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DEVELOPMENT OF *TRICHOMASTHUS FRONTALIS* ALAM, (HYMENOPTERA; ENCYRTIDAE) AND ITS BEHAVIOUR IN RELATION TO ITS HOST, *GREENISCA PLACIDA* (GREEN) (HOMOPTERA; COCCOIDEA; ERIOCOCCIDAE).

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Abstract

This paper reports observations made by the author on the Encyrtid parasite *Trichomasthus frontalis* Alam, during the course of a study of the Eriococcid, *Greenisca placida* (Green) at Rough Common, Wytham, Berkshire, England. Like its host, *Trichomasthus* is univoltine adults appearing in the field in summer. The egg is of the stalked type. There are four larval instars. The first and second instar larvae are metapneustic and the third and fourth instar larvae are peripneutic with nine pairs of spiracles. At Wytham the larval period lasted for about eight months.

Analysis of field data showed that *Trichomasthus* tends to avoid superparasitism in its host, and exercises very little effect upon the fecundity of the host. *Trichomasthus* suffered a very high mortality by the hyperparasite *Pachyneuron concolor* Forster.

Introduction

The larval forms of entomophagous insects are interesting in that they depart radically from the adult enabling the two stages to occupy completely different environments. In the Encyrtidae, however, even the structure of the egg has a distinctly peculiar significance, the stalk of the egg serving for fixation to external or internal organs and in some cases for obtaining atmospheric air for respiration. This paper reports observations made by me on the Encyrtid parasite *Trichomasthus frontalis* Alam, during the course of a study of the Eriococcid *Greenisca placida* (Green) at Rough Common, Wytham, Berkshire, England.

Greenisca placida (Green) is univoltine with four stages in the female, namely, egg, two nymphal instars and the adult but six stages in the male : egg, two nymphal, one prepupal, one pupal and the adult male. The overwintering, diapause eggs are laid within an ovisac secreted by the mother in late July–August (summer) sometimes continuing into the first week of September, depending on the prevailing weather; they remain there until the following, spring when they hatch, i.e., for a period of about nine months. The first instar lasts from mid-May to mid-June, second instar from early June to late July and the adult females from late July to late September. The first instar nymphs and all the stages of the male were never observed to be parasitised. The number of second instar nymphs parasitised was negligible. It is the young and adult females that are usually attacked.

Materials and Methods

At Wytham, the exclusive host plant of *G. placida* is *Brachypodium pinnatum* (L.) Beauv. which is locally abundant in Rough Common. It forms tussocks with other taller grasses, especially *Molinia caerulea* (L.) Moench, *Zerna* (Bromus) *erectus* Huds. and *Dactylus glomerata* (L.).

Sampling was done for two complete generations of *Greenisca placida* commencing from October 1968. The sampling unit was a rectangle of 4 x 25 sq. cm. which could be dismantled easily. All the grass including dead blades that came within the 100 sq. cm. area was brought to the laboratory for examination. Sampling was done at two-week intervals from August until December, and a final set of samples was taken in the following May, just before the eggs hatch, to get an idea of winter mortality of host eggs and parasite larvae. Young host adults (females) were sampled in July. Regular sampling was not done during the nymphal instars, but random counts of nymphs on grass blades were made without disturbing them, to estimate the first and second instar nymphs per 100 blades of grass or per 100 sq. cm. area. The grass blades in sample were brought to the laboratory, counted and the mean number of blades/100 sq. cm. sample was calculated. The counting of grass blades made it possible to estimate the number of first and second instar nymphs/sample and also to make a correction whenever a 100 sq. cm. included a bare patch or burrow made by voles.

The results were recorded in a tabulated form. Each blade was examined and the number of *Greenisca* (host) on each blade was noted. The individuals parasitised by *Trichomasthus* were distinguished by the presence of the stalk of the parasite egg jutting out from the dorsum and by the mummified nature of the parasitised females. Individuals harbouring the Pteromalid *Pachyneuron concolor* (a hyperparasite) showed no external signs of this. Each parasitised specimen was kept in a separate specimen tube (1 x 1/3 in.) which was plugged with a cork. All the parasitised specimens (marked and separated in tubes) of each sample were placed in a container on which was marked the number of the sample and the date on which it was collected. These were examined at weekly intervals to determine the emerged parasites and predators associated with *Greenisca* until emergence was completed. Adult females of *Trichomasthus frontalis* were dissected to study the ovarian egg. The developmental stages of the parasite were studied by dissecting and making permanent stained preparations of the parasitised individuals of *Greenisca*.

