Egyptian Journal of Biological Pest Control

RESEARCH Open Access

Endo- or ecto-parasitism with the cluster fly, *Pollenia dasypoda* Portochisky (Diptera: Calliphoridae), based on the diameter of its host body, the earthworm *Allolobophora caligonosa* (Sav.)

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Abstract

Parasitism with the cluster fly, *Pollenia dasypoda* Portochisky (Diptera: Calliphoridae), in relation to its host, the earthworm *Allolobophora caligonosa* (Sav.), was studied at Giza region, Egypt. Also, different biological parameters of the parasitoid were studied. Natural parasitism rate during March 2019 ranged between 4 and 10% per 50 earthworms. In 6 samples (300 earthworms), the total rate of parasitism was 12.66%. Experimental parasitism by placing the newly hatched larvae (L_1) of *P. dasypoda*, directly on the bodies of their host worms, *A. caligonosa*, resulted to 100% parasitism in all replicates. An interesting phenomenon by larvae of the last instar (L_3) of *P. dasypoda* appeared during the study. This parasitoid is known as an endo-parasitoid on the earthworms, where the third instar larvae (L_3) complete their development inside the same parasitized earthworm. But under laboratory conditions of the present study, few individuals of L_3 became ecto-parasitoids on the parasitized earthworm. The reason behind this behavior was the diameter of the host (earthworm). Experimentally, the L_1 was placed on young earthworm, and when reaching the L_3, its body diameter became larger than that of its host. To avoid the pressure of the host body around L_3, it moves backward from the host body keeping its head with mouthparts inserted in the same location of initial parasitism, continuing feeding for 4–5 days as ecto-parasitoid, until fully developed. Then, it leaves the host and turns into a pupa adjacent to the corpus of its mostly dead host earthworm.

Keywords: *Pollenia dasypoda* Portochisky, Earthworms, *Allolobophora caligonosa*, Parasitism, Behavior

Background

The dipteran calliphorids, known as "cluster flies," include few endo-parasitoid species, usually recorded on different earthworm and snail species (Tawfik and El-Husseini 1971). As far as recorded in the international literature, there are more than 100 calliphorid species around the world in Palaearctic, Nearctic, Oriental, and Australasian Regions (Dear 1986; Rognes 1991, 1998). Earlier records showed that the cluster fly, *Pollenia rudis* (F.), parasitized earthworms in the USA and Europe, while *Onesia accepta* Mall. parasitized earthworms in Australia (Keilin 1911, 1915; Webb and Hutchinson 1916). In all studies, the cluster flies were found as endo-parasitoids during their larval stage of earthworms and snails. Among the available literature, less than 10 species were studied for their life cycles as predators or endo-parasitoids on earthworms (Keilin 1915; Tawfik and El-Husseini 1971; Yahnke and George 1972; Richards and Morrison 1972; Szpila 2003; Grzywacz et al. 2012).

In the present study, an astonishing phenomenon was noticed during studying the parasitism rate of *P. dasypoda* on *A. caligonosa* under laboratory enforcement. As usual, all the third larval instars of the parasitoid, *P. dasypoda* complete their development inside the body of the host. In some cases, the third instar larvae come out of the host body and complete their larval development feeding externally on the same host for 4–5 days. Thus, we looked after the reason(s) behind this phenomenon in which this endo-parasitoid acted as an ecto-parasitoid.

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Material and methods

Large numbers of the earthworm *A. caligonosa* were collected in March 2019 by digging in freshly irrigated soil cultivated with the Egyptian clover, *Trifolium alexandrinum* L., at the Experiment Station, Faculty of Agriculture, Cairo University, Giza, Egypt, and transferred to the Center of Biological Control for the study. The collected earthworms (300 individuals) were divided into 6 groups, each of 50 worms. The earthworms were examined individually under a stereoscope to separate those parasitized by larvae of the fly. The natural parasitism rate was estimated. Each parasitized earthworm was kept in a moistened field soil in a glass container covered with muslin cloth fitted in place with a rubber band until the parasitoid larvae left their hosts and pupate in the soil. The pupae were then collected by soft forceps and placed vertically in a moistened soil with the cephalic ends upwards and kept in caged until adult emergence. The emerged flies were kept in cloth ovipositional cages (20 × 20 × 30 cm) supplied with 5 Petri dishes (5 cm) filled with moistened field soil, as sites for oviposition, and a wetted piece of cotton wool soaked in a diluted bee honey (1:5 honey:water) as nutrient for the adult flies. They were left under laboratory conditions (25 °C) for incubation and hatching. The newly hatched larvae (similar to *Planidia*) were active in searching for their hosts (*A. caligonosa*) in the soil. Each larva was picked up by a tip of wetted fine camel brush and placed onto the body of a healthy earthworm, kept individually with moistened field soil in Petri dishes, where parasitism took place. Five replicates, each of 50 exposed earthworms, were used for the test besides the control (50 healthy untreated earthworms). Different biological parameters of *P. dasypoda* were measured: egg incubation period, larval durations and measurements, pre-pupal and pupal periods, adult fecundity, longevity, and sex ratio. The treated earthworms, as well as those of the control, were not all at similar sizes and/or ages because they were collected as wild individuals from the irrigated fields. Rate of parasitism among exposed earthworm individuals to the parasitoid larvae was under laboratory conditions calculated. Cases of ecto-parasitism by the third instar larvae (L_3) of *P. dasypoda* were counted, and the diameter/width of both the larva and its host (earthworm) was measured.

