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ON THE DEFENCES OF LEPIDOPTEROUS PUPÆ IN RELATION TO THE OVIPOSITION BEHAVIOUR OF CERTAIN ICHNEUMONIDÆ

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Anyone who has collected pupæ of Lepidoptera in the field knows only too well that from many of his finds will emerge ichneumon flies, some of which will have developed from eggs laid in the larvæ, others from eggs inserted into the pupæ. In this account I hope to draw attention to some of the defences of pupæ against the attempts of these insects to deposit eggs within them. I shall not be concerned with the concealment of the pupa or with its physiological reaction to the introduction of an egg, but with the course of events between its being discovered by an Ichneumonid and its being either penetrated by the ovipositor or abandoned unscathed.

With the diversity of pupation sites in the Lepidoptera is associated a wide variety of habitat preferences and searching behaviour in the Ichneumonidæ. A searching Ichneumonid of a given species will find a certain more or less restricted range of species of pupæ which evoke the series of actions leading to oviposition. These can be arranged in an order of vulnerability peculiar to that Ichneumonid's species. At one end of the scale will be those species of pupae that, though found and attacked, are impregnable, and at the other, those that always succumb. Between the extremes are those whose individuals are capable of delaying and sometimes averting their being stabbed by the ovipositor of the attacking female, even though their species may be regular hosts of that species of Ichneumonid.

Without further research it is impossible to assess the advantage to an individual pupa of merely delaying the consummation of an Ichneumonid attack. The obvious direct advantage is that an attack of long duration is more likely to be interrupted by some outside agent such as a predator of the Ichneumonid. Besides this, however, there is a practical justification for taking the power of prolonged resistance in a pupa as an indicator of the effectiveness of its defences. Females of an Ichneumonid species vary from time to time and from individual to individual in their ability to overcome pupal defences. A resistance by which a pupa delayed oviposition in the attack actually observed would perhaps have ensured complete success had the pupa been found and attacked by another Ichneumonid female, or by the same female at a different time. The outcome of an encounter between Ichneumonid

and potential host is not a foregone conclusion but can be influenced by (a) external agents, (b) characteristics of the host individual, and (c) characteristics of the Ichneumonid female, mainly differences in size and persistence. Persistence is itself affected by many little-known factors, which might include, for instance, the condition of the ovaries, or the number and species of hosts successfully attacked in the past.

Despite the importance of the host's means of protection as a component of host selection in the wide sense (*vide* Salt, 1935), and despite the number of lepidopterous pupæ that are killed by Ichneumonid parasites, the nature and effectiveness of pupal defences in this direction have received very little attention. HINTON (1955), reviewing the known protective devices of endopterygote pupæ, makes no particular reference to the Ichneumonidæ. JACKSON (1937) mentions a female of *Pimpla turionellæ* (L.) [=*examinator* (F.)], capable of piercing a soft-cuticled pupa in 30 seconds, that remained for two hours drilling a hole in a pupa of the Geometrid *Selenia*

