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A Contribution to Parasitism of Egg Batches of *Thaumetopoea pityocampa* (Denn. & Schiff.) (Lepidoptera Thaumetopoeidae) on the Peloponnes (Greece)

INTRODUCTION

For five years we have been interested in the forest pest *Thaumetopoea pityocampa* which is widely distributed in Greece. The first report on the structure, the hatching of the caterpillars and parasitism in the south of Greece was given by Schmidt (1988). Egg batches from Kalogria (West Peloponnes) and Delphi were investigated. The length of the egg batches showed a largely linear correlation with the number of deposited eggs. A batch length of 1 cm contained 86.6 eggs, on the average. The mean egg number per batch was 208.3 ± 64.8. The eggs of a batch can be parasitized to more than 60%. A mean of 17% of the eggs per batch was parasitized at Kalogria but only 2.9% at Delphi. The parasitoids belonged to the genera *Tetrastichus* and *Ooencyrtus* (Chalcidoidea) (Schmidt, 1988).

The caterpillars of an egg batch hatched within a period of 4-8 days at 20-22°C. Most of the parasitoids hatched before the caterpillars did. But the hatching time of the parasitoids was much longer than that of the caterpillars. Kailidis (1962) observed two further hatching periods in spring and summer, the latter during the next egg deposition of *T. pityocampa* in the following year. This matter we studied in this paper.

MATERIAL AND METHODS

From August to November 1987 we collected egg batches of *T. pityocampa* (Denn. & Schiff.) at Korinthos, near Patras-Rion and on the way to Tripolis and in March 1988 some batches at Kalogria near the beach. All egg batches were found on *Pinus halepensis* Miller.

In all batches caterpillars had already hatched, thus the first hatching period of the parasitoids during the development of the caterpillars in the eggs could not be investigated.

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After removal of the scale cover all egg batches were isolated with the needles in polystyrol tubes closed by a Ceapren stopper and stored in the laboratory at room temperature (22-27°C) with and without water at normal daylight periods from November 1987 to June 1989. During this period, all hatching parasitoids were counted ([1]).

RESULTS

1. Observations on egg batches about one year after deposition.

In the beginning of August 1987 the eclosion of *T. pityocampa* moths had not started at Korinthos and in other parts of the Peloponnes. In Öros Olimbos we could collect *T. pityocampa* moths (males and females) at 1000 m NN by light trapping (only males) on 28.VII.1987, they died on sea level within 24 hours without copulation and egg laying. Light trapping near Korinthos from 4th to 8th of August 1987 was unsuccessful. Therefore, all egg batches collected near Korinthos before this time should have been deposited in 1986, thus they overlay about one year. On 15.IX.1988 we received the following results (Table 1).

Table 1: Characterization of the egg batches of T. pityocampa *collected on 30.VII.1987 at Korinthos studied on 15.IX.1988.*

Number	Total eggs	eggs not developed	Remarks
1	191	6	wrapped on 4 needles, not destroyed, from most of the eggs caterpillars hatched
2	173	31	wrapped on a twig, not destroyed, besides caterpillars many parasitoids had hatched
3	90	28	wrapped on a twig, partly destroyed, from most of the eggs caterpillars hatched
4	104	23	wrapped on a twig, highly destroyed
5	234	5	wrapped on one needle, much destroyed, from most of them caterpillars hatched

([1]) Miss Beate Volke, Hannover, gratefully supported the investigations by technical assistance.

Table 2: Characterization of the egg batches and eggs collected on the Peloponnes on 23.X.1987 (T) near the road cross from Patras to Tripolis, on 25.X.1987 (U) and 03.XI.1987 near Patras-Rion (UR).

