TRICHOGRAMMA PRETIOSA RILEY. OVIPOSITION,—A RÉSUMÉ.

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LINTNER (1883), in Psyche, has given an accurate and somewhat detailed account of the oviposition of this minute and very interesting egg-parasite, which has also been copied *verbatim* in his second report on the injurious and other insects of the state of New York (Lintner, 1885). The host was *Pteronus ribesii* Scopoli, formerly *Nematus ventricosus* Klug.

This is the first account of its oviposition published, and although lacking in many details, the best one so far placed on record. Others have observed and briefly recorded this habit. Riley (1885), quoting Hubbard, states briefly that the mother parasite works chiefly by day, depositing two ova in each egg of *Alabama* or *Heliothis*; often, but a single adult emerged from the host eggs, and occasionally three were obtained. Nothing is said in regard to the manner of oviposition. F. W. Mally (1902), briefly states the manner of oviposition, with some details; probably he had not seen Lintner's account. The host was *Heliothis obsoleta* Fabricius, and the account is accurate so far as given. Finally, Quaintance and Brues (1905), give a brief, condensed account of its oviposition, as a result of recent studies. The following is but an enlargement of their account.

During the recent investigations on the cotton bollworm (*Heliothis obsoleta* Fabr.) by the United States Department of Agriculture, in Texas, during 1904 the writer had occasion, in studying its parasites in the laboratory at Paris, to make detailed studies on this species of *Trichogramma*, the most common and useful of the many parasites attacking that important pest. Hundreds of specimens were reared in confinement, and ample opportunity offered to observe its habits closely. As shown below, specimens were placed on the stage of the microscope, and there watched at leisure, while they ran busily about among the host eggs, depositing into them.

These observations but serve to confirm those made by Lintner; and it is through the kindness of Dr. L. O. Howard, Chief of the Bureau of Entomology, that the writer is able to offer these more detailed notes on this little egg-parasite. They are also offered as an addition to our knowledge of the habits of the smaller parasitic Hymenoptera, and to sum up, as it were, what is now known in regard to the oviposition-habit of *pretiosa*. Further results of the studies on this insect will be offered as time permits.

1. *General manner of oviposition.* This has been aptly described by Lintner (1883); details are here added.

Females taken from jars in which confined, several hours after emergence, were transferred by means of a camel's hair brush to a portion of a blade of corn upon which had been deposited, thickly, eggs of *Heliothis*, by moths also in confinement. The blade of corn was then placed on the stage of the microscope, and the insects watched, while they deposited egg after egg. These observations were repeated from time to time, with and without the aid of the microscope. The latter, however, was necessary, on account of the microscopic size of the insect.

When placed on the leaf of corn, the insects immediately began to crawl rapidly about, showing no indications that they were aware of the presence of suitable hosts, until they chanced upon one. At this, the female stopped and sensed the egg with its antennae, recognizing it. Searching is evidently the only way in which the insect finds its hosts, and in nature they doubtless travel many hours, at times, before finding a suitable place in which to deposit their eggs. After stopping and recognizing the host, and satisfying itself of its suitability, the female crawls upon it, and begins a minute, though rapid, examination of its surface with the brush-like clubs of the antennae, travelling generally, when doing this, transversely around the egg in a direction perpendicular to the polar ridges, and taking a variable, though short, period of time.

Becoming suddenly satisfied, she stops short, advances the length of her body, and raising the latter, applies the point of the ovipositor to the spot last sensed by the antennae. The tip of the abdomen now forms a caudal support, and the legs are well out and propped. Thus gripping the host egg, pressure is brought to bear on the ovipositor, which being resisted by the tough chorion, now bends and twists like a good rapier, which it much resembles in miniature. The parasite is now trying to force the ovipositor into the egg. In this it often fails (cf. 6), though generally successful. After its point has pierced the chorion, the ovipositor is slowly and firmly pushed in to its entire length, which is about half of the greatest diameter of the host egg, about 0.24 mm., or until the ventum of the female is in contact with the surface of the host.

Slight spasmodic contractions of the abdomen then ensue, doubtless sending the egg through the oviduct and ovipositor into the host, where it is deposited centrally. The ovipositor is then as slowly withdrawn, the female arching its abdomen very high, almost straining, until it is entirely out, when she regains her feet, and darts off in search of another egg, or simply changes position on the same egg, and repeats the operation; or else rests, according to circumstances. After beginning

to insert the ovipositor, the antennae become non-vibratile, lying against the face; and in some observed cases, just as the ovipositor was wholly within the egg, the wings were lifted straight up over the body, perhaps to help in preserving a balance. The ovipositor is inserted always between the polar ridges on the host egg.

