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Augmentation of managed populations of *Osmia cornuta* and *O. rufa* (Hymenoptera: Megachilidae) in Southeastern Europe

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Abstract. We describe augmentation of managed populations of *Osmia cornuta* and *O. rufa* in the vicinity of Belgrade (Serbia). Annual augmentation of *O. cornuta* populations was more than five-fold during the six years of our study. This was achieved by watering the soil near the *Osmia* augmentation shelters used for nest building. However, populations of *O. rufa* under the same treatment only doubled annually. Data are also presented on sex ratios in these managed populations, the sex ratio being an important factor in increasing population numbers and raising pollination efficacy. Sex ratio values (♂ : ♀) varied from 1.46 : 1 to 3.22 : 1 in the populations of *O. cornuta* and from 1.35 : 1 to 2.68 : 1 in those of *O. rufa*.

INTRODUCTION

A number of species of orchard bees are used commercially as pollinators in many countries. However, in view of its effectiveness, the practice can still be considered inadequately employed, especially in the Southeastern Europe, where – in contrast to the United States (Torchio, 1976; Batra, 1998; Bosch & Kemp, 2002) and Japan (Maeta & Kitamura, 1974; Sekita, 2001) – the tradition of commercial use of bees as pollinators is weakly developed at this time.

Previous to the current study, we attempted to augment *Osmia cornuta* and *O. rufa* populations in orchards. After efforts lasting several years, our population oscillated within the limits of 20,000 to 30,000 cocoons (Krunić et al., 1987). Reproduction of *O. cornuta* and *O. rufa* requires the presence of suitable nesting material [bundles of the common reed *Phragmites australis* (Cav.) ex Steud. and lamellar boxes (Krunić et al., 1995)] and abundant pasture (mainly fruit trees with extended flowering periods) in places less exposed to winds. However, even though we carefully selected such places over a long period of time (several years), we did not succeed in increasing the population to a level higher than about 50,000. It seemed at the time that dispersion of females was a significant constraint on population during this period (Bosch, 1994; Krunić et al., 2001). We chose to test this assumption by studying augmentation of *O. cornuta* and *O. rufa* in the vicinity of Belgrade (Serbia) by examining whether, alternatively, the sex ratio of both species in the managed populations is a factor important for augmentation.

MATERIAL AND METHODS

Cocoons were collected from naturally occurring populations of *O. cornuta* (Latr.) and *O. rufa* (L.) in places where they are usually found in abundance. This was most often around houses and other buildings roofed with the reed *P. australis*. Empty nesting material was placed as trap nests under the eaves of such buildings. The trap nests most often consisted of wooden blocks with inserted paper tubes (8 mm in diameter and 15 cm in length) and bundles of common marsh reeds.

We found it easiest to achieve augmentation of our populations of *O. cornuta* by forming shelters out of numerous bundles of the marsh reed *P. australis* and lamellar boxes. The shelters were put in places protected from strong winds among an abundance of fruit trees blooming at different times, including almond, apricot, cherry, plum, pear, apple, medlar, and quince. We called such a shelter an "Osmia augmentation shelter" (OAS). At the Grocka locality, an OAS with total height of 3 m was composed of four wooden stakes driven into the ground. Stakes were placed in each corner of each rectangular shelter (0.6 m in width and 3 m in length). Within the corners formed by these stakes were two planks, the first of which was positioned 1 m above the ground and supported by one central stake, while the second one was 1 m above the first. The entire structure was covered with a roof which protected nesting material from direct sunlight, rain, and wind (Fig. 1). During each season, 200 bundles of reeds were put on the first plank of the shelter; each consisted of 100 tubes with nodes in the middle and openings on both ends. The reed tubes differed in diameter and in depth, which ranged from 5 to 9 mm and from 15 to 25 cm, respectively. Twenty lamellar boxes each measuring 15 cm in width, 20 cm in height, and 12 cm in depth were put on the second plank and contained 120 tubes which were 8 mm in diameter and 12 cm in length (Krunić et al., 1995).

