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Strepsiptera larvae in pollen collected by *Apis mellifera* L. (*)

Abstract - The finding of Strepsiptera first instar larvae in pollen loads of *Apis mellifera* L. is reported. The biology of some Strepsiptera involves, indeed, that the larvae are laid upon flowers and so they may be picked up by workers of honeybees collecting nectar and pollen. The samples of pollen loads were taken from hives situated in two sites of the central Italian Alps by means of special traps, which were inspected weekly from mid-April to mid-October over two years (1992 and 1993). The pellets were subjected to filth-test using an analytical method especially devised for the purpose, which permitted the extraction of Arthropods (insects and mites) and their fragments included in the pollen loads. Most of the specimens found were Strepsiptera first instar larvae, and they varied in number according to the season. In no case were observed stylopized bees: this would suggest that the presence of Strepsiptera larvae in the pollen loads is merely a causal consequence of the bees' antophylous behaviour.

Riassunto - *Larve di Strepsitteri in polline raccolto da* Apis mellifera *L.*

Si riferisce del ritrovamento di larve di prima età di Strepsitteri in pallottole di polline raccolte da *Apis mellifera*. La biologia di alcuni Strepsitteri prevede, infatti, che le larve siano deposte sui fiori e possano così essere raccolte dalle operaie di ape durante le loro visite per prelevare nettare e polline.
Il polline studiato proveniva da alveari situati in due località dei dintorni della città di Bergamo, ispezionati settimanalmente dalla metà di aprile sino alla metà di ottobre di due annate (1992 e 1993). I campioni così ottenuti sono stati sottoposti a filth-test applicando un metodo analitico che ha consentito di estrarre dalle pallottole di polline gli Artropodi (insetti e acari) e loro frammenti ivi inclusi. La maggior parte di questo materiale era composto da larve di prima età di Strepsitteri, in numero variabile di esemplari secondo la stagione. In nessun caso si sono osservate api stilopizzate: ciò fa ipotizzare che la presenza di larve di Strepsitteri nelle pallottole di polline sia soltanto casuale conseguenza dell'attività delle api.

Key words: *Apis mellifera*, Strepsiptera first instar larvae, pollen.

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INTRODUCTION

Several species of Arthropoda dwell in nests of *Apis mellifera* L.; they are parasitoid or prey on larvae, pupae and adult bees; they can be also hyperparasitoid, or commensal living off the society's production and waste. There are also various mites and insects that do not invade bee colonies actively and systematically but are often found there as occasional guests.

All these species together go to make up a rich and complex biological association whose components are linked up in a sort of constellation that varies according to the time of year and the different physical situations (Kistner, 1982; Kaczmarek, 1984, 1991; Schousboe, 1984).

This biocenosis has been studied little and published information on it is scarce, whereas, obviously, there is a wide range of biological and applied research regarding the parasites that endanger the survival of the colonies.

Among the various cases, a quite characteristic type of relationship can be set up between worker bees gathering pollen and Strepsiptera first instar larvae that Authors have called triungulin by merit of their similarity to the first larva stage of Coleoptera Meloidae and Ripiphoridae, though there is no morphological analogy as the Strepsiptera larvae have no claws (Kinzelbach, 1978; Kathirithamby, 1989; Luna de Carvalho & Kogan, 1991).

The relationship comes about during the flights that the bees make from flower to flower in their search for pollen. Indeed, the biology of many Strepsiptera decrees that the first instar larvae are laid by the females in large numbers on flowers, to await the arrival of insects, predestined victims for parasitization. When the bees collect the pollen and roll it into the characteristic pellets that they carry off to their nest, these larvae are included. In some cases, larvae of Strepsiptera were found on the floor of hives (Bolchi Serini & Sommaruga, 1983; Orantes Bermejo & Garcia Fernandez, 1995)

Little is known about the possibilities of parasitization of bees on the part of Strepsiptera. Some Authors (Borchert, 1970; Caron, 1978; Bailey, 1981), in specialized textes about honeybee pathology, report there are no sure data and list the species *Stylops aterrimus* Newport and *S. melittae* (Kirby) as occasional bee pests without further documentation. In particular, Adlakha and Sharma (1976) reported evidence of *Apis cerana* Fabr. parasitism by stylops. They found these bees in the Kangra Valley and Jamalpur (India) infested by stylops. The infested bees had a tendency to desert combs and formed small clusters on the bottom board or hive walls; they could not fly or sting. Heavily infested colonies were likely to perish.

