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Reproduction of the red mason solitary bee *Osmia rufa* (syn. *Osmia bicornis*) (Hymenoptera: Megachilidae) in various habitats

Monika FLISZKIEWICZ, Anna KUŚNIERCZAK and Bożena SZYMAŚ

Department of Apidology, Institute of Zoology, Poznań University of Life Sciences, Wojska Polskiego 71C, 60-625 Poznań, Poland;
e-mails: monef@up.poznan.pl; aniajuliak@gmail.com; beszymas@up.poznan.pl

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Abstract. *Osmia rufa* L. (*Osmia bicornis* L.) is a species of a solitary bee, which pollinates many wild and cultivated plants. A total of 900 cocoons containing mature individuals of *Osmia rufa* L. (450 females and 450 males of a known weight), were placed in each of four habitats (orchard, mixed forest, hay meadow and arboretum of the Dendrology Institute of the Polish Academy of Sciences at Kórnik). These bees were provided with artificial nests made of the stems of common reed. The following parameters were calculated: reproduction dynamics, total number of chambers built by females, mean number of breeding chambers per reed tube and mean number of cocoons per tube. Included in the analysis were also the nectar flowers and weather conditions recorded in each of the habitats studied. General linear mixed models indicated that the highest number of chambers was recorded in the hay meadow (6.6 per tube). However, the number of cocoons per tube was similar in the hay meadow, forest and orchard (4.5–4.8 per tube) but was significantly lower in the arboretum (3.0 cocoons per tube on average). Also, the highest reproduction coefficient, 317.5%, was recorded in the hay meadows. Some nests were also parasitized and the highest mean number of infected chambers per tube (0.68) was recorded in the forest. The number of chambers was positively related to temperature. These results indicate that habitat had a significant effect on the reproduction of the red mason bee.

INTRODUCTION

A dramatic decrease in the abundance of pollinators has been reported worldwide over the past decade (Watanabe, 1994; Matson et al., 1997; Delaplane & Mayer, 2000; Aizen et al., 2008; Spivak et al., 2011). This decline is caused by various factors (Brown & Paxton, 2009), including habitat loss, urban development (McIntyre & Hostetler, 2001; Zanette et al., 2005), high use of chemicals in agriculture (Johansen et al., 1983; Pham et al., 2002; Mullin et al., 2010) as well as the spread of diseases and parasites of bees (Cox-Foster et al., 2007; Johnson et al., 2009; Dietemann et al., 2012). Bees are important for the human economy (Cheung, 1973; Allen-Wardell et al., 1998; Knight et al., 2005; Klein et al., 2007; Kremen et al., 2007; Aizen et al., 2008). On the one hand, the number of species described grew due to the increasingly better and improved methods of apid fauna analysis (McGinley, 1986; Williams et al., 1991; Gräfenhan et al., 2001) and research conducted in areas that had previously been unexplored or little studied (Celary, 2007).

The decrease in bee populations could be alleviated by breeding honey bees and some species of solitary bees of the superfamily Apoidea (Michener, 2000). A number of methods of breeding wild bees have been developed for commercial use; i.e. pollinating fruit, seed and greenhouse crops (Wójtowski, 1971; Raw, 1972; Parker et al., 1987; Torchio, 1991; Bosch, 1994b; Delaplane & Mayer, 2000; Kemp, 2000). Such bees complement honey bees in their pollination of plants (Franklin, 1952). Controlled breeding allows not only for effective reproduction but also reintroduction of many species of bee (Wójtowski et al., 1991; Goulson et al., 2005). However, the effectiveness of such controlled breeding may depend on the habitat or type of crop. This has yet to be investigated.

The red mason bee (*Osmia rufa* L. – syn. *Osmia bicornis* L.) is a solitary, univoltine species of bee, which is an important pollinator of many wild and domestic plants (Holm, 1973; Bosch & Kemp, 2002; Biliński & Teper, 2003). Both adult males and females diapause in cocoons. In spring, males and females emerge from their cocoons, mate, and females then start nest building. Nests are usually built in irregular cavities in stones and old walls or in the hollow stems of plants, particularly the stems of reeds. The female bees build linear nests comprised of chambers, separated by clay mortar mixed with saliva. Osmia is a progressive provisioner, which means that females complete each cell (including provisioning and egg laying) before starting the next chamber (Delaplane & Mayer, 2000). Offspring complete their development before autumn (Tasey, 1973; Radmacher & Strochm, 2011).

On average, males live for 4–6 weeks and females for 8–10 weeks (Raw, 1972; Delaplane & Mayer, 2000)

The aim of this paper is to study the effect of different habitats on reproduction of the red mason bee using artificial nests.

