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SHORT COMMUNICATION

Supercooling points and diapause termination in overwintering adults of orchard bees *Osmia cornuta* and *O. rufa* (Hymenoptera: Megachilidae)

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Abstract

The orchard bee species *Osmia cornuta* (Latreille) and *O. rufa* (Linnaeus) are both European in distribution. *Osmia cornuta* is distributed in the central and southern half of the continent, while *O. rufa* is distributed additionally in more northern parts of Europe, including Great Britain. These bees over-winter in cocoons as diapausing adults. The freezing temperatures of overwintering adults of *O. cornuta* and *O. rufa* during diapause are similar: −30°C to −24°C and −31°C to −26°C, respectively. However, there are differences in the termination of their diapause. Under natural conditions in the wider area of Belgrade, *O. rufa* appears in spring about two weeks later than *O. cornuta*.

Keywords: diapause, supercooling, *Osmia*

Introduction

Osmia cornuta (Latreille) and *O. rufa* (Linnaeus) (Hymenoptera: Megachilidae) overwinter as diapausing adults and in that stage possess a significant ability to supercool (Stanisavljević, 2000). Supercooling refers to the maintenance of body water in the liquid state at temperatures below its freezing point. Both species belong to the group of freeze-avoidance insects (Salt, 1957, 1969; Zachariassen, 1985), that is they cannot survive freezing. Although their ranges coincide over wide areas, *O. rufa* is additionally distributed in more northern parts of Europe, including Great Britain (Peters, 1977). The fact that *O. rufa* is also found in colder parts of Europe suggest to us that it possesses certain adaptations to harsher climatic conditions which distinguish it from *O. cornuta*.

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The species *Osmia cornuta* and *O. rufa* represent recently commercialized orchard pollinators. In the wider area of Belgrade, *O. cornuta* and *O. rufa* have been raised for pollination of orchards since 1986 (Krunić *et al.*, 1987). The ratio of *O. cornuta* to *O. rufa* in natural and managed populations in the wider area of Belgrade was constant at about 95:5% in favour of *O. cornuta* in the period of 1987–2000 (Stanisavljević, 2000).

Osmia cornuta and *O. rufa* are highly specialized orchard pollinators with certain advantages (but also disadvantages) in relation to the honeybee, which is a universal pollinator. They are more effective than honeybees as pollinators of fruit trees. The *O. cornuta* female pollinates more than 90% of all visited flowers in an orchard, e.g. as much as 98.7% was recorded in the case of almond flowers in Spain. *Apis mellifera* Linnaeus (Hymenoptera: Apidae) nectar foragers pollinate only 10–40% and nectar-pollen foragers up to 70% of flowers visited (Bosch & Blas, 1994).

Instead of hundreds of honeybee workers, only a few nesting females of *Osmia* spp. are needed to pollinate a single blooming fruit tree. For example, a single hectare of apple or pear trees requires from two to four strong honeybee

colonies (McGregor, 1976; Free, 1993), but only 355–500 nesting females of *O. cornuta* (Vicens & Bosch, 2000; Ladurner *et al.*, 2004; Monzon *et al.*, 2004), 500–600 females of *O. cornifrons* (Radoszkowski) (Maeta, 1990; Batra, 1998), or 600–1800 females of *O. lignaria* Say (Rieckenberg, 1994). *Osmia cornifrons* is individually 80 times more efficient as a pollinator of the Delicious variety of apple than are honeybees (Maeta & Kitamura, 1981). Although these bees are more efficient orchard pollinators than honeybees, they are still not adequately managed in south-eastern Europe.

The species *O. cornuta* and *O. rufa* differ from honeybees in having a solitary way of life: they are gregarious and univoltine. Immediately following emergence in early spring (from February to April) and after mating, females start to establish nests. After building a nest and leaving their progeny in it, the females of *O. cornuta* and *O. rufa* die before their progeny emerge the following spring (Tasei, 1973a,b; Stanisavljević, 2000).

