Influence of the presence of *Cacoxenus indagator* Loew. parasite larvae in brood chambers on the emergence rate and size of red mason bees

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Summary

Cacoxenus indagator is one of cleptoparasites most frequently found in the nests of *Osmia bicornis* L. The goal of this experiment was to examine the influence of the presence of 2-3 *C. indagator* larvae in the brood chamber on the cocoon mass, on the mass and size of bee imagines, and on their emergence rate. During the analysis of red mason bee nest material, 200 cocoons were taken from brood chambers, each of them also containing 2-3 larvae of *C. indagator* (CC). The control group consisted of 200 randomly chosen cocoons from brood chambers with no parasites inside (CFFC). The cocoons and the emerged bees were weighed, and then the size of the bees was determined by the microscope image analysis software Axio Vision Rel. 4.0 coupled with a Stereo Lumar V12 stereoscopic microscope (Carl Zeiss, Germany). This involved measuring the sum of the widths of tergites 3 and 4, the distance between the wings and the forewing length and width. It was found that the presence of 2-3 *C. indagator* larvae in the brood chamber had no impact on the mortality of bees in cocoons. The research demonstrates that CC cocoons do not have to be removed when collecting cocoons from artificial nests in managed *O. bicornis* populations, as bees emerging from such cocoons are fully developed.

Keywords: *Osmia bicornis* L., *Cacoxenus indagator* Loew., cleptoparasites, body weight, cocoons

The body size of solitary bees is positively correlated with their fitness and has influence on the provisioning rate, provision mass, fecundity, offspring size and sex ratio (7, 11, 15-17). The body size of bees is strongly dependent on conditions prevailing during their larval period, most significantly on the provision weight (13, 16, 22), temperature (9, 13, 21), tunnel diameter and the natal nest size (12, 23).

Nests of solitary bees are occupied by characteristic accompanying and parasitic fauna, including the fly *Cacoxenus indagator* (Fig. 1). The number of *C. indagator* larvae in brood chambers varies. When the chamber is occupied by several or more fly larvae, the bee is unable to develop and dies. *C. indagator* might be called a facultative parasite which restricts the population of red mason bees (*Osmia bicornis* L.) (20) and other bees from the *Megachilidae* family,

Fig. 1. Larva, pupa and imago of *Cacoxenus indagator*

O. ligniaria (5) and *O. cornuta* (3, 4). *C. indagator* may cause significant damage, affecting as many as 30% of the chambers (8).

The mass of the cocoons and adult females and males of bees (*O. cornuta*) is strongly correlated with the provision weight (6). The research assumption was that bees coming from chambers containing cleptoparasite larvae (restricting the amount of provision for bee larvae) would be lighter and smaller. The aim of this experiment was to determine the influence of 2-3 *C. indagator* larvae present in the brood chamber on the cocoon mass, on the mass and size of bee imagines, and on their emergence rate.

Material and methods

The research was divided into two stages. The first stage was conducted in 2012 at the Apiculture Division of the Warsaw University of Life Sciences. The subject of the analysis were artificial nests made of common reed with a diameter of 7-8 mm, nested by *O. bicornis* in spring. The tubes were slit and 400 fully formed cocoons were taken out. Two hundred of them were taken from brood chambers which, apart from the cocoon, contained 2-3 larvae of *C. indagator* (CC – chambers with *Cacoxenus*) (Fig. 2.), whereas the other 200 were taken from brood chambers without any cleptoparasite larvae (CFFC – chambers with fully formed cocoons).

The cocoons were kept in cold storage at ca. 4°C until the end of April 2013 (until the end of the natural diapause). Next, each cocoon was weighed and put in a separate transport cage (cages used for transporting *Apis mellifera* L. queens, with two chambers, 3.5 cm × 4 cm and 2.5 cm × 3.5 cm with 27 slides (1 × 9 mm) in one of the sides. The cages with cocoons were stored at a temperature of 24°C and air humidity of 65%. After 3 days, bees started to emerge from the cocoons. The cages were checked twice a day. The emerged bees were weighed after excrete mycodium and then put back in the cages and killed by freezing. The emergence of the bees was observed for 3 weeks. After this period, the emergence rate was determined.