Results and discussion

Biological parameters

Egg stage

Laid eggs could be seen with naked eyes or small lens (× 10) characterized by their Perl-white color; when magnified, two longitudinal aeroscopic structures could be seen on the ventral side (Fig. 1a) through which the respiration of the embryo is facilitated in the moist soil.

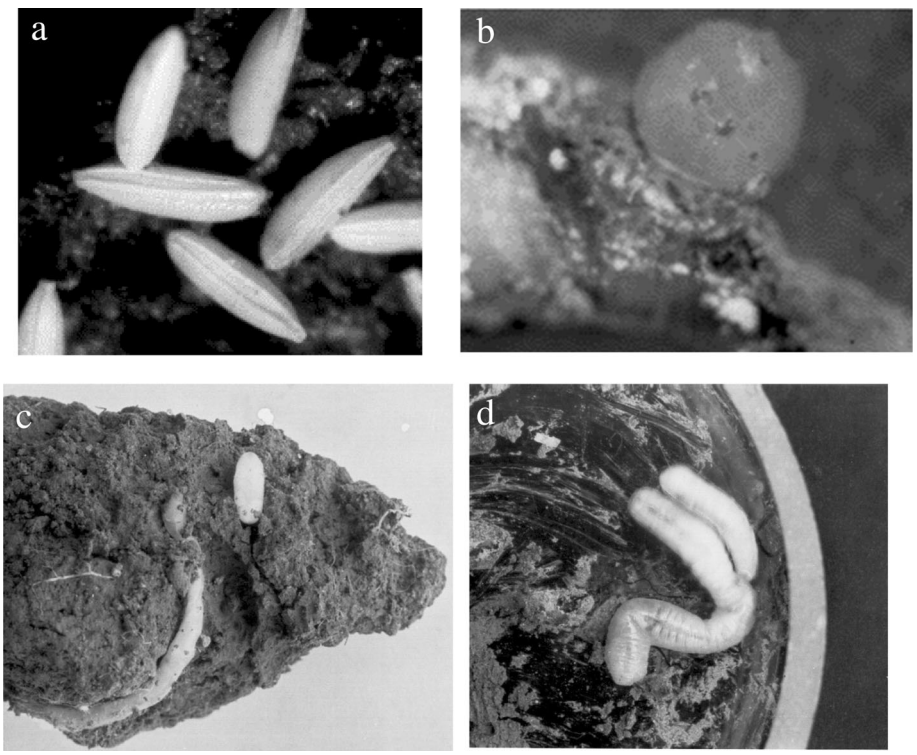

Fig. 1 *Pollenia dasypoda*. **a** Eggs magnified showing aeroscopic structure on the ventral side. **b** Posterior end of larva showing posterior spiracles protruding outside the host body (*Allolobophora caligonosa*) (endo-parasitism). **c** Freshly formed fly pupa adjacent corpus of the parasitized host. **d** Full-grown parasitic L_3 feeding externally on the earthworm (ecto-parasitism)

Table 1 Durations and measurements of the different immature stages of *P. dasypoda* ($n = 20$)

Developmental stages	Durations (/days)			Measurements (/mm)					
	Min.	Max.	Aver.	Length			Width		
				Min.	Max.	Aver.	Min.	Max.	Aver.
Egg	3	5	4.4	1.03	1.05	1.04	0.29	0.33	0.31
First larval instar (L_1)	4	6	5.2	1.03	1.05	1.04	0.29	0.33	0.31
Second larval instar (L_2)	3	4	3.5	2.56	2.86	2.60	0.80	0.91	0.83
Third larval instar (L_3)	6	7	6.8	40.23	43.22	41.55	4.11	4.89	4.44
Pre-pupal period	1	2	1.4						
Pupal period	19	23	20.6	34.2	39.4	37.45	3.11	3.45	3.14
Total period	36	47	41.9						