Batch No.	Total egg number per batch	Number of eggs without hatching hole		Number og eggs from which hatched			% of undeveloped eggs	
				larvae	parasitoids		until Nov. '87	up to Nov. '88
		Nov. '87	Nov. '88		until Nov. '87	in 1988		
T 1	160	14	14	140	6	0	8.7	8.7
T 2	199	1	1	195	3	0	0.5	0.5
T 3	218	6	6	210	2	0	2.7	2.7
U 1	221	83	30	101	37	35	37.6	13.6
U 2	163	29	24	85	49	5	17.8	14.7
U 3	268	51	51	203	14	0	19.0	19.0
U 4	173	12	8	137	24	4	7.0	4.6
U 5	225	1	1	205	19	0	0.4	0.4
U 6	192	110	53	52	30	57	57.3	27.6
U 7	235	21	21	198	16	0	8.9	8.9
U 8	150	82	15	33	35	67	54.7	10.0
UR 1	201	34	34	156	11	0	16.9	16.9
UR 2	210	90	81	80	40	9	42.9	38.6
UR 3	149	68	68	66	15	0	45.6	45.6
UR 4	138	95	2	30	13	2	68.8	67.4
UR 5	199	81	76	88	30	5	40.7	38.2
UR 6	150	53	44	61	36	9	35.3	29.3
UR 7	140	38	38	79	23	0	27.1	27.1
UR 8	189	24	20	128	37	4	12.7	10.6
UR 9	213	4	4	195	14	0	1.9	1.9
UR 10	172	61	53	88	23	8	35.5	30.8
UR 11	104	12	12	87	5	0	11.5	11.5
UR 12	156	1	1	139	16	0	0.6	0.6
UR 13	234	86	79	98	59	7	35.4	33.8
UR 14	149	2	2	140	7	0	1.3	1.3
Mean:	184.3	42.4	29.5	119.8	22.6	8.5	23.6	18.6
± SD:	38.7	35.8	26.8	56.8	14.9	17.7	20.4	17.1

Up to June 1989 there was no hatching of parasitoids from the egg batches. It could be demonstrated that in the field a high percentage of eggs do not produce neither caterpillars nor parasitoids during the first year after deposition. Almost all these eggs showed a black dot on the shell demonstrating the micropylar area. During the second year after deposition no hatching of parasitoids could be observed although many undestroyed eggs were present. Thus, an overwintering of parasitoids of more than one winter period inside the egg shell could be found.