The area of ground close to the shelter was heavily watered every morning and evening during periods of bee nesting activity. When the fruit trees started to bloom, cocoons were transferred from a cold room to OASs at intervals of 7–15 days (on March 24 and April 2 in 1994; on March 10 and 25 as well as April 4 and 18 in 1995; on April 10 and 17 in 1996; on March 3, 16, and 25 in 1997; on April 5 and 20 in 1998; and on April 2 and 10 in 1999). The total number of cocoons per monitored shelter was about 10,000 to 15,000 (Table 1). Storing of cocoons at appropriate temperatures during the period of hibernation is of great importance not only for emergence dynamics, but also for individual viability, on which both successful pollination and augmentation depend (Bosch & Blas, 1994; Bosch & Kemp, 2000; Kemp & Bosch, 2000; Krunić et al., 2001). Temperatures in the cold room where cocoons were stored ranged from 2–6°C, and RH was about 50–60%. When cocoons were transferred to OASs at the end of February or in the first half of March, at which time almond and apricot trees often started blooming, they were stored at 4–6°C. The cocoons used at the

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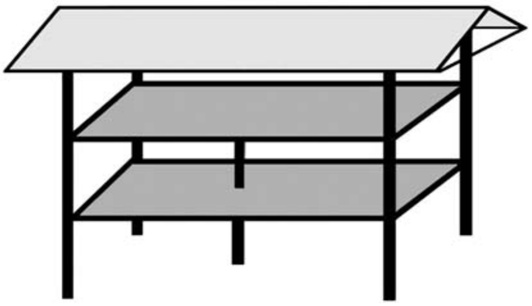

Fig. 1. Drawing of *Osmia* augmentation shelter (OAS).

end of March or in April, when apple trees most often bloom, were stored at 2–3°C. A week before transferring cocoons to OASs, the temperature in the cold room with cocoons was raised to about 10°C.

Cocoons were transferred to shelters in cardboard boxes with slits through which the bees could fly out. The boxes with cocoons in the shelter were protected from direct sunlight, rain, insectivorous birds, and mice. Smaller numbers of cocoons were transported in cardboard boxes because bees defecate immediately at emergence, which could foul unemerged and newly emerged bees. The cocoons were carried in layers of 3 to 4 cm. After sampling emergence rates, boxes with empty cocoons were burnt because their odour attracted many predators. By successively transferring cocoons several times within a short period (7 days), we were able to avoid the adverse effects of unfavourable weather conditions, which could sometimes decimate emerging females. This approach to augmentation was applied to manage populations from 1994 to 1999 for *O. cornuta* and from 1996 to 1999 for *O. rufa* in the vicinity of Grocka.

The total number of stripped *O. cornuta* cocoons from each OAS was estimated by randomly selecting 10 samples (100 ml) and extrapolating for the entire volume of cocoons in the OAS. Results are presented as values of the sample mean and the standard error.

The sex ratio in *O. cornuta* was followed at Grocka during the period of 1994–1999, while in *O. rufa* it was monitored at the same locality from 1996 to 1999. Sex ratios were determined on samples of cocoons randomly taken from the same OAS; each unemerged cocoon was placed in a small chamber (1×1×1 cm), and incubated. The sex ratio was calculated after this method was repeated several times.

RESULTS

After accidental bursting of a water main and subsequent moistening of the soil surrounding a building with managed populations of *O. cornuta* and *O. rufa* in the vicinity of Belgrade, a large number of females were observed taking mud and carrying it off to their nests. Reeds left under eaves of that building in only a week's time after the previous checking had tunnels that were almost 90% completed. Just a week before, the same reeds were only about 10% filled. That same day, around buildings several hundreds of meters away from the indicated place (where pasture conditions were similar, but the soil was not moistened), the filling of nesting material was found to range from 10 to 30%. All this happened during a period of warm and sunny weather without precipitation, when the soil in blooming orchards was fairly dry. In subsequent years, the method of soil watering was used to produce *O. cornuta* in the hundreds of thousands from relatively small initial populations. Evidently, abundant pasture and fair, warm weather ordinarily are not enough to produce optimal circumstances for augmentation of *O. cornuta* under our climatic conditions.

Augmentation of managed populations of *Osmia cornuta* and *O. rufa* was very different during the period of investigation (Table 1). That of *O. cornuta* increased more than five-fold (5.36- to 7.79-fold). On the other hand, *O. rufa* numbers only doubled at the same locality. The augmentation of *O. cornuta* in OASs during the study was never less than fivefold in relation to the number of cocoons taken outdoors (Table 1). In some years, the majority of bundles and lamellar boxes set out in OASs exhibited almost 100% nest fill. Under conditions of constant watering of the soil (several times a day when warm dry winds were blowing), the population of *O. cornuta* in our OASs multiplied as much or more than sevenfold in some years (Table 1).

After several years of practice in propagating these bees with supplementary watering of the soil, only *O. cornuta* reproduction can be elevated through augmentation, which can be seen from Table 1. Of all cocoons stripped in the fall from OASs, only up to 4% were cocoons of *O. rufa*.