While engaged in oviposition, the female is very intent on her work, and is not easily disturbed. Indeed, large things do not seem to frighten or annoy these minute parasites, and no indication is given that they are aware of the presence of an observer, even when this presence is forced upon them. When they are sufficiently disturbed, however, they remain perfectly motionless, very seldom resorting to flight. On account of this apparent nonchalance, they are, in spite of their minuteness, very easy to breed.

2. *Oviposition in regard to time.* The time taken for single depositions averages about two (2) minutes, and is fairly constant in a large number of cases. The following observations were made on this point.

On June fourth, at 3 P. M., four females and one male, issuing from host eggs during the morning of the same day, were confined beneath a large bell-jar of convenient size, with eighty-one (81) fresh ova of *obsoleta* deposited in confinement during the afternoon and evening of June third, on a large piece of bristol-board. The latter was simply covered by the jar and the parasites then introduced.

After crawling about for several minutes, two (2) of the females found the eggs, and without hesitation, began to oviposit. They were so intent on their work, that the jar was removed and the individuals closely watched for a period covering two hours. This whole time they made no attempts whatever to escape, even showing no disposition to do so. They were so absorbed, indeed, that the piece of bristol-board was cut to a convenient size with a pair of scissors, and moved about freely, most of the time being on the stage of the microscope.

Oviposition was almost continuous for the first half-hour (3.00-3.30 P. M.), both females working actively. Then the paper was moved to the stage of the microscope. The parasites became perfectly motionless when this movement was made, remaining in their respective positions for another half-hour, doubtless, at first, slightly annoyed. At 4 P. M., one again began to oviposit, and soon the other joined her, though both showed tendencies to wander, or rest, and seemed to be more or less jaded. They continued thus until 5 P. M., when observations were suspended.

During the first half-hour, observations were made with a hand lens (aplanatic, triplet, $\frac{1}{2}$-inch, Bausch and Lomb), but the latter was then discarded because the ovipositor, though visible, was difficult to follow, and the time of oviposition had to be judged in most cases by the movements of the insect. Hence, the time varied

considerably as may be expected. The range was from fifteen (15) to one hundred and fifty (150) seconds, the smaller time-periods doubtless due to the fact that mere attempts to oviposit were mistaken for accomplishments of the act itself. With the lens, the following periods of time were obtained.

Deposition number.	Time, seconds.
1.	70.
2.	115.
3.	80.
4.	80.
5.	15.
6.	15.
7.	15.
8.	25.
9.	120.
10.	150.
10.	85.6, avg.

Observations with the microscope were made with the $\frac{2}{3}$-inch objective, Bausch and Lomb, which was admirably fitted for the purpose. Every movement of the insect could be plainly seen, and therefore the time for each deposition could be accurately measured. The following time is to be accepted as that which is correct, obtained from many subsequent observations. The time was taken from the beginning of the insertion of the ovipositor.

Deposition number.	Time, seconds.
1.	120.
2.	120.
3.	150.
3.	130., avg.

From this, it is safe to say that two (2) minutes, more or less, is the average time taken to deposit a single egg. Individual differences cause the variation. From fifteen (15) to forty-five (45) seconds is normally required for insertion of the long ovipositor. (Vide seq., 6.)

3. *Faulty instinct.* This is doubtless present in some form in all insects. In *Trichogramma pretiosa,* when in confinement, some very interesting observations were made on this.

On the fourteenth of June, a single female without hesitation, oviposited twenty (20) times into infertile host eggs which were supplied her (Vide seq., 6). The eggs of the parasite hatched, and a generation successfully developed in these infertile ova. This being the case, it is doubtful whether or not this oviposition was due to faulty instinct, although the parasite was, seemingly, not conscious that the hosts were infertile. The fact is a very interesting one. It is worthy of note that these infertile eggs when thus parasitized, did not shrivel at all, but remained as full in appearance as fertile and normally developing eggs. The checks, however, shriveled completely.

On the first of October, a tenth reared generation of *Trichogramma* was obtained from infertile hosts under similar conditions.