Besides, in a study concerning research into impurities present in pollen for human consumption, Locatelli et al. (1992) found, among others Arthropoda, Strepsiptera first instar larvae. On the basis of this indication, we decided to exa-

mine pollen pellets from bees belonging to colonies situated in a hill zone of the central Alps, in order to extract arthropods, adult and preimaginal instars, or any fragments that might be included.

MATERIALS AND METHODS

The pellets of pollen for analysis were taken by means of appropriate traps at hives in two places near Bergamo city (Northern Italy). The samples were taken at weekly intervals over periods of varying duration from the beginning of April to the beginning of October during 1992 and 1993. Each sample was composed of 20 g of fresh pollen, consisting of about 2500 pellets.

To get out the Strepsiptera larvae and any eventual other Arthropoda or fragments included, we used the filth-test method (table 1), widely used for the extraction of impurities in a wide range of food products. In this case, the method applied was contrived by Locatelli et al. (1992).

Table 1 - Passages of the filth-test method adapted for pollen loads (Locatelli et al., 1992)

- Bring to the boil a solution of 400 ml of water and 35 ml of HCl 37%
- Add 10 g of pollen and boil for 30'
- When cold put in a 2 litre Wildman trap flask, adding 50 ml of a mixture of paraffin (85%) and n-heptane (15%)
- Subject to slow magnetic agitation for 2' and top up with water
- Allow to separate for 30', and vacuum filter the oily layer onto filter paper
- Top up again with the paraffin/n-heptane mixture, and filter after separation.

Apart from the method described, since the process may damage the specimens and because we sometimes noticed the tiny Strepsiptera larvae sticking out of the pellets, we tried to get them out by hand, in order to connect the number of the specimens with the blossom. Nevertheless, the technique was too arduous and laborious to be statistically reliable.

RESULTS AND DISCUSSION

The filth-test technique as applied to the pollen loads enabled us to get out a large number of Arthropoda, both adult and preimaginal instars.

Specific classification of the specimens is hardly ever possible, either because of the immaturity of the stage or because of damage caused either by the bees du-

Table 2 - Number of Strepsiptera first instar larvae included in pollen loads collected by workers of Apis mellifera *near Bergamo (Northern Italy) (Site A, southern suburb; site B, northern suburb).*

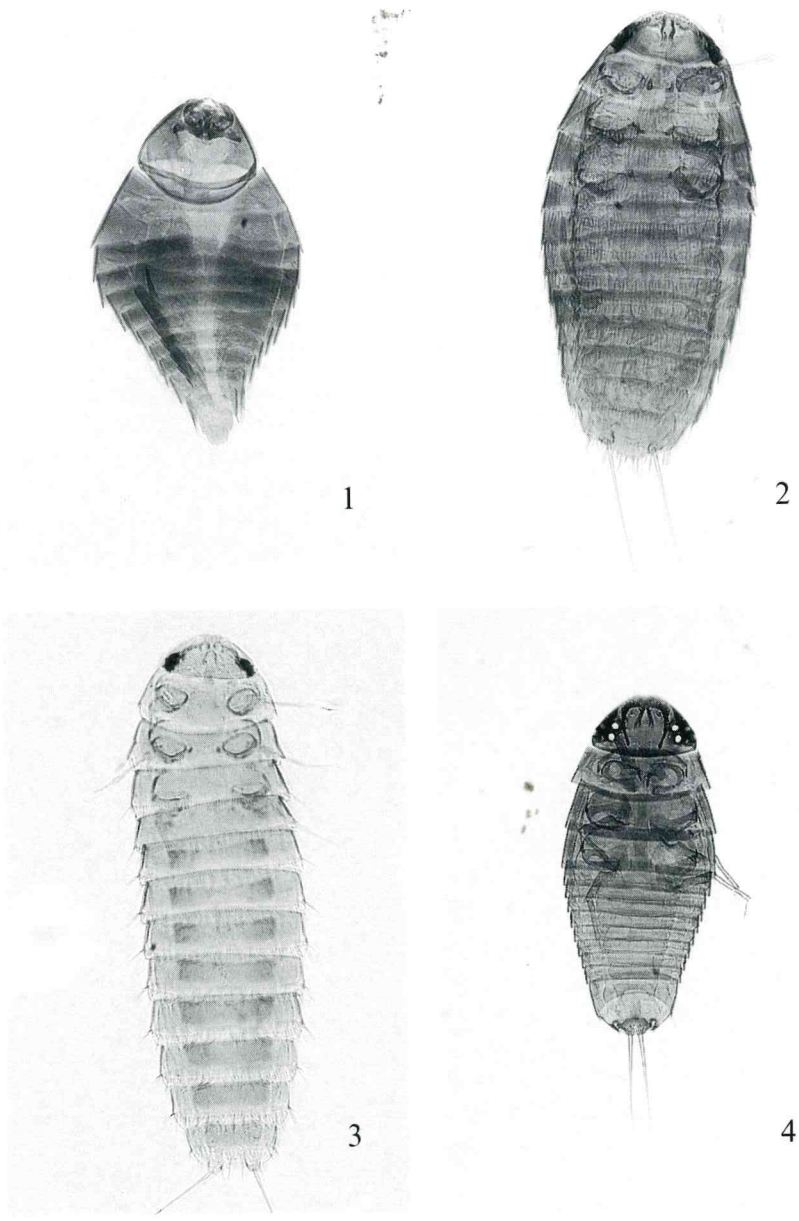
Year	Site A		Site B	
	1992	1993	1992	1993
Week				
14th (April)	—	0	—	—
15	—	0	—	—
16	—	0	3	—
17	5	4	4	34
18	3	25	40	83
19	2	11	19	362
20	6	22	776	980
21	0	18	761	464
22	2	6	44	13
23	3	2	4	0
24	0	0	1	2
25	3	8	0	4
26	22	2	0	0
27	0	9	0	0
28	2	3	0	3
29	69	24	0	14
30	203	140	—	31
31	84	47	—	19
32	13	19	—	1
33	6	19	—	0
34	3	6	—	0
35	0	0	—	0
36	0	0	—	0
37	0	0	—	0
38	0	0	—	0
39	—	0	—	0
40th (Oct.)	—	0	—	0