MATERIAL AND METHODS

The study was carried out in 2010, in four different habitats (orchard, mixed forest, hay meadow and the arboretum of the Institute of Dendrology, Polish Academy of Sciences) in the Kórnik commune, district of Poznań. Table 1 presents descriptions of the four habitats, including their area and flora. The distance between each of them was approximately 8 km. This is the first time the red mason bee has been bred in these habitats. Three field shelters were placed 50 m apart in each habitat (Fig. 1). In the previous year, several groups of empty nests were placed in each of the habitats in order to check for the presence of a natural *Osmia rufa* population. Only 2 to 4% of the nests were occupied, which indicates there were no large, stable populations in the area taking into account the fact that *Osmia rufa* usually nests in the vicinity of their maternal nesting area (Raw, 1972). Therefore, we assumed that in the course of the experiment, the red mason bee females provisioning the nests are those that emerged from the cocoons we placed in each habitat.

In each field shelter there were 4 groups of nests made of 18 cm long sections of stems of the common reed [*Phragmites australis* (Cav.) Trin. ex Steud]. The cross-section of the reed stems was about 6 to 9 mm. In one group of nests, there were about 80 to 90 empty stems, whose inlets pointed south-east. Artificial aggregations of the red mason bee (cocoons with mature individuals), consisting of 300 individuals (150 females and 150 males of a determined weight), were placed in cardboard incubators (Wójtowski et al., 1991) at all locations on 30 April. One aggregation was placed in each field shelter. The cocoons used in this study were produced by the Department of Apidology, Institute of Zoology Poznan University of Life Sciences. In total, we used 12 field shelters, 48 groups of 78 nests, 3600 cocoons and 4080 empty reed stems. The groups of nests were monitored once a week to check for nesting activity. The reed stems sealed by female bees were counted and marked with paint. The first stems nested in were recorded on the 22nd of May and the last on the 3rd of July. This provided the information for determining the reproductive dynamics of the red mason bee in each of the habitats. The period of observation covered the entire period of the red mason bee's activity; i.e. until the end of the flight period in the first ten days of July.

As the maximum flight range of *Osmia rufa* females is 600 m (Gathmann & Tscharntke, 2002; Raw, 1972) we recorded all the nectar producing plants in each habitat within a radius of 500 m. In addition, we recorded the average daily temperature (checked daily between noon and 1 p.m., and then estimated a monthly mean), cloudiness (%) and precipitation (mm) during the experiment.

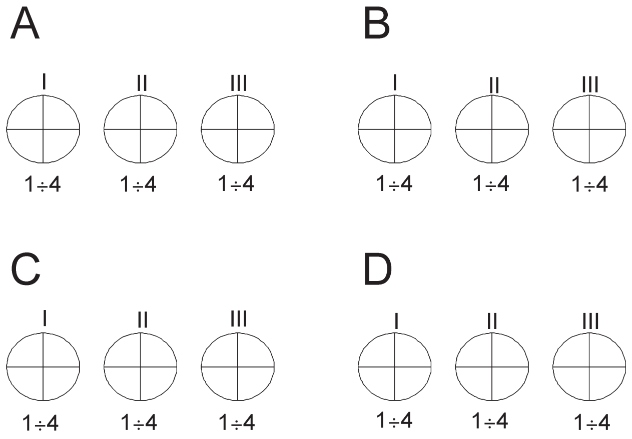

Fig. 1. Experimental design. A – arboretum; M – meadow; C – forest; D – orchard; I, II, III – field shelters; 1÷4 – nests.

The analysis of the nests was carried out at the end of the bees' development period (October). We determined the number of reed stems in each nest, and number of breeding chambers and number of fully formed cocoons of *Osmia rufa* L. in each stem. This data was later used to determine the nesting dynamics and reproduction coefficient. Nesting dynamics was defined in terms of the number of chambers constructed per week and the reproduction coefficient in terms of the number of fully formed cocoons of *Osmia rufa* L. divided by the number of cocoons placed initially in each habitat, which was expressed as a percentage. We also determined the number of brood chambers attacked by parasites and tried to identify them. To test if temperature, rainfall and date in the season affected the number of chambers built we used a general linear model (GLM). This model included the weekly averages of temperature, rainfall and number of chambers built. The quadratic term of date in the season was included in this model. We used backward stepwise selection of variables based on the Akaike information criterion (Burnham & Anderson, 2002) corrected for small sample size (AICc) to identify the most

TABLE 1. Habitat characteristics.