The chorion is sculptured with tiny pits and ridges forming a reticulate pattern. A minute funnel-like area at the anterior pole of the egg contained the micropile. Eggs were laid on soil surface on their ventral side which is flattened. The dorsal side of the egg is concaved. Data in Table 1 show that laid eggs measure 1.03 mm long and 0.31 mm in width. The incubation period under the experimental laboratory conditions ranged between 3 and 5 days with an average of 4.4 days (Table 1).

Larval stage

Morphological description of the three larval instars of *P. dasypoda* was provided by Tawfik and El-Husseini (1971). Data presented on Table 1 show that the duration of the first larval instar (L_1) ranged between 4 and 6 days with an average of 5.2 days. Its length and width averaged 1.03 and 0.31 mm, respectively. The second instar larva (L_2) developed in a period of 3–4 with an average of 3.5 days. On the average, it measured 2.60 and 0.83 mm in length and width, respectively. Meanwhile, the third instar larva (L_3) durated 6–7 days with an average of 6.8 days. It was the largest larval instar, and its length measured 41.55 mm on the average (40.23–43.22 mm) and 4.44 mm (4.11–4.89 mm) in width.

Table 2 Oviposition activity, fecundity, and longevity of *P. dasypoda* ($n = 20$)

Parameters	Min.	Max.	Aver.
Pre-oviposition period (/days)	8	21	11.36
Oviposition period	19	56	31.5
Post-oviposition period	0	54	18.9
Number of daily eggs laid	96	206	165.36
Total number of eggs laid	338	531	456.5
Female longevity (/days)	48	96	70.55
Male longevity	40	61	56.7

Pre-pupal and pupal stage

As seen in Table 1, the pre-pupal period from the time the third instar larva left its host to transform into pupa lasted 1–2 days with an average of 1.4 days. Turned into a pupa, its length measured 34.2–39.4 mm with an average of 37.45 mm. Its width ranged between 3.11 and 3.45 mm with an average of 3.14 mm. The pupal period ranged 19–23 days and averaged 20.6 days.

The total developmental period of the immature stages of *P. dasypoda* ranged between 36 and 47 days with an average of 41.9 days (Table 1).

Oviposition activity and adult longevity of P. dasypoda

The pre-oviposition period ranged between 8 and 21 days with an average of 11.36 days (Table 2). Meanwhile, this period was 19–56 (31.5) and 0–54 (18.9) days for the oviposition and post-oviposition periods, respectively. During a longevity period of 48–96 days (aver. 70.55 days), a single female laid a daily number of eggs ranging from 96 to 206 with an average of 165.36 eggs/day. The total number of eggs laid per female was 338–351 with an average of 456.5 eggs. Meanwhile, males lived shorter than females showing longevity of 40–61 days with an average of 56.7 days.

Sex ratio

Additional sampling of the earthworm *A. caliginosa* collected from *T. alexandrinum* fields resulted the emergence of 39 adult males and 51 adult females of *P. dasypoda.* Accordingly, the sex ratio is apparently 1:1.3.

Natural rate of parasitism

The field-collected individuals of the earthworm *A. caligonosa* showed a rate of 4–10% individuals parasitized by larvae of the cluster fly *P. dasypoda* during March 2019 (Table 3).

The total numbers of parasitized earthworms recorded 34 individuals out of the 300 field-collected individuals, representing 12.66% parasitism. Although the adult female of the cluster fly has a life span of 2 months on the average and a high fecundity (530 eggs) (Tawfik and El-Husseini 1971), this rate of parasitism seems to be less

Table 3 Numbers of healthy *A. caligonosa* and those naturally parasitized by *P. dasypoda* in March 2019 ($n = 300$)

Samples ($n = 50$)	1	2	3	4	5	6
Healthy	47	48	45	47	46	48
Parasitized	3	2	5	3	4	2
Parasitism % in samples	6	4	10	6	8	4
Total parasitism %	12.66 (38 parasitized earthworms out of 300 ones)					

than could be expected. But in nature, the first instar larva dies within 2–4 days, when failed to reach an earthworm. Thus, *A. caligonosa* poses such a high fecundity to keep existing in nature through the survived progeny. In all parasitized earthworms collected from the field, each harbored only one parasitoid larva. Thus, it seems that *P. dasypoda* so far is a solitary parasitoid.