The life cycle of these species encompasses three periods: summer development (from eggs to adults), pre-wintering (adults at relatively high temperatures in the autumn), and wintering (adults at relatively low temperatures in winter) (Kemp *et al.*, 2004). Species of the subgenus *Osmia* (*Osmia*) overwinter exclusively as adults and are active in early spring (Bosch & Kemp, 2000). The length of the period of overwintering under different temperature conditions is of crucial significance for successful emergence and utilization of these bees as pollinators (Bosch & Blas, 1995; Stanisavljević, 2000; Bosch & Kemp, 2003).

The species *O. cornuta* is able to fly and successfully pollinate at temperatures of about 10–12°C in sunny, rainy, and windy weather (Vicens & Bosch, 2000, Stanisavljević, 2000), whereas it is known that honeybees and *O. rufa* do not forage at all at these temperatures. In the vicinity of Belgrade, *O. rufa* females are active at temperatures above 15°C (Stanisavljević, 2000). Honeybees start to forage only at temperatures above 13°C, while even higher temperatures, calm conditions, and sunny weather are required for successful pollination by honeybees (Free, 1993).

In the present paper, we compared supercooling points of *O. cornuta* and *O. rufa* during the adult diapause, as well as the nature of their diapause termination. An attempt is made to explain the differences in the time of their spring appearance in the wider vicinity of Belgrade.

Materials and methods

Cocoons of *O. cornuta* and *O. rufa* were collected from managed populations in the vicinity of Belgrade during the autumn of 1994, 1997, and 1998. The samples of cocoons were stored during overwintering periods as follows: during 1994/1995, under natural conditions in the vicinity of Belgrade (average temperature for this period was +5.2°C) and in a cold chamber at +4°C from 1 November 1994 to 1 May 1995; during 1997/1998, in a cold chamber at +3°C from 1 October 1997 to 15 April 1998; and during 1998/1999, in a cold chamber at +2°C from 1 October 1998 to 5 April 1999. Bees during the overwintering period were exposed to different temperatures in different seasons because only one cold chamber was available. The supercooling points were recorded for samples of 20 specimens (10 males and 10 females) of both species during intervals of approximately 15 days, from 26 December 1994 to 27 April 1995; 29 October 1997 to 6 April 1998; and 28 October 1998 to 17 March 1999. The cocoons with specimens were carefully affixed with glycerol grease to a Copper-Constantan (type T) thermocouple connected to a one-channel data logger (Honeywell, Electronik 15). After that, the temperature was decreased at a cooling rate of approximately 1°C min^{-1}, and supercooling point values were recorded at the beginning of the exothermy (release of heat) caused by the crystallization of supercooled body fluids (Lee, 1989). Cocoons were taken out of the cryostat after reaching the temperature of crystallization. Individuals were extracted from cocoons, weighed, and left in 70% ethyl alcohol in a freezer at −18°C.

Table 1. Supercooling points of *Osmia cornuta* and *O. rufa* during the overwintering period in natural conditions in the area of Belgrade (average 5.24°C) or in a cooling chamber at 2, 3 or 4°C.