Habitat	Characteristics	Flora
Arboretum (1)	The arboretum of the Institute of Dendrology, Polish Academy of Sciences comprises an extensive collection of woody plants and bushes from the temperate zone of the northern hemisphere (Europe, Asia, North America), and covers an area of 50 ha.	*Cotoneaster lucidus* (Schltdl.), *Viburnum fragrans* (Bunge), *Viburnum Sieboldii* (Miq.), *Syringa vulgaris* (L.), *Syringa meyeri* (Palibin), *Azalea pontica* (L.), *Rhododendron* sp. (L.), *Eranthis hyemalis* (L.), *Fagus sylvatica* (L.), *Betula* (L.) sp., *Quercus* (L.) sp., *Tilia* (L.) sp., *Aesculus hippocastanum* (L.), *Cerasus* (Miller) sp., *Myosotis arvensis* (L.), *Laburnum* (Fabr.) sp., *Ranunculus acer* (L.), *Magnolia acuminata* (L.), *Magnolia × soulangeana.* (Soul-Bod.), *Daphne mezereum* (L.), *Laburnum anageroides* Medic.
Hay Meadow (2)	Semi-natural hay meadow in the second year of use (about 0.5 ha)	*Ranunculus acris* (L.), *Taraxacum officinale* (L.), *Lychnis flos-cuculi* (L.), *Leucanthemum vulgare* (Lam.), *Knautia arvensis* (L.), *Leontodon hispidus* (L.), *Tragopogon pratensis* (L.), *Vicia cracca* (L.), *Daucus carota* (L.), *Urtica dioica* (L.), *Campanula patula* (L.), *Myosotis arvensis* (L.), *Trifolium repens* (L.), *Trifolium pratense* (L.), *Cardamine pratensis* (L.), *Bellis perennis* (L.)
Forest (3)	Mixed forest, mostly with coniferous trees; chiefly pine-trees. Relatively numerous bushes in lower layers, herbaceous plants in the understorey and groundcover.	*Pinus* (L.) sp., *Picea* (Dietr) sp., *Abies* (Mill.) sp., *Fagus* (L.) sp., *Betula* (L.) sp., *Quercus* (L.) sp., *Populus* (L.) sp., *Frangula alnus* (Mill.), *Euonymus* (L.) sp., *Corylus* (L.) sp., *Rubus fruticosus* (L.), *Hypericum perforatum* (L.), *Convallaria majalis* L., *Hierocilum murorum* (L.), *Scorzonera humilis* (L.), *Oxalis acetosella* (L.)
Orchard (4)	Trees planted in five rows, each 200 m long (about 0.5 ha). Lawn of grass covered the ground between the trees.	Trees: *Persica vulgaris* (L.) aged 12 years, *Prunus domestica* (L.) aged 2 years and *Cerasus vulgaris* (Mill.) aged 8 years.

Table 2. Weather conditions during the experiment.

Observation period	Average daily temperature °C	Number of rainy days	Number of cloudy days
May 1st–31st 2010	18	21	19
June 1st–30th 2010	25	6	7
July 1st–10th 2010	27	1	6

parsimonious model. We considered models with ΔAICc values lower than 2 as equally good (Burnham & Anderson, 2002).

In order to estimate possible differences in (1) the mean number of sealed brood chambers per reed stem, (2) the mean number of fully developed cocoons in chambers per stem built by the females and (3) number of parasitized chambers per stem in different habitats, we used a general linear mixed model (GLMM). In this model type of habitat was a fixed categorical variable. Shelter and particular groups of nest were included as random factors. When analyzing the mean number of fully developed cocoons and the mean number of parasitized chambers we also included the total number of chambers built by the females in a stem as a covariate. This was done because the number of cocoons and parasitized chambers is constrained by the total number of chambers built by the female in a stem. We used contrast analysis to determine the means that differed significantly.

We used chi-square tests to determine whether there were differences between reproductive coefficients. We calculated expected values by summing the records for all the chambers built in the four habitats and dividing this value by four (assuming null hypothesis that there is no difference in the number of chambers built in the four habitats).

All means and function slopes are presented with standards errors (SE). We used the SPSS19 package for our analysis.

RESULTS

The dynamics of reproduction of the red mason bee was similar in all four habitats in peaking in the sixth week of female activity (Fig. 2). At that time, the bees sealed the highest number of stems in all four habitats. The most parsimonious model contained (AICc = 439.292) the effect of temperature and a non-linear effect of date. Other models lacked support (Δ AICc > 3). The number of chambers built increased with temperature (GLM $F_{1,33} = 4.54$, slope = 15.70 ± 6.80, $r^2 = 0.13$) after controlling for the non-linear effect of date (GLM $F_{1,33} = 5.99$, slope = –49.30 ± 20.19, $r^2 = 0.20$).