The second stage of the research was conducted in 2013 at the Department of Apidology of the Poznań University of Life Sciences. Morphometric measurements of all emerged bees were determined with a Stereo Lumar V12 stereoscopic microscope (Carl Zeiss, Germany) coupled through a camera with the microscope image analysis

software Axio Vision. The measurements were conducted at a magnification of 16-20 ×. The length and width of the left forewing, the width of dissected 3rd and 4th abdomen tergites and the distance between the wings were measured (1, 2, 22) to determine the size of the insects. Statistical analyses were performed with the statistical software SPSS 17. The distributions of the results were checked by the Kolmogorov-Smirnov and Shapiro-Wilk tests. One-way Anova and Univariate Anova were used for results with a normal distribution, and the Chi Square test was used for results which did not meet the normal distribution criteria.

Results and discussion

The emergence rate and sex distribution of bees from brood chambers infested by the cleptoparasite *C. indagator* (CC) and from those containing fully formed cocoons (CFFC). The presence of the cleptoparasite *C. indagator* in the brood chamber had no significant impact on the bees' emergence rate (Chi-Square test, $\chi^2 = 1.197$, $df = 1$, $p = 0.27$). More males than females emerged from cocoons in chambers infested by the cleptoparasite (CC). On the other hand, more females than males emerged from cocoons taken from CFFC (Tab. 1). After the bee emergence period ended, it was observed that parasite flies *Anthrax anthrax* emerged from some cocoons – 8 (4%) from CC and 1 from a CFFC (0.5%). Levene's test confirmed that in the CC and CFFC groups the homogeneity of variance was preserved ($L = 0.843$, $df_1 = 1$, $df_2 = 314$, $p = 0.359$). One-way ANOVA showed that the mean mass of randomly chosen fully formed cocoons from CFFC was significantly lower than the mean mass of cocoons taken from brood chambers containing 2-3 larvae of *C. indagator* (CC), ($F_{1, 316} = 7.149$, $p \leq 0.00$), (Tab. 2).

The influence of the presence of a parasite in the brood chamber on the cocoon mass and body mass of emerged bees, broken down by sex. The mean mass of cocoons taken from brood chambers containing 2-3 larvae of *C. indagator* (CC) did not vary significantly from the mean mass of cocoons from chambers free from the cleptoparasite (CFFC) for both sexes. Similarly, the body mass of the females and males which emerged from the CC cocoons did not differ significantly from the mass of females and males which emerged from CFFC cocoons (Tab. 3).

The influence of the presence of a parasite in the brood chamber on the size of emerged bees, broken down by sex. Anova did not show any differences in the mean distance between the wings or in the wing length and width of females and males emerged from CC and CFFC cocoons. Only the sum of the widths of the 3rd and 4th abdomen segments was significantly higher for females emerged from CC cocoons than for those from CFFC cocoons, and significantly lower for males emerged from CC as compared to those from CFFC (Tab. 4).

Provision mass is one of the most important factors influencing the offspring body size (7, 11, 15, 16, 18)

Fig. 2. Chambers with 3 larvae of *Cacoxenus indagator* and a fully formed cocoon

and sexual size dimorphism of Hymanoptera, including *O. bicornis* (19). On average, female larvae receive 89 mg of pollen, while males 49 mg (22). Larger bees are more fecund and lay larger eggs than smaller bees (11). Costs of reproduction are the main reason for female body loss, which is why at the end of the season larger bees have greater reproductive chances (19).

Earlier observations shows that the presence of 2-3 larvae of the *C. indagator* fly in the brood chamber influence larva development (14) and cocoon size (10). The present research has demonstrated that the presence of 2-3 larvae of *C. indagator* in the brood chamber

Tab. 1. Bee emergence rates for cocoons taken from infested chambers (CC) and from chambers free from the cleptoparasite *C. indagator* (CFFC)

Chambers	Number of cocoons	Number of emerged bees	Percentage of emerged bees	Number of emerged females	Number of emerged males
CC	200	144 ^a	73	62	82
CFFC	200	171 ^a	86	100	71
Total	400	316	79	162	153

Explanation: the same letters in columns indicate no significant differences in mean ranks of the groups, ChiSquare test, $p > 0.2$

Tab. 2. Mass of cocoons from infested chambers (CC) and from chambers free from the cleptoparasite *C. indagator* (CFFC)

Chambers	No.	Mean	SD	SE	Range	Skewness
CC	171	10.40 ^a	2.95	0.22	4.30-17.10	0.11 +/- 0.19
CFFC	145	9.45 ^b	3.34	0.58	3.20-19.20	0.58 +/- 0.20
Total	316	9.97	3.17	0.18	3.20-19.20	0.31 +/- 0.14