The sex ratio (♂ : ♀) in our managed populations of *O. cornuta* and *O. rufa* at the monitored locality (Table 1) varied from 1.46 : 1 to 3.22 : 1 and 1.35 : 1 to 2.68 : 1, respectively. It can

TABLE 1. Multiplication and sex ratios in managed populations of *Osmia cornuta* and *O. rufa* in Grocka (20 km southeast of Belgrade); (Ni – number of introduced cocoons, RE – rate of emergence, Ns – number of stripped cocoons, RM – rate of multiplication, $\bar{x}$ – mean, s.e. – standard error of the mean, (N) – number of samples, (n) – size of samples.

Year	Ni	RE (n)	Ns	RM	$\bar{x}$ ± s.e. (N)	♂ : ♀ (n)
Osmia cornuta						
1994	~10000	71.35 (1522)	77910	~7.79	245.00 ± 5.81 (30)	1.58 : 1 (400)
1995	~10000	82.51 (1738)	61997	~6.19	251.00 ± 4.50 (30)	1.97 : 1 (400)
1996	~14000	93.99 (1381)	75081	~5.36	258.90 ± 4.68 (30)	3.22 : 1 (400)
1997	~14000	78.42 (2534)	102092	~7.29	248.40 ± 4.17 (30)	1.46 : 1 (2500)
1998	~14000	89.57 (2723)	77924	~5.57	253.00 ± 3.33 (30)	2.24 : 1 (1000)
1999	~14000	92.34 (3245)	84886	~6.06	250.40 ± 5.52 (30)	1.72 : 1 (500)
Osmia rufa						
1996	~1000	94.32 (856)	1832	~1.83	53.40 ± 1.08 (10)	2.68 : 1 (200)
1997	~1000	92.51 (928)	2237	~2.24	52.30 ± 1.37 (10)	1.35 : 1 (1200)
1998	~1000	85.32 (1000)	2008	~2.01	51.00 ± 1.24 (10)	2.03 : 1 (500)
1999	~1000	83.15 (1000)	2143	~2.14	52.80 ± 1.07 (10)	1.64 : 1 (300)

be seen from Table 1 that the ratio of sexes varied significantly from year to year.

DISCUSSION

Both bee species utilize similar nesting niches. In the wider area of Belgrade, *O. rufa* appears in nature about 2 weeks later than *O. cornuta*, so that about 2/3 of the periods of their activities overlap. We still have no way of explaining why *O. rufa* in OASs did not increase significantly. It would seem that the available floral resources during our investigations were insufficient for significant augmentation of managed *O. rufa* populations. In Italy a managed population of *O. rufa* underwent multifold increase on rape plants, which was not the case with *O. cornuta* (M. Pinzauti, pers. commun.). We did observe that the more robust female of *O. cornuta*, when appropriating tunnels for nest establishment, sometimes takes over a nest already occupied by an *O. rufa* female. In this way, it probably forces the *O. rufa* female to establish its nest in a new empty reed or else leave the site. But that could not have been the main factor hindering augmentation of *O. rufa*, which was low even when the shelters contained enough narrow tubes suitable for its smaller females.

More than 10,000 cocoons should not be transferred to a single OAS because of the possibility of inadequate pollen levels from surrounding fruit trees at a particular site. Rather, it is more expedient to have several OASs at a distance of at least 500 m apart from each other, rather than one shelter with an excess of cocoons.

Depending on weather and availability of suitable floral resources in different orchards, two- to three-fold increases have been reported for *O. cornifrons* in apple orchards (Maeta, 1990), two-fold increases for *O. cornuta* in almond trees (Bosch, 1994), and five-fold increases for *O. lignaria* in cherry orchards (Bosch & Kemp, 1999).

The ratio of sexes in populations of *O. cornuta* and *O. rufa* is favourable if the number of males is between 3 : 1 and 1 : 1. Table 1 indicates that the sex ratio was unfavourable in both species in 1996 and 1998, since the number of males was more than twice as great as the number of females. In *O. cornuta* at the Grocka locality, the ratio in 1996 even achieved a value of 3.22 : 1. This result means that the season during which cocoon formation occurred was very unfavourable.

In conclusion, populations of *O. cornuta* can be reproduced and used as pollinators in the desired numbers in the vicinity of Belgrade only if suitable soil next to the OASs is constantly watered throughout the period of their activity. This presumes that sufficient pasture is available during that period, which lasts about 3 to 4 weeks. For reasons unknown to us, even with this approach, we did not achieve equally successful augmentation of *O. rufa* numbers. Sex ratios should also be considered, if possible, in establishing augmentation protocol for these species.

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