Ovarian Egg

The ovarian egg (Fig. 1A) is quite large and its neck is about 1.5 times the length of egg proper. The bulb is smaller than the egg proper. Measurements are : length of bulb, 0.135 mm.; width, 0.060 mm.; length of neck, 0.294 mm.; width of neck, 0.012 mm.; length of egg proper 0.195 mm.; width of egg proper 0.087 mm. A distinct surface rib termed the 'band', 'aeroscopic plate' or 'respiratory plate' by Silvestri (1919) extends the whole length of the stalk and the greater portion of the egg itself, which in certain respects resembles that of *Ooencyrtus johnsoni* (How.) (Maple, 1947). The marginal areas of the band on the main body are composed of minute cells. Along the centre of the band the cells are large. At the apex of the neck the cells completely surround the neck and as the band narrows the cells underlie a thickened wall, which covers the rest of the neck, upto its junction with the egg (Fig. 1C).

Deposited Egg (Fig. 1B)

In the process of oviposition the egg proper is placed within the host, the distal portion of the neck and the crumpled bulb at its tip projecting through the dorsal derm of the host to the exterior. This portion remains colourless,

and appears as a clubbed white seta. Melanization was limited to those portions within the host and was confined to the band. Throughout the neck and upper part of the egg proper the band becomes brown. The position of the egg in the host is such that the band is directly opposed to the surface of the host.

Developmental Stages (Fig. 1D, E, F)

The larva on hatching feeds on the body fluids of the host, and subsequently devours all the contents of the body. There are four larval stages, moulting taking place three times, which is extremely gradual. The first and second instar larvae are metapneustic with one pair of spiracles. After two ecdyses the larva becomes peripneustic with nine pairs of spiracles. Each of the cast-off skins with the mandibles is seen in close contact with the previous skin, forming a sort of a cap surrounding the hind end of the body (Fig. 1E). Subsequently, after the last moult the larva loses its attachment to the cast-off skins and comes to lie free in the body cavity of its host. A membranous sheath was observed surrounding the third instar larva. The fourth instar larva is larger in size, and was seen to possess nine pairs of spiracles. This condition appears to be an adaptation to the new environment within the host, which at this time contains a good supply of air, among its tissues.

The number of parasite larvae harboured by any individual *Greenisca placida* does not vary very much, very often being either one or two and very occasionally three, so that it appears that the parasite tends to avoid superparasitism in this host. Very often only one adult emerges from an individual host scale and less often two adults. Where more than one parasite develops the body cavity of the host is chambered depending on the number of larvae developing inside. Once the host dies the body wall of the host as well as the internal partitions become rigid and form oblique bulges in the body wall of the host.

The parasite larva when disturbed or irritated, is capable of some slight movement, both the head and anal ends admitting of a certain amount of protrusion and retraction. At times also a kind of slow 'peristaltic.' movement passes over the body from one end to the other. Under ordinary circumstances, however, the fully grown larva appears practically motionless, with the head end somewhat retracted into the region which immediately follows.

The pupal stage is passed within the host. When fully fed the larva discharges the contents of the alimentary canal, which are usually observable as small, ovoid, dark brown pellets (meconium), clustered around the posterior region of the pupa, lying along the sides of the chambers. No excreta are voided earlier in life and in fact, their presence would have had deleterious effects on its host, perhaps involving the death of both the host and the parasite.

The duration of the larval and pupal stages may depend upon the prevailing climatic conditions provided the suitable host is available. At Wytham the larval period lasted for about eight months. The adult emerges making a circular hole with its mandibles in the dorsum of the host's body. The average duration of adult life of the parasite in the laboratory was about two weeks; but when fed with honey they were able to live for about a month.

Effect of parasite on the host

Trichomasthus exercised very little effect upon the fecundity of its host the death of the latter seldom occurring until after it has deposited its eggs. The fact that many parasitised hosts deposited no eggs at all, affords no certain evidence that the presence of *Trichomasthus* larvae exercises an inhibitory effect upon oviposition. This conclusion is supported by evidence derived from my examination of 2681 unparasitised hosts 8.95% of which failed to lay any eggs, whereas 40% of the 7.2% parasitised hosts laid from 2 to 75 eggs, the average being 10.9 eggs per parasitised female. It is difficult to account for these facts except upon the supposition that they were unfertilised individuals, as reproduction in *Greenisca placida* is exclusively bisexual. In the vast majority of instances *Greenisca* was attacked by *Trichomasthus* so late in life that little or no interference with egg-production occurred. The parasite larvae only assume their sarcophagous habit at a time when their hosts have laid the greater proportion of their eggs. The parasite larvae did not become predacious upon the eggs of the host, after the death of the latter, as is the case with some parasites. However, there were indications that the adult parasite has the important habit of destroying hosts, by host feeding or sucking. Punctures similar to those made by the ovipositor were observed in the dorsum of some females, with solidified body fluid forming plugs. Several of them were found dead before oviposition commenced.