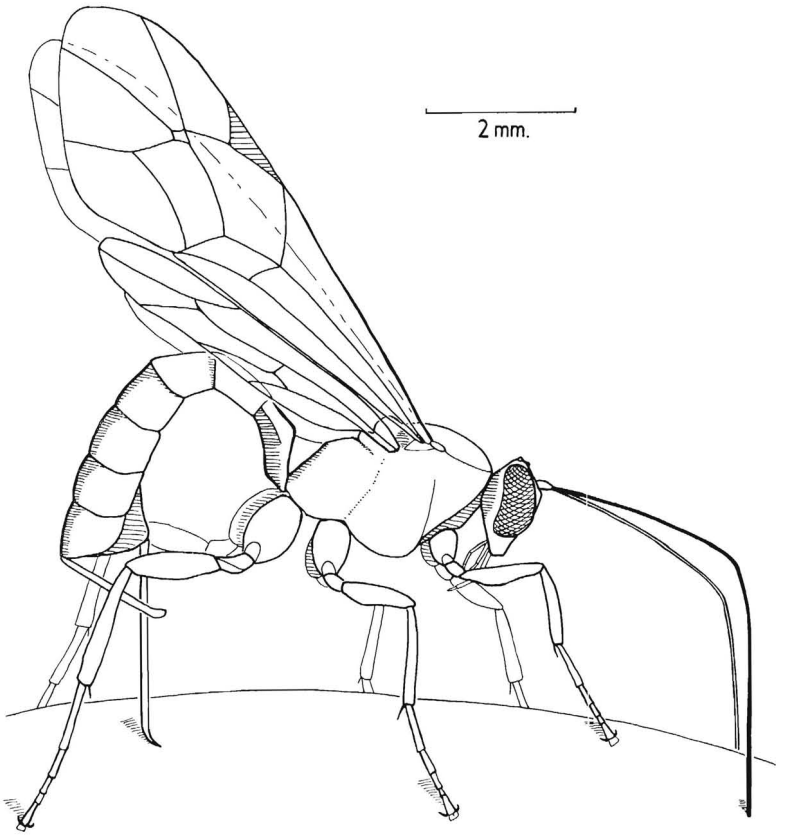

Fig.1. *Apechthis resinator* female about to pierce a folded leaf.

bilunaria (Esp.), and another that failed after repeated attempts to puncture a pupa of the Notodontid *Cerura vinula* (L.) though it succeeded in piercing the hard cocoon.

The Lepidoptera referred to below were encountered in the first place as known or suspected hosts during a biological study of three species of the Ichneumonid tribe Pimplini [=Ephialtini of some American authors]. They are to be found, either naked or in cocoons in folded leaves, in the places where these Ichneumonids search, viz., amongst the foliage of trees and undergrowth in woods and hedgerows. The Pimplini in question, *Itoplectis maculator* (F.), *Apechthis rufata* (Gmel.), and *A. resinator* (Thunb.), all operate in much the same way, by alighting on the pupa or on its cocoon and examining it with the antennæ, then flexing the abdomen and thrusting the stout ovipositor through the cocoon if present and into the pupa (Fig.1). In the field they attack a wide range of hosts. In laboratory experiments females of *Apechthis* would usually attack any of the Lepidoptera mentioned in this paper, while those of *I. maculator* were offered only Tortricids, which they accepted readily. The ovipositor in the genus *Apechthis* differs from that of other Pimplini in being decurved at the tip (fig. 1). The significance of this will be discussed later. Ovipositor lengths vary from 3.3 to 5.6 mm. in *Apechthis rufata,* 2.5 to 4.3 mm. in *A. resinator,* and 2.8 to 4.0 mm. in *I. maculator.*

It is clear from the mode of attack of these ichneumon flies that hard cuticles and thick cocoons are potentially valuable defences, as will be any feature of the pupa making the attacker's grip less secure or causing its ovipositor to slip. The cocoon may in fact be a liability, for it can provide an excellent foothold. While other predators must get inside it to reach the pupa, a Pimpline Ichneumonid can easily push its ovipositor through even a very hard cocoon.

Defences of Naked Pupæ

The first examples are chrysalids of three species of butterflies. They are freely suspended, without cocoons, so that Ichneumonids must alight on them in order to oviposit. Laboratory observations suggest that the females of *Apechthis* species are often unsuccessful against fully hardened chrysalids and that concealment is not the only means of defence for pupæ not enclosed in cocoons. If chrysalids of the Nymphalid *Aglais urticæ* (L.) were gently squeezed or stroked with a camel hair brush they gave a few sluggish movements of the abdomen. But the slightest touch of antenna or tarsus of an *Apechthis* female resulted in movements so violent that the Ichneumonid was often thrown into the air as soon as it had alighted. A persistent attacker was usually able to stab a newly formed pupa with the cuticle still soft, but when a pupa was more than 24 hours old the movements of the abdomen, so rapid as to be almost vibrations, together with the burnished surface of the cuticle, made it impossible to press the ovipositor against any part for more than a moment, or to get any anchorage with the tarsi. Only very large fe-

males, able to clasp their tarsi around the pupa, met with any success. Parasitization of the most intractable pupæ could, however, usually be secured in the laboratory by restricting their movements, either by removing them from their pads and laying them on the floor, or by holding them with forceps. The provision of a foothold for the Ichneumonid by covering the pupa with muslin still further facilitated the operation. This state of affairs recalls that described by PICARD (1922), who found that captive females of *Pimpla instigator* (F.), a species with oviposition habits similar to those of *Apechthis*, were usually unable to pierce suspended pupæ of *Vanessa* [=*Pyrameis*] *cardui* (L.) because of the rapid vibrations of the abdomen that were produced at the slightest touch.