The nesting effectiveness in the four habitats is presented in Table 3.

As a similar number of reed stems were provided for the red mason bees there was a clear effect of habitat on the mean number of chambers built by females (GLMM $F_{3, 42} = 2.544$, P = 0.048) with the highest values recorded in the hay meadow and lowest in the forest (Table 3).

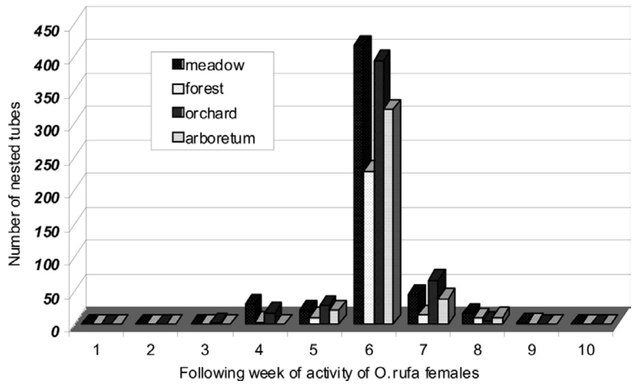

Fig. 2. Dynamics of reproduction of *Osmia rufa*.

The mean number of fully developed cocoons, which indicates the size of the next generation of the red mason bee, also differed between habitats (GLMM $F_{3,25} = 10.151$, P < 0.001). It can be concluded that the orchard (2858), hay meadow (3522) and forest (1793) provided similar conditions for bee development with the mean number of cocoons significantly higher than in the arboretum (1522) (Table 3). The lower number of cocoons in the arboretum does not appear to be associated with availability of flowering plants as blossom was abundant there throughout the entire period of the red mason bee's activity, which was very high (high number of breeding chambers supplied with pollen). Only half of the chambers in this habitat harboured fully developed insects of the next generation of bees.

The reproduction coefficient, which is the percentage growth of the population relative to the initial population in the different habitats, is presented in Table 4. The highest reproduction coefficient was recorded in the hay meadow (391.3%), was slightly lower in the orchard (317.5%) and lowest in the forest (199.2%) and arboretum (169.1%), where it was less than half of the value recorded in the hay meadow. These differences were statistically significant (χ^2 = 503.4, df = 3, P < 0.001).

Parasitization was highest in the forest, i.e. 9.5%, and significantly different from that recorded in the other habitats. The second highest was recorded in the hay meadow, where parasites were found in 5.9% of chambers, which is statistically different from that recorded in the other three habitats. On the other hand, in the arboretum and orchard the percentage of chambers attacked by parasites was 1.1% and 2.4% respectively, and these values do not differ from each other, but are different from those recorded in the hay meadow and forest (Table 5). The species composition of

Table 3. Effectiveness of nesting in the four habitats studied.

Habitat	Number of reed stems in nests	Number of stems used for nesting	Mean and total (in brackets) number of breeding chambers constructed	Mean and total (in brackets) number of fully formed cocoons
Arboretum	1075	530	6.3 ± 0.3 (3283)ab	3.0 ± 0.3 (1522)a
Hay meadow	1089	710	6.6 ± 0.3 (4734)a	4.6 ± 0.3 (3522)b
Forest	1083	420	5.6 ± 0.3 (2455)b	4.5 ± 0.3 (1793)b
Orchard	1093	631	6.0 ± 0.3 (3750)ab	4.8 ± 0.3 (2858)b

*Different lower-case letters indicate significant differences between means at α = 0.05 in GLMMs.

Table 4. Reproduction coefficient. Percentage growth of experimental populations of the red mason bee (*Osmia rufa* L.) in the different habitats.

Habitat	Initial number of cocoons (a)	Final number of cocoons (b)	Percentage growth (a/b)*100
Arboretum	900	1522	169.1%
Hay meadow	900	3522	391.3%
Forest	900	1793	199.2%
Orchard	900	2858	317.5%

the parasites recorded in the brood chambers was similar in all four habitats, where the following insects prevailed: *Chrysis ignita* L., *Monodontomerus obscurus* W. and *Sapyga quinguepunctata* F. (Hymenoptera), and: *Hemipenthes morio* L., *Antrax antrax* Sch. and *Cacoxenus indagator* L. (Diptera).