	Osmia cornuta		*Osmia rufa*	
Date and temperature	$\overline{X}$(°C)	±SE	$\overline{X}$(°C)	±SE
Natural conditions, average +5.24°C				
26 Dec. 1994	−26.68	±0.33	−27.34	±0.39
12 Jan. 1995	−28.25	±0.36	−28.45	±0.35
30 Jan. 1995	−29.15	±0.46	−29.45	±0.39
15 Feb. 1995	−28.75	±0.44	−31.25	±0.42
01 Mar. 1995	−28.55	±0.42	−30.45	±0.20
19 Mar. 1995	−27.90	±0.41	−29.50	±0.39
05 Apr. 1995	−22.56	±0.30	−27.25	±0.43
19 Apr. 1995	−18.51	±0.36	−23.60	±0.33
27 Apr. 1995	−14.62	±0.37	−17.00	±0.30
Cold chamber +4°C				
26 Dec. 1994	−29.75	±0.31	−28.55	±0.19
12 Jan. 1995	−28.45	±0.29	−28.78	±0.30
30 Jan. 1995	−28.50	±0.21	−30.10	±0.13
15 Feb. 1995	−28.00	±0.10	−30.20	±0.11
01 Mar. 1995	−26.75	±0.15	−29.65	±0.26
19 Mar. 1995	−27.55	±0.25	−28.50	±0.28
05 Apr. 1995	−26.60	±0.13	−28.20	±0.30
19 Apr. 1995	−24.50	±0.20	−27.60	±0.24
27 Apr. 1995	−23.45	±0.28	−26.90	±0.32
Cold chamber +3°C				
29 Oct. 1997	−26.45	±0.32	−25.75	±0.31
12 Nov. 1997	−26.38	±0.31	−26.45	±0.41
26 Nov. 1997	−28.45	±0.33	−27.50	±0.21
11 Dec. 1997	−29.00	±0.33	−29.85	±0.32
29 Dec. 1997	−28.20	±0.37	−28.05	±0.26
19 Jan. 1998	−27.25	±0.43	−27.90	±0.24
09 Feb. 1998	−27.55	±0.27	−28.50	±0.35
23 Feb. 1998	−27.70	±0.36	−28.75	±0.40
10 Mar. 1998	−27.30	±0.45	−28.22	±0.26
06 Apr. 1998	−24.25	±0.38	−26.65	±0.27
Cold chamber +2°C				
28 Oct. 1998	−25.85	±0.44	−25.25	±0.29
15 Nov. 1998	−26.31	±0.17	−26.55	±0.26
01 Dec. 1998	−28.25	±0.36	−27.83	±0.38
15 Dec. 1998	−29.40	±0.17	−29.95	±0.33
30 Dec. 1998	−28.75	±0.23	−29.05	±0.38
15 Jan. 1999	−27.80	±0.41	−28.90	±0.35
30 Jan. 1999	−27.40	±0.38	−28.80	±0.25
15 Feb. 1999	−27.10	±0.29	−29.50	±0.40
01 Mar. 1999	−26.60	±0.27	−28.61	±0.37
17 Mar. 1999	−25.40	±0.33	−27.80	±0.27

Sample size: $n = 20$ for each date. $\overline{X}$, sample mean; SE, standard error.

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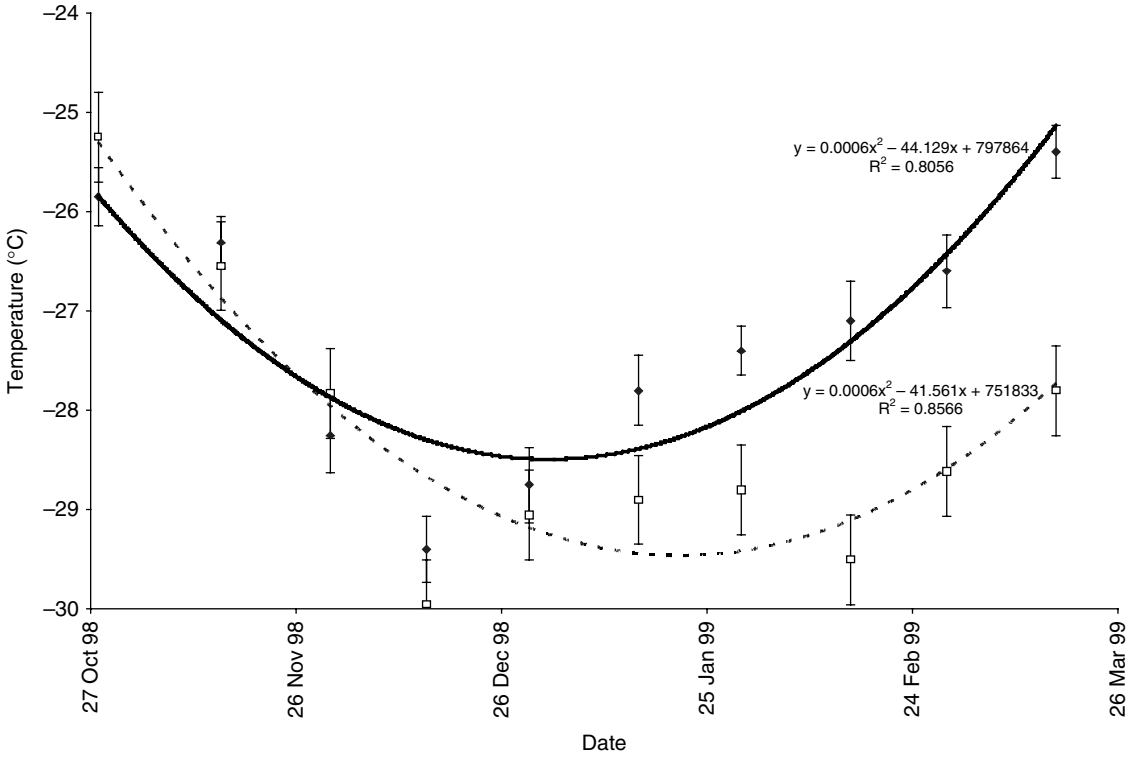