The chrysalis of the Satyrid *Pararge ægeria* (L.) is a winter host of *Apechthis resinator* (Cole, 1957), and its cuticle is easily pierced by females of this species in its first 24 hours. Later the cuticle becomes very hard. Though the abdomen is, unlike that of the Nymphalids, capable of only slight movements, my attempts to get oviposition by *Apechthis* females in older pupæ often failed. When the attack was successful it was usually because the ovipositor had been thrust through the flexible cuticle between the fourth and fifth abdominal segments. Less often penetration was through the wing-case. The Ichneumonids would sometimes persist for half an hour or more, though they usually abandoned their attempts before this. The effectiveness of this passive resistance is due to the smooth, rounded surface of the cuticle, as well as to its thickness. The tarsi and ovipositor of the Ichneumonid slip continually, so that the necessary pressure cannot be brought to bear. In this connection a waxy bloom which appears on the surface of the pupal cuticle after 24 hours may help to prevent the adhesion of the tarsal pads. The importance of the smoothness of the surface was often demonstrated when, on my placing a previously abandoned chrysalis in a fold of muslin, the Ichneumonid's tarsal claws found anchorage, and the cuticle was pierced in a minute or two.

The chrysalis of the Pierid *Pieris brassicæ* (L.) is in contrast fairly easily pierced by captive females of *Apechthis*. Though its cuticle is thick it is also rough enough to afford an adequate grip for the tarsi. Moreover the movements of the abdomen are not sufficiently strong to dislodge the attacking female. According to PICARD (1922) females of *Pimpla instigator*, also, have little difficulty in ovipositing in this species.

EXPERIMENTS WITH COCOON-SPINNING TORTRICIDÆ

The examples given in the rest of this paper are all cocoon-spinners. The following three species of Tortricidæ pupate amongst foliage, usually spinning their cocoons in leaves that they have folded or fastened together. They are parasitized in the field by all three species of Ichneumonid, to which the pupæ fall easy prey when the cuticle is still soft. In casual observations the female Ichneumonids often became involved in prolonged struggles with the pupæ in their cocoons, suggesting that the pupæ in their exposed leaf-envelopes on the trees were not quite as helpless in face of attack as might

be supposed. In early June, 1958, I carried out a series of small experiments to investigate the outcome of encounters between females of *I. maculator* and of *A. resinator* and pupæ of *Tortrix viridana* (L.).

MATERIAL. (a) Pupæ. Folded oak leaves containing cocoons and pre-pupæ of *T. viridana* were collected in Wytham Wood, near Oxford, and kept in plastic bags out-of-doors. The contents of each cocoon were examined twice daily through the anterior end, left unsealed by the larva. This made necessary some tearing of the cocoon so that, although the elasticity of the leaves returned the openings almost to their original sizes, the material as used was not quite in its natural condition. Further, some wilting of the leaves was inevitable, though surprisingly little in three days. The time of pupation of each individual was recorded so that pupæ of known age were available.

(b) Ichneumonids.

1. A rather small female *I. maculator,* caught flying in oak foliage on 25th May 1958. Wing length: 5.6 mm.; ovipositor length: 3.0 mm.

2. A medium-sized female *A. resinator,* from a chrysalis of *P. ægeria,* 2nd May 1958. Wing length: 7.5 mm.; ovipositor length: 2.8 mm.

3. A large female *A. resinator,* caught in oak foliage on 23rd May 1958. Wing length: 10.6 mm.; ovipositor length: 3.8 mm.

PROCEDURE. The folded leaves containing pupæ of the required age were offered singly to the Ichneumonids, each of which was confined by itself in a glass cylinder of dimensions 17 cms. by 8 cms. The folded leaves were held near them with forceps. In all cases they readily accepted the offered hosts and began their attacks without delay. An attack was timed from the moment the female flexed her antennæ to examine the leaf until she either succeeded in penetrating the pupa, or left the pupa by walking or flying away, or was removed by the experimenter. The experiments extended over seven days, and no more than four pupæ were offered to each female in a day.

RESULTS.