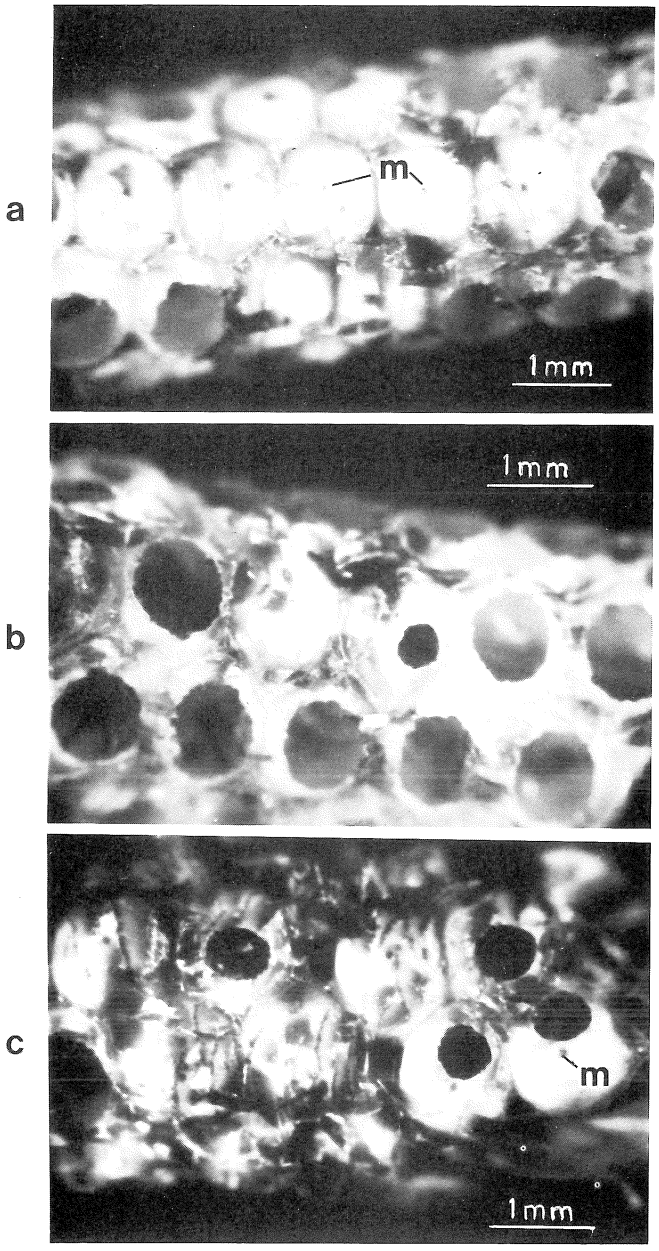

Fig. 1 - Parts of egg batches of *Thaumetopoea pityocampa* (Denn. & Schiff.): a) undeveloped eggs with black dots on the white shell demonstrating the micropyle (m); b) one egg shell with a small hole from which a parasitoid hatched and 8 large holes made by the hatched caterpillars; c) 4 egg shells with holes of parasitoids from which two additionally show a black dot (micropyle).

In April 1988 we collected 4 egg batches at Kalogria. In all of these some tapped eggs were present. But all of them were showed considerable damage, so that they could not be evaluated for this purpose.

2. Characterization of the egg batches after the hatching of the caterpillars.

25 egg batches were collected near Patras-Rion and on the road to Tripolis in October to November 1987. In all batches the caterpillars had already hatched and many eggs were parasitized. From a lot of eggs parasitoids had already hatched, too, indicated by a small round hole (Fig. 1). The numbers are given in Table 2. The evaluations took place during 20 - 26.XI.1987 and on 15.XI.1988.

In T 1 - T 3 collected near the road cross from Patras to Tripolis, no parasitoids had hatched in 1988 as in some other batches collected near the University campus of Patras.

Interestingly there sometimes was a very high percentage of undeveloped eggs. Almost all of these had also a black dot on the white shell surface (Fig. 1a). After the hatching of the caterpillars it could not be seen (Fig. 1b). On the other hand, in the shells of those eggs from which parasitoids had hatched a black dot was present, besides the smaller hatching hole (Fig. 1c).

It is remarkable that most of the parasitoids had hatched before caterpillars did, but in some cases there was a high percentage of parasitoids which hatched during the following year, sometimes even higher than that before caterpillar's hatch (U 6 and U 8).

3. Hatching of parasitoids after the caterpillars' hatch

After the first examination of the egg batches at the end of November 1987 further examinations took place on 11.I.'88, 06.III.'88, 05.V.'88, 15.VI.'88, 20.VII.'88, 10.VIII.'88, 5 - 28.IX.'88 and 10.XI.'88. At all dates a lot of parasitoids had hatched except on 20.VII. and 10.VIII.'88, some of them were even alive. With the exception of 2 specimens all of the parasitoids belonged to the genera *Tetrastichus* and *Ooencyrtus*. *Ooencyrtus* sp. hatched predominantly in May and June and *Tetrastichus* sp. almost exclusively in September. Table 3 indicates the detailed results.

Apart from one *Tetrastichus* male all parasitoids were females. The last *Ooencyrtus* hatched on 15.VI.'88. Up to this date only 4 *Tetrastichus* had hatched. It can be concluded that *Tetrastichus* sp. can overlay about ten months to parasitize the next generation without a second host. *Ooencyrtus* sp. survived inside the eggs only for up to a maximum of about 9 months. First hatching occurred after 3 months. Therefore, this species needs other hosts for multiplication.

Additionally three egg batches were investigated collected at Kalogria at the end of March 1988. From these only 4 parasitoids of the genus *Ooencyrtus* hatched.

The egg batches were again examined on 10.VIII.'88 and on 03.X.'88. The results are given in Table 4. In October and November 1988 no further of parasitoids could be observed. Also, up to June 1989 no parasitoid hatched.

Table 3: Egg batches collected in the fall of 1987 from which parasitoids hatched during 1988; n: numbers not counted.