Experimental rate of parasitism

A pale pink-colored spot appeared on the body of the earthworm indicating the site of penetration by the like-planidium stage (first instar larva) of the parasitoid. At the middle of the pale spot, the end of the anal portion of the parasitic larva protruded visibly with its pair of spiracles from the host skin permitting direct air respiration (Fig. 1b). The third larval instars were differentiated from each other, under a stereoscope, through the number of openings on the posterior visible spiracles. Each spiracle was structured by 1, 2, and 3 openings in the first, second, and third instar larvae, respectively (Tawfik and El-Husseini 1971). The five replicates of the directly exposed earthworms (*A. caligonosa*) to hatch larvae of the parasitic cluster fly (*P. dasypoda*) showed a 100% successful parasitism, examined through 10 days after exposure under laboratory conditions. Such a high rate of parasitism is related to the active mobile or migratory first instar larva (L_1), which is similar in the habit of the "planidium stage" of other parasitoids. Here, the first instar larvae were placed directly on the skin of the earthworms in which the parasitoid larvae started boring into the body wall of the earthworm by their sharp mouthpart structure. Completely developed L_1 molts into L_2 without changing the location of parasitism with its posterior end bearing the spiracles protruding at the body surface of the host. Usually, the L_3 is found in the same location until completing its development and leaves the body of the host to pupate in the soil adjacent to the corpus of its host (Fig. 1c).

Endo- and ecto-parasitim by *P. dasypoda* in *A. caligonosa*

Data presented in Table 4 realize that the diameter of the host body was the factor influencing the type of parasitism (endo- or ecto-parasitism?). The mean diameter of the endo-parasitized earthworm (3.85 mm) was found always larger than that of the parasitoid L_3 (2.90 mm), which allowed the parasitic L_3 larva to live inside its host as endo-parasitoid. In the case that the diameter of the parasitized earthworm (2.35 mm) was less than that of the newly molted parasitoid L_3, and due to the narrow space pressuring around the parasitoid larva (L_3), it crawled backwards out of the pressure of its host body, but kept its head with mouthparts inserted in the same opening. Thus, it fed for 4–5 days on the tissues and fluids of its host, while its body located outside, i.e., behaving as an ecto-parasitoid until it fully developed. Then, it separated itself from the host worm for pupation adjacent to the corpus of its mostly dead host (Fig. 1d). Available literature revealed that this phenomenon in which the early immature feeding stage (L_1 and L_2) behaves as an endo-parasitoid and during the late stage (L_3) of the same species, it behaves as an ecto-parasitoid because of the host narrow diameter than its body. Such behavior has not been previously recorded among the calliphorid parasitoids.

Conclusion

The cluster fly, *Pollenia dasypoda* Portochisky, is known as endo-parasitoid on the earthworms, where all the three instar larvae complete their development inside the same parasitized individual of the earthworm. Under laboratory conditions, the present study revealed the phenomenon in which the early immature feeding stage (L_1 and L_2) behaved as an endo-parasitoid and during the late stage (L_3) of the same species and behaved as an ecto-parasitoid because of the host narrow diameter than its body. Such behavior has not been previously recorded among the calliphorid parasitoids.

Table 4 Numbers of appeared endo- and ecto-parasitism by third instar larvae (L_3) of *P. dasypoda* in *A. caligonosa* related to diameter of both parasitoid larva and host body ($n = 250$)

Replicates ($n = 50$)	1	2	3	4	5	Control
Endo-parasitism	50	49	50	49	49	50
Ecto-parasitism	0	1	0	1	1	0
Ecto-parasitism %	0	2	0	2	2	0
Mean diameter of L_3	2.90 mm					
Mean diameter of host (endo-parasitized)	3.85 mm					
Mean diameter of host (ecto-parasitized)	2. 35					
Total ecto-parasitism %	2.4					

Abbreviations
L_1: First instar larva; L_2: Second instar larva; L_3: Third instar larva

Acknowledgements
The author thanks and highly appreciates the assistance of Eng. Ata A. Ata at the Center of Biological Control.

Author's contributions
The author read and approved the final manuscript.

Funding
This work was not supported by any funding body, but personally financed.

Availability of data and materials
All data are available at the article, and the materials used in this work are of high transparency and grade.

Ethics approval and consent to participate
I agree to all concerned regulations.

Consent for publication
I agree to publish this scientific paper at the EJBPC.

Competing interests
The author declares that he has no competing interests.

Received: 5 April 2019 Accepted: 22 July 2019
Published online: 05 August 2019

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