1. Of 12 pupæ with ages ranging from 2 to 5 days offered to *I. maculator* all survived without being stabbed. The attacks lasted from 1 to 2½ minutes. Three of these pupæ, removed from their cocoons immediately after being abandoned and offered again in a fold of muslin, were stabbed in 3, 1¾ and 4¼ minutes respectively.

2. Of 4 pupæ aged 24 hours or less offered to *I. maculator* 3 were stabbed, in 1, ½ and 2¾ minutes respectively. A fourth had not been pierced after 19 minutes, when the experiment was stopped.

3. All 9 pupæ aged from 2 to 5 days offered to the large *A. resinator* were stabbed, though 4 of them withstood the attack for over 2 minutes.

4. Of 7 pupæ with ages ranging from 2 to 5 days offered to the small *A. resinator* 4 had not been stabbed after 10 minutes, when the experiments were stopped. By this time the Ichneumonid had made between 15 and 24 attempts to impale each of them.

CONCLUSIONS. Remembering that only three individual Ichneumonids were used in these experiments and that the *I. maculator* was a small one of its kind, one may nevertheless conclude that the defences of the fully hardened *T. viridana* pupa free in its cocoon are capable of delaying an attacking female and can often frustrate its attempts, whereas young pupæ are easily overcome while their cuticles are still soft. It is evident also that the defences are less effective against *A. resinator* than against *I. maculator,* which apparently is rarely able to oviposit in hard-cuticled *T. viridana* pupæ unless an artificial foothold is provided. In the field, on four occasions I have seen *I. maculator* females attack soft pupæ of *T. viridana,* and each time the pupa was stabbed in less than thirty seconds. I have witnessed one attack on an older pupa in which a female of this species persisted for fifteen minutes, piercing the leaf over fifty times before impaling the victim. Both species of *Apechthis* are much scarcer than *I. maculator* in early summer, and I have not been able to observe their oviposition in *T. viridana.*

Watching these encounters, one soon realizes the great importance, after its hardness, of the pupa's agility. It is well known that by movements of the abdomen, which has four free segments in the male, the pupa of *T. viridana* works its way forward to protrude from the cocoon before the emergence of the moth. But throughout life the abdomen is capable of strong movements which cause the pupa to rotate or move about within its spacious cocoon. Now when the attacking *Apechthis,* say, has located with the antennæ the pupa through the enveloping leaf it quickly inserts the ovipositor in this place, but often suceeds only in scraping the pupa, not striking it squarely. At once the pupa wriggles out of reach of the probing ovipositor so that the Ichneumonid must withdraw its weapon, re-locate the pupa, and stab again. The pupa may even leave its cocoon and fall to the ground though if, as sometimes happens, it should become stuck half way out it is an easy victim. Sometimes, when the cocoon is spun in the half-eaten stub of a rolled leaf, there is not enough room for such manœuvres. But still the rotary movements may delay or prevent its being stabbed by causing the threatening ovipositor to slip. There is, however, a conflict between the requirements of a continuous thick cuticle and the mobility necessary in the first place to escape from the cocoon before emergence of the moth. A mobile abdomen must have

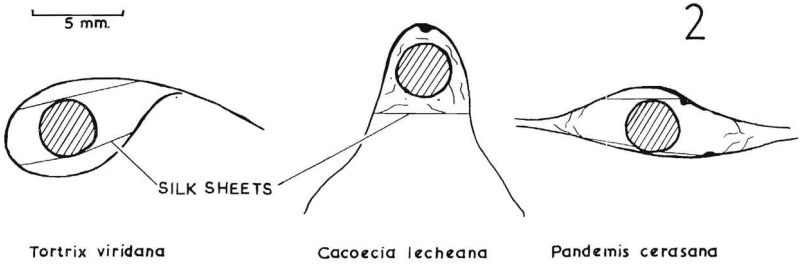

Fig.2. Sections across the cocoons of three species of Tortricidæ.

extensive areas of flexible cuticle between its segments and though the mobility can be turned to advantage as a means of taking evasive action, as described above, it may be that a smooth, completely obtect pupa would be even safer. In the experiments, if an ovipositor did penetrate the pupa it was almost always thrust through these flexible areas. The cuticle here is armoured with small polygonal sclerotized plates and can be completely covered by the overlapping edges of the segments on one side of the abdomen at a time (Fig. 3).