Number of batch	dates of the hatching control						
	11.I'.88	6.III.'88	5.V.'88	15.VI.'88	20.VII.'88	10.VIII.'88	5.-28.IX.'88
U 1	—	n	n	6	0	0	53
U 2	—	n	n	1	0	0	5
U 3	—	n	n	1	0	0	0
U 4	—	0	0	3	0	1	4
U 6	—	n	n	5	0	0	57 (1♂)
U 7	—	0	n	0	0	0	0
U 8	—	0	0	1	0	0	67
Total:	?	?	?	17	0	1	186
UR 1	1	0	0	15	0	0	0
UR 2	1	n	6	8	0	0	9
UR 3	1	n	5	2	0	0	0
UR 4	3	n	9	1	0	0	2
UR 5	5	n	4	21	0	0	5
UR 6	1	n	4	21	0	0	9
UR 7	1	0	2	9	0	0	0
UR 8	0	n	1	2	0	0	4
UR 10	0	n	1	7	0	0	8
UR 12	0	0	1	0	0	0	0
UR 13	0	n	2	10	0	0	7
Total:	13	?	35	96	0	0	44

In the batches UR 6 and U 8 more parasitoids were counted than the differences of the figures on 11.I.'88 and 03.X.'88 of undeveloped eggs demonstrated (Table 4).

In all egg batches the number of undeveloped eggs were reduced when parasitoids had hatched. At the beginning of the year the hatching parasitoids were rare. During the first part of June and in September two hatching peaks could be observed.

DISCUSSION

Huchon & Demolin (1971) reported that the egg batches of *T. pityocampa* collected in the south of France consisted of 70-300 eggs per batch covered by scales.

In the south of Greece 93-349 eggs per batch were counted (Schmidt, 1988). In this paper we investigated egg batches of 104-268 eggs each. They were wrapped on 2 to 8 needles or on small twigs.

As Kailidis (1962) reported we could demonstrate in former investigations that a relatively high number of eggs did not develop (Schmidt, 1988). After hatching of the caterpillars, up to a maximum of 69% eggs of one batch remained, from which neither caterpillars nor parasitoids had hatched. On the white shell of almost all of these undeveloped eggs there was a black dot demonstrating the micropylar area which can be also seen in Masutti's paper (1964; Fig. 9+10) and close to that sometimes very small black points similar to a closed punctured hole of a female parasitoid. In the Braconid Family *Aphelinidae* (Wilbert, 1964; Michel, 1967; Hamilton, 1973) it is well known that egg parasitoids puncture host eggs to get some yolk which they need for their own egg production. These eggs did not develop and were not parasitized.

Table 4: Number of undeveloped eggs of the investigated batches after the hatching periods of parasitoids; n: numbers not counted.

No. of batch	Not developed eggs on			Parasitoids counted
	11.I.88	10.VIII.88	03.X.88	
UR 1	34	15	15	16 + n
UR 2	90	51	42	24 + n
UR 3	68	37	37	8 + n
UR 4	95	56	54	15 + n
UR 5	81	31	26	35 + n
UR 6	53	32	23	35 + n
UR 7	38	17	17	12 + n
UR 8	24	18	14	7 + n
UR 9	2	2	2	0
UR 10	61	27	19	16 + n
UR 11	12	12	12	0
UR 12	1	0	0	1
UR 13	86	55	48	19 + n
UR 14	1	1	1	0
U 1	83	66	19	59 + n
U 2	29	9	5	7 + n
U 3	51	22	22	1 + n
U 4	12	0	0	8 + n
U 5	1	1	1	0
U 6	110	94	36	62 + n
U 7	21	3	3	n
U 8	82	68	23	68 + n

If the parasitic female lays an egg inside the host egg it has to puncture the latter in a similar way. On many shells of host eggs from which parasitoids hatched

there was a small black dot besides the micropyle near the hatching hole. The number of the black-dotted closed eggs was reduced considerably in 1988 until egg deposition of the new generation by hatched parasitoids.