Parasitised hosts undergo no further growth or moulting when they are attacked during their second nymphal instar. They continue to feed on the host plant, but invariably die as a result of parasitism.

Effect of host on parasite success

During the course of dissections of field collected specimens it was found that in spite of being parasitised by *Trichomasthus* some of the hosts behaved normally and laid either the full quota or a good proportion of eggs. Dissections also showed that sometimes death of the parasite occurred before it hatched. The parasite eggs were observed as black lumps and blood cells were seen surrounding each egg including the whole of the neck. Once parasite larva had hatched the host appeared to be unable to kill it, though there was a low winter mortality during the early larval instars and a high mortality due to parasitism by *Pachyneuron concolor* during the late larval instars of *Trichomasthus*. The stage of the host during which it is parasitised was also found to be critical for the parasite's success. If at the time when it is parasitised, the host had partly shrivelled up, the parasite egg very often failed to hatch. Some of those eggs were not even encapsulated by blood cells.

Site of oviposition by the parasite

Although *Trichomasthus* eggs have been found to be deposited through all parts of the dorsum including the marginal areas, there appeared to be a definite preference for the middorsal region of the body. The site of penetration could easily be located by the colourless projecting stalk of the egg and the dried up brownish fluid surrounding the stalk forming a sort of a plug.

Pachyneuron concolor in relation to *Trichomasthus* and *Greenisca*

P. concolor lays its eggs on the mature larvae or the third instar larvae of *Trichomasthus*, at a time when there is very little or no fluid contents at all within the scale's body. Very often several *Pachyneuron* larvae feed on one *Trichomasthus* larva. When a scale is about 6–8 chambered the larvae at the peripheral chambers were seen to be smaller than the others. Sometimes all of them die due to starvation, at different stages before emerging. Very often only one, and less often two or three *P. concolor* adults emerged from a batch of 6–8 larvae. Cannibalism was not observed among the larvae. Under crowded conditions all that emerged were males, which were generally smaller than the females; the female *Pachyneuron* appears to be solitary in development. The remains of the host larva is found at one end of the scale.

It is almost impossible to distinguish between hosts parasitised by *Trichomasthus* only, and those parasitised by both *Trichomasthus* and *Pachyneuron* in the early developmental stages of the latter because it leaves no indication of the host being parasitised. It is only when the larval development has proceeded to a considerable extent so as to show the small semitransparent chambers, that some of them (not all) can be distinguished. Still there is a good proportion of parasitised hosts which cannot be separated as either parasitised by *Trichomasthus* only or both by *Trichomasthus* and *Pachyneuron* until the latter is reared, because those do not give any external indications of what is inside, being non-transparent.

Discussion

When compared with other mortalities the loss due to parasitism was small, but was more important in 1969–1970 than in 1968–1969. The important parasite, *Trichomasthus* suffered an extremely high percentage mortality due to hyperparasitism by *Pachyneuron concolor*, without which *Trichomasthus* probably would have been very much more effective as a potential controlling agent of *Greenisca*. In 1969–70, out of 1031 *Greenisca* parasitised by *Trichomasthus* 80 individuals, i.e., 7.56% produced adult parasites; 138, i.e. 14% produced *Pachyneuron*, the rest were dead *Pachyneuron*. So, the mortality of *Trichomasthus* for 1969–70 generation was 92.44%. *Trichomasthus* larvae suffer very little other (additional) mortality, the greater proportion of the deaths being due to hyperparasitism by *Pachyneuron* which in turn, appears to suffer a very high degree of winter mortality, during the early larval stages.

The field data on parasitism showed that the percent parasitism (affecting females only) has increased year after year; it was 7.2% for 1968–69; 15.6% for 1969–70; 29% for 1970–71. There was an increase in the area of discovery of *Trichomasthus* for *Greenisca* from 1969 to 1970; i.e. a = 0.0984 for 1969, and a = 0.2616 for 1970. The area of discovery was calculated using the formula given below. This is a modification by Varley (1947) of that given by Nicholson and Bailey (1935) :–

$$a = \frac{2.3}{p} \log \frac{u_1}{u}$$

where a = area of discovery, p = adult parasite density, u_1 = initial host density before parasitism and u = surviving host density after parasitism.

The above information suggests that this parasite is slowly gaining importance in the natural regulation of the population of *Greenisca placida* at Wytham Wood.

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Legend for Figure 1

Developmental stages of *Trichomasthus frontalis* Alam.

A. Ovarian egg.
B. Deposited egg.
C. Part of the neck of egg.
D. First instar larva.
E. Third instar larva.
F. Fourth instar larva.
G. Mandibles of first instar larva.
H. Mandibles of second instar larva.
I. Mandibles of third instar larva.
J. Mandibles of fourth instar larva.