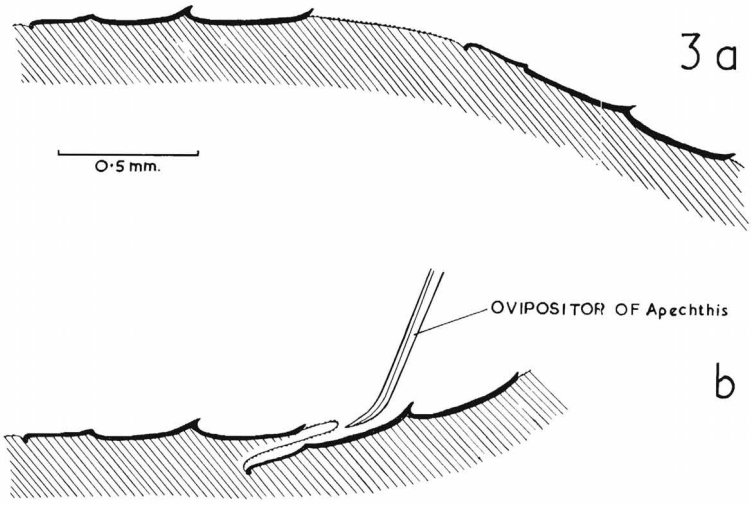

Fig.3. Longitudinal section of dorsal cuticle of pupa of *Tortrix viridana,* showing flexible ring between two abdominal segments. Abdomen flexed (a) ventrally, (b) dorsally.

This at least protects it from the thrusts delivered at right angles to the surface by the species of *Pimpla* and *Itoplectis,* which have straight ovipositors. It is much less effective, however, against the hook-tipped ovipositors of the species of *Apechthis,* which I interpret as an adaptation towards the inserting of the ovipositor between such overlapping segments (Fig. 3b). Females of *I. maculator* and *Apechthis* species tend to thrust forward when attempting to stab pupæ, and since they turn about frequently during the operation, at least some of the thrusts will be towards the anterior end of the pupa, so taking advantage of the direction of overlap of the segments. Now the more nearly parallel the ovipositor is to the surface of the pupa the more likely it is to penetrate. The decurved ovipositor tip enables its owner to press it forward almost horizontally without sacrificing depth of penetration. Although the leaf-envelope is very thin-walled and the cocoon a mere sheet, an ovipositor must be inserted perhaps two millimetres before it can press against the pupa owing to the way in which this is held in the middle of the chamber by the cocoon (Fig. 2).

The Tortricid *Pandemis cerasana* (Hübn.) [=*ribeana* (Hübn.)] spins a very flimsy cocoon, sometimes in a folded leaf, more often between leaves (fig. 2). The pupa is very active indeed and again seems quite impregnable, once fully hardened, to *I. maculator*. Four cocoons containing pupæ were collected in the field and offered singly to the larger *A. resinator* used in the *Tortrix* experiments, with the following results: —

1. The first pupa was stabbed after a struggle lasting 10 minutes, in which the Ichneumonid pierced the enveloping leaf 19 times. The pupa finally wriggled half way out of the cocoon, when the Ichneumonid mounted it and stabbed it at the junction of wing-case and abdomen.

2. The second pupa wriggled out of the cocoon and dropped to the ground after the *resinator* had pierced the leaf 6 times in 1½ minutes. The Ichneumonid continued to examine and stab the empty chamber.

3. The *resinator* female attacked the third pupa for 6 minutes, piercing the leaf 9 times before abandoning the attempt.

4. The female succeeded in stabbing and ovipositing in the fourth pupa in 2¼ minutes.

The Tortricid *Cacœcia lecheana* (L.) has in the pupal stage an even harder cuticle than has *T. viridana*. It is then capable of extremely vigorous rotary movements, and its cocoon differs from the two above in many respects (Fig. 2). The pupa is suspended in the centre of the triangular chamber by strands of silk. I have several casual records of captive females of the Pimplini unsuccessfully attacking the hardened pupæ, defeated, it seems by the hardness, activity and loose suspension.