On the morning of the twenty-fifth of June, the last of the newly emerged females of the third reared generation were found still wandering about the bell-jar under which confined, in search of fresh hosts in which to deposit; as there were only eleven (11) of the latter present, not already blackened by parasitism, it was highly probable that all of them had been deposited into.

In spite of this, the females were ovipositing continuously, refusing, in every case observed, the blackened hosts. To the contrary, depositing from three to five (3–5) times in the yet pale hosts. At times, these were refused.

In one of these pale eggs (which as stated, were also evidently parasitized but not yet blackened) which had been isolated, a female deposited about four (4) times; this egg turned black two (2) hours afterwards, proving beyond doubt that that female had oviposited into a host already parasitized about forty-eight (48) hours previously, as the black color does not appear until after the parasite's egg hatches, at this time of the year, slightly under three (3) days. It is probable that some of the pale eggs were not parasitized.

Hence, apparently, the female recognizes hosts which have already been deposited into, not until they have blackened, or by their color.

4. *Number of eggs deposited; calculation of the possible maximum number deposited.* The highest number of eggs observed to be laid was thirty-three (33). On the twenty-seventh of September, a single female issuing from *obsoleta* eggs collected on corn, in a small glass vial, at 3.40 P. M., was at once confined in another glass vial, closely stoppered, with portions of cotton leaves containing many ripe host eggs. She oviposited as follows.

Host number.	Time, P. M.	Number depositions per egg.
1.	3:46	1; 1; 1 (4:59); 1 (5:02).
2.	3:52:30.	1; 1 (4:58); 1 (5:10); 1 (5:12).
3.	3:54–3:55:30.	1; 1 (5:40).
4.	3:56:30	1.
5.	3:58.	1; 1.
6.	4:00.	1.
7.	4:05:30.	1.
8.	4:50–4:54.	1; 1.
9.	4:57.	1; 1; 1; 1; 1;
10.	5:13.	1; 1 (5:43).
11.	5:17.	1; 1.
12.	5:20.	1; 1 (6:05); 1 (6:10).
13.	5:25–5:27	1; 1.
14.	6:20.	1; 1. Final observation.

On July first, twenty-two (22) parasites emerged from hosts exposed to a single female; she therefore deposited at least twenty-two (22) eggs.

For other important observations on this, vide seq., 6.

From the fact that these observations were made under artificial conditions, that is, that the host eggs were supplied gratuitously, the female not having to search for them as they occur in nature, it is rather difficult to base calculations upon them. However, the following calculation is based on several apparent facts,— that the average time for single depositions is two minutes; and that, after females had been laying eggs continuously for a half-hour they appeared to be more or less exhausted, and deposition occurred at intervals of time only thereafter, for the space of about two hours. Hence the period of oviposition appears to be at its height during the first half-hour after commencement, and continues with more or less persistency for about two hours.

Without going into details, the average number of eggs for two hours would be sixty (60) eggs; and for one-half hour, fifteen (15) eggs. This calculation agrees with conditions found in confinement. Two females in the time of two hours, parasitized twenty-four (24) eggs, out of many supplied, averaging three depositions to a host. This would make thirty-six (36) eggs deposited by each. Repeated observations seemingly confirm this, and indicate forty (40) eggs, or less, as the average number deposited.

A calculation of the possible, but improbable and obviously absurd, maximum

number of eggs laid, leads to the enormous number of one thousand and eighty (1,080). The average length of life of a female was here limited to two days, the average length of the period of oviposition to thirty-six hours, and the average time for single depositions to two minutes.

It is important to take into consideration the fact that in nature the host eggs are well scattered, and that the little *Trichogramma* has to take considerable time and much pains to find them. For such a minute insect, the examination of a whole leaf of corn is no small undertaking.

5. *Number of eggs deposited into single hosts.* This was determined both by direct observation and by rearing experiments.

By direct observation, much data was obtained but was hardly worthy because of the conditions prevailing in confinement. Females were observed several times to deposit as many as five eggs into a single host, but all of these did not successfully develop.

On this point, the following results were obtained by rearing experiments made in the laboratory at Paris. The hosts were obtained from corn and cotton.

Date, 1904.	*Host No.*	*Number parasites emerging.*
May 6th.	1.	4.
May 15th.	2.	3.
	3.	3.
May 16th.	4.	4.
	5.	1.
	6.	1.
	7.	2.
	8.	5.
	9.	3.
	10.	2.
	11.	4.
	12.	4.
	13.	2.
	14.	2.
	15.	3.
	16.	4.
	17.	5.
May 31st.	18.	1.
	19.	4.