In two cases we found that more parasitoids hatched than holes were present which is in agreement with Schmidt (1988). These findings also agree with the observation of Halperin (1970) that sometimes two *O. pityocampae* eggs can develop in one host egg, but then parasitoids are very small.

Most of the parasitoids which hatched after the caterpillars did were observed in May-June of the following year. Kailidis (1962) found this hatching period in April-May 1961 in Attica and near Thessaloniki in higher regions (200-900 m NN) and Halperin (1970) in April to June for *T. wilkinsoni* in Israel. For this, the eggs of the parasitoids have to overlie many months, and have to find a second host or they have to survive many months by feeding on honey dew produced by aphids to be able to parasitize *T. pityocampa* eggs of the next generation. The parasitiods that hatched in May-June belonged to the genus *Ooencyrtus* as it was stated by Halperin (1970) for *T. wilkinsoni*. It is also well known that *O. pityocampae* is polyphagous and can parasitize eggs of many other insect species living on pine trees (Ceballos & Sandoz, 1962; Masutti, 1964; Halperin, 1970). If a parasitoid has developed inside the host egg shortly before hatching the egg looks dark coloured. At this stage some parasitoids can die. In contrast to our observations, Kailidis (1962) also found many *Tetrastichus* sp. which hatched during spring time from *T. pityocampa* eggs at Attica near Athens.

It is unknown if the parasitoids that hatched in the closed tubes are able to parasitize undeveloped eggs of the same batch and if super- or hyperparasitism can take place after caterpillars' hatch.

A second high number of parasitoids hatched at Hannover in September 1988. It was at the same time when egg deposition of *T. pityocampa* started in Greece at Kalogria. Kailidis (1962) found these hatches in August-September, but also during egg deposition. We observed a very good synchronization of parasitoid hatch at Hannover under laboratory conditions and the egg deposition of the moths in Greece at Patras-Rion and other parts near sea level. But all these parasitoids belonged to the genus *Tetrastichus*. This is in contrary to the findings of Kailidis (1962) who also found *Ooencyrtus* at that time. Species of the genus *Tetrastichus* can overlie for almost one year to parasitize the next generation of *T. pityocampa* egg batches as Masutti (1964) reported. We have to assume that the parasitoids had an obligatory, differently timed diapause. The investigations of Kailidis (1962) give no information about the cause of the periodic hatching of the parasitoids, but Halperin (1970) found that *O. pityocampae* (Mercet) has 3-4 generations per year in nature and *Tetrastichus servadeii* Dom. two generations in Israel, the second was synchronous with the life cycle of *Thaumetopoea*.

For determination of the species further investigations have to be carried out. Males of the parasitoids were very rare; only one *Tetrastichus* male could be counted. The multiplication of *T. servadeii* Dom. results from thelytoke parthenogenesis

(Masutti 1964). In his unpublished dissertation Halperin (1970) demonstrated that in *O. pityocampae* sex development depends on temperature. Up to 32°C only females hatched and above 34°C only males developed. Males of *T. servadeii* were very rare.

After a period of one year no hatch of parasitoids could be observed although many undeveloped eggs were present. This was also not possible by increasing the humidity inside the covered glasses. Thus, an average of 18% of the eggs did not develop into caterpillars or into parasitoids. About one third of the parasitoids hatched after the hatching of the caterpillars. In the fields, most of the egg batches are destroyed during winter time by birds (*Parus* species), perhaps because many undeveloped eggs are present. That means, that most of the diapausing parasitoids inside the eggs are eaten by their predators and lost for parasitizing the new generation of egg batches.

SUMMARY

Laboratory studies were carried out with egg batches of *Thaumetopoea pityocampa* (Denn. & Schiff.) collected on the Peloponnes/Greece to study why many eggs do not develop and to find out the role of the parasitoids. The investigations started when all caterpillars had hatched and lasted for about two and a half years. During this time the hatching periods of the parasitoids were recorded. All egg batches were laid on needles of *Pinus halepensis* Miller. From batches which had overlain one year no parasitoid hatched during the second year although many undeveloped eggs were present. Hatching of parasitoids could be observed only up to one year after egg deposition. Two hatching periods of parasitoids were observed during the first year after egg deposition. One was found in May-June for *Ooencyrtus* sp. and the other in September for *Tetrastichus* sp., the latter was synchronized with the egg laying of the host. The parasitoids developed at 22-27°C. At these temperatures only females hatched apart from one male of *Tetrastichus* sp.. In two egg batches more parasitoids were counted than hatching holes were present. In nature most of the diapausing parasitoids are eaten by predatory birds during winter time.