The hyphen indicates 'sample not collected'.

ring the collection of the pollen or by the subsequent extraction operation. In any case, we managed to separate the material into groups.

Nearly 79% of the material comprised first instar larvae of Strepsiptera; the remaining part included preimaginal instars of Thysanoptera (10%), planidia of parasitoid Hymenoptera (5%) (fig. 1), then, in the same proportion (about 2% each), preimaginal instars of Psylloidea and Aphidoidea, mites (*Prostigmata Tarsonemidae, Mesostigmata Gamasida* and *Cryptostigmata*).

Table 2 shows the trend regarding the larvae of Strepsiptera: it will be noted that most of them were found during May and at the end of July.



Figs. 1-4 - Planidium of parasitoid Hymenoptera (fig. 1); first instar larva of: *Stylops melittae* Kirby (fig. 2), *S. nitidiusculae* Poluszinsky (fig. 3) and *Pseudoxenos hydeni* Saunders (fig. 4).

Table 3 - Species of Strepsiptera found in pollen loads.

Stylops melittae Kirby (fig. 2)
Hosts: *Andrena nigreaenea* Kirby *A. haemorrhoea* (F.), *A. hatterfiana* (F.), *A. barbilabris* Kirby, *A. cineriana* (L.), *A. proxima* Kirby, *A. tibialis* Kirby, *A. wilkella* Kirby

Stylops nitidiusculae Poluszinsky (fig. 3)
Host: *Andrena nitidiuscula* Schenk

Stylops pasteelsi Luna de Carvalho
Host: *Andrena ramlehiana* Pérez

Pseudoxenos hydeni Saunders (fig. 4)
Host: *Odynerus deflendus* Saunders

The difficulty in the classification enabled us to determine for the greater part of the specimens examined only the fact of their belonging to the Stylopidae family. Certain specimens were attributed to genus *Stylops*. Finally, the species pointed out in table 3 were recognised. The table shows their hosts also, as taken from the literature (Hofeneder & Fulmek, 1942-1943; Ulrich, 1964; Luna de Carvalho, 1974; Darchen 1993). Amongst these only *S. melittae* Kirby, as already stated, can be considered a likely parasite of the *A. mellifera*. But as far as we are concerned, over a long period of observation we have examined and dissected many worker bees without ever having come across a stylopized one.

In conclusion, our observations offer a base for a series of possible developments. The analytical method makes possible taking out from the pollen loads brought home by worker bees the microarthropods that are wrapped up in them.

A census of the arthropodological fauna included in pollen loads of honey bees, if extended to cover other geographical areas, could prove to be the means of creating a network of information concerning species that are normally difficult to collect, and so to follow their biological evolution.

Furthermore, and more specifically, it may be presumed that in this way a more precise understanding of the relationship between *Apis mellifera* L. and Strepsiptera may be reached.

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