Fig. 1. Supercooling points of *Osmia cornuta* (◆) and *O. rufa* (□) during the overwintering period 1998/1999 at +2°C, and regression curves for the same (—, *O. cornuta*; - - -, *O. rufa*). ($\overline{X}$(°C) ± SE (n = 20)).

Results and Discussion

The species *Osmia cornuta* and *O. rufa* are resistant to low temperatures during hibernation and are even able to survive in laboratory conditions at temperatures far below −20°C. Overwintering adults of *O. cornuta* are cold tolerant and able to survive all temperatures above their supercooling points for a long time period (for example, one week at −15°C without any evident cold injuries (Krunić *et al.*, 1976)). Both species have approximately the same supercooling points (table 1). Their supercooling point values decline after the onset of diapause in October and during December and January. After that, when the diapause is probably completed, supercooling point values gradually increase until March (fig. 1).

The link between cold hardiness and diapause in many insects has not yet been fully elucidated, and it remains unclear whether they are independent or dependent or whether cold hardiness is simply part of the diapause syndrome (Denlinger, 1991). In many cases, cold hardiness ensures successful diapause during overwintering, thereby enabling the insect to survive the cold winter months. When diapause is completed, the concentration of cryoprotectors (for example, glycerol) and tolerance of low temperatures decline significantly, even under constant external temperature conditions. Induction of diapause is linked with increase in the level of glycerol, while termination of diapause results in rapid loss of glycerol, which is linked with freezing temperatures (Denlinger, 1991). Earlier investigations (Krunić *et al.*, 1976) indicated the presence of glycerol in amounts constituting 1.8% of fresh bodyweight in overwintering adults of *O. cornuta*. Further research on changes in the concentration of this and other cryoprotectors would be of great significance for clarifying the transitional period, i.e. the period during which diapause is completed, in these bees.

Adults of *O. cornuta* have the lowest supercooling point values of about −30°C during the winter period, and about −24°C in March, whereas these temperatures for *O. rufa* are about −31°C and −26°C, respectively. The supercooling point values for these two species slightly differ towards the end of diapause. With the approach of spring, the supercooling point values of *O. cornuta* increase faster than those of *O. rufa*. As a result, they probably complete their diapauses at different times. *Osmia cornuta* completes its diapause more abruptly in comparison to *O. rufa*. In *O. rufa*, supercooling point values increase gradually over a longer period (fig. 1). These supercooling point values explain why we find emerged specimens of *O. cornuta* but not *O. rufa* in the vicinity of Belgrade during spring after only a few days with daily temperatures above 15°C. Emerged specimens of *O. rufa* appear only when higher temperatures persist over a longer period of time.

The investigation of Kemp *et al.* (2004) involving measurement of oxygen consumption in adults of overwintering *O. lignaria* enabled them to conclude that the diapausing period for that species lasts about three months and is followed by a post-diapause period. This is in agreement with the lowest measured supercooling point values for *O. cornuta* and *O. rufa*. It follows that overwintering adults of *O. cornuta* and *O. rufa*, after a slight increase of supercooling point values, probably pass over into a

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post-diapause period during which they can be successfully incubated and used.

Taking into account all facts regarding advantages, shortcomings and differences in geographical distribution of the discussed pollinators, we recommend to fruit growers that honeybees and orchard bees in south-eastern Europe should be managed in the same environment, if possible. Only an adequate number of honeybees and native bees (solitary bees and bumblebees) can establish the kind of equilibrium of pollination in nature that existed before the excessive degradation of the environment caused by modern man, especially during the past hundred years.

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