DISCUSSION

Beekeepers around the world are considering bees other than the honey bee as alternative pollinators of entomophillic plants (Torchio, 1991). In many countries there have been attempts to breed various species of bees and use them as alternative pollinators (Wójtowski, 1971; Raw, 1972; Parker et al., 1987; Torchio, 1991; Maeta, 1978; Bosch, 1994a; Delaplane & Mayer, 2000; Kemp, 2000). It is thus crucial for breeding to be as effective as possible. Our research has shown that one of the major factors determining the successful reproduction of the solitary bee, *Osmia rufa* L., is the habitat. Of the four habitats we analyzed, the hay meadow was the best in terms of the number of cocoons produced. It would take a few years for a naturally occurring red mason bee population to achieve the same result, even in a very favourable environment (Steffan-Dewenter & Schiele, 2008). Meadows are generally regarded as typical habitats of various pollinating insects in the temperate zone. Usually there is an abundance of flowering plants in meadows, which provide the bees with nectar and pollen (Moroń et al., 2009) and may account for their higher reproductive success there.

Bees of the family *Megachilidae* are commonly used as fruit tree pollinators (Ruszkowski et al., 1997; Delaplane & Mayer, 2000; Steffan-Dewenter, 2003). An orchard was included in our study and the estimated reproduction coefficient for *Osmia rufa* there was the second best. This indicates that this bee may not only be an important pollinator of fruit trees but also that orchards are a good habitat for this bee.

Osmia rufa is a polylectic bee, feeding on 23 to 30 plant species (Hallmenn & Leeuwen, 1990; Wilkaniec & Warakomska, 1992; Teper, 2007). Of the habitats studied the highest number of plant species was recorded in the arboretum, in which the bees had the lowest reproduction coefficient. One reason for this might be the abundance in this habitat of plants toxic to bees; e.g. *Thymelaeaceae* (Juss.), *Rhododendron* (L.), *Laburnum* (Fabr.) and *Aesculus* (L.) (Palmer & Jones, 1964; Ott, 1998). We did not identify the pollen in breeding chambers however, these plants were very common within the flight range of female bees, which is 500 to 600 m (Raw, 1972; Gathmann & Tscharntke, 2002). The analysis of nests in this habitat revealed that a high percentage of the larvae were dead or parasitized (Fliszkiewicz et al., 2012).

The lowest reproduction coefficient was recorded in the forest, most probably due to the deep shade and high humidity prevailing there. It was also in this habitat that we recorded the highest percentage of breeding chambers invaded by other insects and attacked by parasites. This result was unexpected. However, forest edges in some cases are ecological traps for some insects due to the greater activity there of predators and parasites (Ries & Fagan, 2003). Also, certain species may disperse along forest edges (Lenda & Skórka, 2010). In addition, forests may offer protect from wind and as a result flight activity of parasites there is higher than in open landscapes (Öckinger & Smith, 2006). Parasitism is a serious threat to man-controlled production of solitary bees (Wójtowski & Szymaś, 1973; Szymaś, 1991; Krunić et al., 1995; Goodell, 2003). The other insects and parasites recorded in the in nests in this study were similar to those recorded in nests of other species of solitary bees (Wójtowski & Szymaś; 1973; Eves et al., 1980; Szymaś, 1991; Bosch, 1992; Felicioli & Pinzauti, 1998; Krunić et al., 2005).

Environmental conditions significantly affect the development of populations of bees belonging to the family Osmia (Ulbrich & Seidelmann, 2001). The numbers of females inhabiting nests was similar in the four habitats studied. The highest number of stems with chambers was recorded during the sixth week (June) of the period when the red mason bee females were active, coincided with an improvement in the weather, which indicates its importance for the successful reproduction of this species (Raw, 1972; Seidelmann, 1995). During the first 4 weeks of the

Table 5. The percentage of brood chambers parasitized and colonized by other faunal elements in the four habitats.

Habitat	Total number of brood chambers	Mean number ± SE and total percentage (in brackets) of brood chambers attacked by parasitoids and other faunal elements (%)
Arboretum	3283	0.07 ± 0.13 (1.1)a
Hay meadow	4734	0.35 ± 0.14 (5.9)ab
Forest	2455	0.68 ± 0.14 (9.5)b
Orchard	3750	0.13 ± 0.13 (2.4)ab

Different small letters indicate significant differences between means at α = 0.05 in GLMM.

study, the weather conditions were unfavourable because it rained frequently and was very cloudy, which decreased the flight activity of the females and probably caused a decrease in their reproductive activity. Other studies also indicate that the number of breeding chambers produced by females of the family Osmia is highly dependent on weather conditions and the availability of food (Torchio, 1985).

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