OBSERVATIONS ON OTHER COCOON-SPINNERS

Salebria betulæ (Goez) (Phycitidæ) pupates amongst the foliage of birch (*Betula* spp.), suspending its cocoon in a web of silk incorporating several leaves. The diameter of the mass is such that a large captive female of *A. resinator*, removed after five minutes of thrusting its ovipositor into one of these webs, had barely touched the pupa. This species is probably an example of the one extreme of the scale of vulnerability, being unavailable to these Pimplini because of its extensive cocoon. (For comparison, in the Ichneumonid *Ephialtes inquisitor* (Scop.), which sometimes parasitizes the full-grown larva of *S. betulæ* in its web, the ovipositor is about 6.5 mm. in length.) The Pyraustid *Notarcha ruralis* (Scop.), too, seems fairly safe from attack by these Ichneumonids. It spins together the edges of a nettle leaf (*Urtica dioica* L.) and pupates in the pod so formed. In the laboratory an *A. resinator* female could easily reach the pupa with its ovipositor but was not able to pierce it. The smooth, unattached pupa simply rolled away. When the pod was compressed with forceps, however, the Ichneumonid soon stabbed it and deposited an egg, which completed its development in due course. Another *A. resinator* female was removed after five minutes of attempting to pierce a pupa of the Drepanid *Drepana lacertinaria* (L.), when it was evident that she had little chance of succeeding. This species spins a thin cocoon,

usually in a folded leaf of Birch. The pupal cuticle, though rough, is very hard and is armoured at the three flexible abdominal joints with small sclerotized plates. In this and in the overlap of the abdominal segments the pupa resembles that of the three Tortricids, though it is less active. In contrast, the Œcophorid *Diurnea fagella* (Schiff.) seems to have no effective means of protection against the species of *Apechthis*. It is a winter host of *A. rufata* (Cole, 1957), pupating in autumn between thin sheets of silk in a chamber made by spinning two dead leaves together, either on the tree or on the ground. The pupa is capable of rotary movements, but its cuticle is thin and offers little resistance to a thrusting ovipositor. In the laboratory, pupæ of this species have always been parasitized within two minutes of being attacked by *Apechthis* females.

Evidently, there is scope for detailed studies in the morphology and behaviour of pupæ in relation to animals that attack them. Ichneumonids are particularly interesting in this respect since, unlike other predators, those species that oviposit in pupæ attack nothing else (except, sometimes, prepupæ), so that the relation between the adaptations of host and parasite will be especially close. Although most Pimplini parasitize a wide range of pupæ, some Ichneumonidæ are restricted to small groups of taxonomically related hosts. It would be interesting to compare the effectiveness of the host's defences against the more and the less specialized Ichneumonidæ. The subject is important, too, in the study of populations. As might be expected, pupæ are very vulnerable to Ichneumonidæ while their cuticles are soft. But in most examples it turns out that the Ichneumonid female must spend a surprising amount of time and energy in order to oviposit in older pupæ. If the host population all pupate together, the effective oviposition period of the Ichneumonids may be shortened, and so more likely to be affected by, for instance, weather conditions. However, a full investigation of these aspects awaits further advances in the study of Ichneumonid behaviour and physiology.

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Summary

Searching females of an Ichneumonid species attacking lepidopterous pupæ find a certain range of potential host species in which they will oviposit. Some of these are readily parasitized when found, while others always escape by virtue of their defences. Yet others may escape on some occasions only, and the success of attacks on pupæ in this category depends on the individual characteristics of the Ichneumonid female, as well as on the host and on external agents. The power of a pupa to delay an Ichneumonid attempting to oviposit in it can be used as an indication of the effectiveness of its defences.

The defences of the pupæ of ten species of Lepidoptera have been investigated by offering them to females of three species of Pimplini (Ichneumonidæ) of which these Lepidoptera are known or possible hosts. Hardness and smoothness of the cuticle, and sometimes activity, were important defences of the naked pupæ of butterflies. Hardness, activity and certain properties of the cocoons were important to three species of Tortricidæ. The species of Ichneumonidæ differed in their ability to pierce Tortricid pupæ. The defences of four other species of cocoon-spinning Lepidoptera were studied in less detail. It is concluded that some species of pupæ are very vulnerable to attack in their first few hours, but that after the cuticle has hardened Ichneumonid females must spend much time and energy if they are to deposit eggs in them. The main period of oviposition in such cases may be very short.

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