Date, 1904	*Host No.*	*Number parasites emerging.*
May 31st.	20.	3.
	21.	4.
	22.	1.
	23.	4.
	24.	3.
June 12th.	25.	2.
June 27th.	26.	4.
July 23rd.	27.	2.
	28.	2.
	29.	2.
	30.	1.
	31.	2.
	32.	1.
July 30th.	33.	3.
	34.	4.
	35.	3.
	36.	3.
	37.	3.
	38.	1.
October 18th.	39.	3.
	40.	3.
Totals.	40.	111.

Average, 3.

It is thus seen that the number of adults issuing from a single host is not constant, but varies from one to five, averaging from two to three. The number also doubtless varies according to the species of host.

6. *Observations on a single female, bearing on the foregoing.* The following notes are derived from observations made on a single female, supposedly virgin, emerging from an egg of *Heliothis* between the hours of 12:30 and 2 P. M., June 14. The observations continued until 6:35 P. M., when the insect escaped.

a. *Faulty instinct.* At 3:30 P. M., the glass jar containing this female was lifted and the insect transferred by means of a camel's hair brush, to a portion of a corn leaf on which had been deposited thirty-four (34) sterile host eggs. The leaf was transferred to a slide, and then placed on the stage of the microscope ($\frac{2}{3}$-inch objective, Bausch and Lomb).

It crawled about, not in the least disturbed, and soon found the hosts. At exactly 3:33, oviposition began, or about one and one-half hours after birth. There were no indications to show that she was in any way conscious that the hosts were infertile.

Table showing activity in regard to oviposition.

Oviposition from	Resting periods	Number eggs deposited
3:33–3:47	3:47–3:48	
3:48–3:55	3:55–4:12	11 eggs.
4:12–4:27	4:27–4:35	3
4:35–4:44	4:44–5:40	3
5:40–5:42	5:42–6:08:30	1
6:08:30–6:18	6:18–6:35	2
Totals. 56½ minutes	125½ minutes.	20 eggs.

b. *Number of eggs deposited; number of depositions in single hosts.* The actual number of eggs deposited by this female, as shown above is twenty (20). Most of them were deposited during the first hour of the whole time during which the female was under observation, showing, or at least indicating, that by this time the insect was well tired out. This condition may have been brought on by the difficulty in this case experienced in inserting the ovipositor:

From 4:12–4:27 P. M., a period of activity as given in foregoing, the female, after depositing a single egg, made 17 futile attempts in succession to insert the ovipositor in as many places in the same host. Each attempt occupied from 15–35 seconds. Two successful depositions then occurred, followed by a period of 7 minutes rest, when another active period of 8 minutes ensued, during which, after depositing another egg, 3 more successive futile attempts to insert the ovipositor were made, all on the same host. Two successful depositions were then made in a different host. After this oviposition became fitful, but three eggs being deposited in the remaining two hours.

The exact cause of this inability to insert the ovipositor could not be ascertained from the nature of the case, but it was probably caused by weakness. The point of the ovipositor did not slip about during these attempts.

Out of the thirty-four (34) host eggs, but ten (10) were parasitized by this female. Nine of these were noted with especial reference to the number of depositions made in each. In the first host, four eggs were deposited; in the 2nd, 3rd, and 4th, 3 eggs; in the 5th, 1 egg; in the 6th, 2 eggs; and in the 7th, 8th, and 9th, 1 egg. The average for each host is therefore about two (2).

c. *Oviposition in regard to time.* The following time periods for single depositions were noted during these observations.

Deposition number.	Time, seconds.
1.	70.
2.	138.
3.	96.
4.	90.
5.	134.
6.	85.
7.	105.
7.	103, avg.

7. *"Effective" oviposition.* The following interesting experiments were performed to find when, or if ever, there occurred a certain advanced period in the development of the host at which it was immune from the effects of the oviposition of the parasite, because of this advanced development. For instance, perfect embryos just before exclusion from the egg. Can these be effectually parasitized? The hosts used in these experiments were all deposited in confinement.

a. On the 26th of September, six hosts showing advanced signs of development, were exposed to two females of *pretiosa* in a small glass vial. They were deposited into, and the resulting parasites successfully developed. A check lot of eggs from the same source and of the same age, hatched eight (8) hours afterwards. So oviposition was effective up to within eight hours of the exclusion of the host.

b. Two females and one male of *pretiosa* issuing from a single host on the morning of September 26th, were confined in a small glass vial with a piece of a cotton leaf containing four ripe ova of *obsoleta*, deposited in confinement, and now showing very advanced signs of development. A control lot of hosts present.