RIASSUNTO

Parassitismo delle ovature di Thaumetopoea pityocampa *(Denn. & Schiff.) (Lepidoptera Thaumetopoeidae) in Peloponneso (Grecia)*

Sono stati effettuati studi di laboratorio su ovature di *Thaumetopoea pityocampa* raccolte nel Peloponneso per conoscere i motivi per cui diverse uova non schiudono e valutare il ruolo ricoperto dai parassitoidi. Le ricerche hanno avuto inizio alla nascita di tutte le larve e sono proseguite per due anni e mezzo. Durante questo periodo sono state registrate le epoche di schiusura dei parassitoidi. Tutte le ovature provenivano da aghi di *Pinus halepensis* Miller. Dalle stesse, tenute in osservazione per più di un anno, non è sfarfallato nel secondo anno nessun parassitoide sebbene fossero presenti numerose uova non schiuse.

Lo sfarfallamento di parassitoidi è stato osservato dopo un anno dalla deposizione delle uova. Sono stati registrati due periodi di apparizione degli adulti: uno in maggio-giugno per *Ooencyrthus* sp. e l'altro in settembre per *Tetrastichus* sp.; quest'ultimo è sincronizzato con la deposizione delle uova dell'ospite. I parassitoidi sviluppano a 22-27°C.

A queste temperature sono schiuse solo femmine, tranne un unico maschio di *Tetrastichus* sp.. In due ovature sono stati rinvenuti più parassitoidi di quanti punti di sfarfallamento fossero presenti. In natura la maggior parte dei parassitoidi in diapausa sono predati da uccelli durante il periodo invernale.

ZUSAMMENFASSUNG

Ein Beitrag zur Parasitierung der Eigelege von Thaumetopoea pityocampa (*Denn. & Schiff.*) (*Lepidoptera Thaumetopoeidae*) *auf der Peloponnes* (*Griechenland*)

Zahlreiche Eigelege von *Thaumetopoea pityocampa* (Denn. & Schiff.) wurden auf der Peloponnes/Griechenland eingesammelt und im Laboratorium in Hannover untersucht, um festzustellen, warum sich viele Eier nicht entwickeln und welche Bedeutung den Eiparasitoiden zukommt. Die Untersuchungen wurden nach dem Larvenschlupf begonnen und dauerten etwa 2½Jahre. Während dieser Zeit wurden die Schlupfperioden der Parasitoide ermittelt. Alle Eigelege waren an Nadeln von *Pinus halepensis* Miller abgelegt. Von Eigelegen, die vor mehr als einem Jahr angefertigt wurden, schlüpften im zweiten Jahr keine Parasitoide mehr, obgleich noch viele unentwickelte Eier vorhanden waren. Ein Parasitoiden-Schlupf konnte somit nur bis zu einem Jahr nach der Eiablage festgestellt werden. Zwei Schlupfperioden der Parasitoide wurden während dieses ersten Jahres nach Eiablage beobachtet. Die erste lag im May-Juni für *Ooencyrthus* sp. und die andere im September für *Tetrastichus* sp.; die letztere war synchronisiert mit der Eiablage des Wirtes. Die Parasitoide entwickelten sich bei 22-27°C. Bei diesen Temperaturen schlüpften nur Weibchen ausser einem Männchen von *Tetrastichus* sp. Von zwei Eigelegen konnten mehr Parasitoide gezählt werden, als Schlupflöcher vorhanden waren. Im Freiland werden während der Winterzeit die meisten der diapausierenden Parasitoide von Vögeln gefressen.

Key words: *Thaumetopoea pityocampa*, egg parasitoids, hatching periods.

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