestry & Urban Greening*, 64, 127250. DOI: 10.1016/j.ufug.2021.127250

Mój udział był następujący:

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**Jestem głównym autorem koncepcji pracy i wszystkich jej założeń metodycznych. Zrealizowałam terenową część badań, opracowaniu statystycznym wyników oraz ich prezentacji graficznej. Przygotowałam pierwszą wersję manuskryptu, a następnie niosłam poprawki zasugerowane przez współautorów. Jako autor korespondencyjny uczestniczyłam we wszystkich etapach procesu redakcyjnego.**

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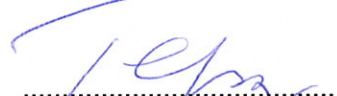
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