Explanation: a, b – means with different superscript letters differ significantly at $p < 0.00$

Tab. 3. Body mass of bees emerged from cocoons from infested chambers (CC) and from chambers free from the cleptoparasite *C. indagator* (CFFC)

Chambers	Sex of bees	Cocoon mass (mg)		Body mass (mg)	
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
CC	Female	122.34 $\pm$ 28.03 ^a	55-192	74.61 $\pm$ 19.32 ^a	25-115
	Male	73.94 $\pm$ 21.76 ^b	32-170	42.45 $\pm$ 12.55 ^b	17-80
CFFC	Female	121.11 $\pm$ 22.47 ^a	68-177	70.56 $\pm$ 13.57 ^a	30-98
	Male	77.86 $\pm$ 16.85 ^b	43-120	45.59 $\pm$ 12.51 ^b	23-88
Total		100.47	32-192	59.13	17-115

Explanation: a, b – means with different superscript letters differ significantly at $p < 0.05$

Tab. 4. Size of bees from infested chambers (CC) and from chambers free from the cleptoparasite *C. indagator* (CFFC), (mean $\pm$ SD)

Chambers	Sex of bees	Sum of widths of 3 rd and 4 th abdomen segments	Distance between the wings	Forewing length	Forewing width
CC	Female	3.86 $\pm$ 0.44 ^a	3.60 $\pm$ 0.35 ^a	8.88 $\pm$ 0.69 ^a	1.95 $\pm$ 0.17 ^a
	Male	3.24 $\pm$ 0.39 ^b	2.96 $\pm$ 0.29 ^b	7.31 $\pm$ 0.63 ^b	1.52 $\pm$ 0.18 ^b
CFFC	Female	3.83 $\pm$ 0.32 ^c	3.57 $\pm$ 0.32 ^a	8.68 $\pm$ 1.13 ^a	2.03 $\pm$ 0.78 ^a
	Male	3.47 $\pm$ 0.37 ^d	3.47 $\pm$ 0.37 ^b	7.63 $\pm$ 0.49 ^b	1.61 $\pm$ 0.15 ^b
Total		3.61	3.33	8.16	1.80

Explanation: a, b, c, d – means with different superscript letters differ significantly at $p < 0.05$

does not result in a decreased body mass of bees, and size differences are found only when measuring the sum of the widths of the 3rd and 4th abdomen tergites. The other parameters (wing length and width and the distance between the wings) show that bees from CC and CFFC cocoons do not vary in size. The results of this work also demonstrate that the presence of several cleptoparasite larvae has no impact on the emergence rate. During the analysis of nest material, it is often observed that chambers with fully formed cocoons also contain a certain amount of pollen, which has not been eaten by the bee larva. It can be conjectured that, under good foraging conditions, the provision mass is so large that the presence of 2-3 larvae of a cleptoparasite in the brood chamber does not impair the normal development of the bee larva. Moreover, the risk of parasitism in open cells is correlated with the provisioning time (17), which means that – if adequate food base is ensured – mason bee females do not leave the nest for long, preventing females of cleptoparasites from laying more than a couple of eggs.

The present study shows that more males than females emerge from CC cocoons. This may be caused by the fact that female eggs are placed at the back of each nest, while male eggs are placed in anterior chambers (14). *C. indagator* is probably more inclined to lay eggs in chambers located closer to the entrance than in those at the far end of the nest hole. As the way to the brood chamber is shorter, the cleptoparasite has better chances of laying eggs. If the nest is abandoned by a mason bee female for a long time, the brood chambers may be infested by two parasite species. As shown by the present research, in chambers with eggs laid by the fly *C. indagator*, imagines of the fly *Anthrax anthrax* were also found in cocoons.

In managed populations of red mason bees, it is possible to eliminate some factors restricting the growth of the population. Opening nest tubes and carefully analysing their contents makes it possible to remove dead or infested cocoons and parasites. Obtaining the largest possible filial generation of bees for pollinating crop plants and further reproduction is the main purpose of breeding this species (22).

Our research demonstrates that during the analysis of the nest material it is not necessary to remove cocoons from chambers containing a small number of *C. indagator* larvae, as fully formed and healthy bees emerge from such cocoons.

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