At 10: 22 A. M., *pretiosa* began to oviposit into the hosts. One of the latter received four (4) of the parasitic eggs, the second, two (2) eggs, and the remaining two, an egg each, when at 10: 38 A. M. they were removed to a separate vial by themselves. By 8 P. M. the same day, the hosts were still more developed, the black head of the embryo perfectly visible through the shell. On the following morning they had not changed, and development had evidently been checked. During late P. M., September 28th, they turned completely black from the effects of the parasitization. Eight parasites emerged from these four hosts during early October. The control lot of hosts hatched at 5: 30 P. M., September 26th, or seven (7) hours afterwards. Hence, oviposition effective up to within seven hours of exclusion of the host.

c. A female of *pretiosa* confined at 3:46 P. M., September 27th with nineteen (19) hosts, then hatching. Thirteen (13) of these hosts were successfully parasitized. A control lot of hosts hatching at 3 P. M., the same day. Hence oviposition effective up to within 4 or 5 hours, at the most, of exclusion of the host.

d. Five (5) hosts nearly ready to exclude, the perfect embryos visible, were exposed to a female parasite at 10:15 A. M., September 28th. One of the hosts excluded at 10:55 A. M. The other four (4) were blackened by the effects of parasitism by October first, or previously. These four yielded adult parasites later. Control lot of hosts excluding at 10 A. M., September 28th. Hence oviposition of *pretiosa* effective up to the moment of exclusion of the host.

These experiments strongly indicate that parasitization is successful up to the very moment of exclusion of the host, despite the fact that the perfect embryos of *obsoleta* are active and very capable of caring for themselves. They are not conclusive, however. Other experiments performed, indicate that when the host embryo is fully formed, parasitization is less successful than otherwise, doubtless due to the greater vitality of the embryo. Hence, there appears to be a certain period in the development of the host, the period between the perfect formation of the embryo and exclusion, in which it is partially, not totally, immune from attack.

8. *Length of the period of oviposition.* It was absolutely impossible to determine this under anything like natural conditions from the very nature of the case. From the evidence gathered in the laboratory, it is certain that the period of oviposition is very short, but it is not felt that this evidence warrants a statement of definite limits.

What conclusions are possible may be induced from what is said in regard to this matter on a previous page.

9. *Changes undergone by parasitized hosts.* After being parasitized, the host eggs retain their normal appearance, until the egg of the parasite hatches, when they become characteristically blackened, an opaque or dull bluish black. They still retain their normal shape and size, and the color persists indefinitely, always serving to distinguish eggs that have been parasitized. After the emergence of the resulting parasite or parasites, through a rounded hole in the side of the host egg, the color and shape still persist, and so on until the empty shell is destroyed through natural agencies. The black color comes on gradually, the host at first becoming dusky, finally opaque and black-blue. Infertile hosts are affected in the same way.

10. *Length of the egg instar.* If it is true that the black color of the host is due to the hatching of the parasite's egg, then the following table shows lengths of the period of incubation at different times of the season of 1904, as indicated by this

change in color. The periods are, of course, but approximately correct, and are probably worthless. It has been indicated, from time to time, that the period of incubation in *Trichogramma* is always several hours longer than that of the host, at any given date. By comparing the records for the periods of incubation of the latter as given by Quaintance and Brues (1905), page 52, and the table following, it is readily seen that this is not borne out by the facts.

TABLE OF THE EGG INSTARS AT DIFFERENT DATES, 1904.

Date hatched.	No. of observations	Length instar. Days.	hrs.	Date hatched.	No. of observations.	Length instar Days.	hrs.	Average.
May 30	6	3	5	Sept. 14	50	2	9	241 observa-
31	4	2	22	24	34	2	17	tions.
June 7	30	2	22	28	9	2	12	
16	14	2	16	Oct. 1	33	3	17	71 hours, or
17	3	2	13	5	5	3	18	2.95 days,
24	28	2	4	17	4	3	9	average.
25	9	2	14	19	12	4	0	

No records, unfortunately, were made during April, July and August, and late October.

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