A Study of Some Factors Governing the Choice of Hosts and Distribution of Progeny by the Chalcid Ooencyrtus kuvanae Howard

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A STUDY OF SOME FACTORS GOVERNING THE CHOICE OF HOSTS AND DISTRIBUTION OF PROGENY BY THE CHALCID *OOENCYRTUS KUVANAE* HOWARD

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I. Introduction

Although the problem of host selection in insects is one which has received considerable attention from various workers within the last twenty-five years, no agreement has yet been reached on the general factors involved in this selection. Apart from its purely academic interest to the insect physiologist, the fact that certain species of insects are restricted to one given host, while others attack numerous species, is one of considerable practical importance. Recently attempts have been made to determine the precise nature of the attraction exercised by hosts on their known insect parasites with a view to the utilization of the knowledge in problems of biological control. Thus, it is thought that if the factors which determine the oviposition response of a given parasite can be found it should be possible to predict the hosts of this parasite in any given environment.

Both monophagous and polyphagous parasites are known to select appropriate hosts for oviposition, but it was assumed that in a population of any single host species, a parasite laid its eggs without reference to the nature of the hosts available. This

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assumption followed from the observed prevalence of superparasitism (the occurrence of more than one immature parasite in one host) in field samples of any given host. This view was shown by Salt (1934) to be incorrect, and he concluded that parasites were capable of distinguishing between parasitized and unparasitized hosts. This worker further showed that previous mathematical treatment of the interactions of host and parasite populations, which assumed a random distribution of the progeny of the parasite in the host populations, was invalidated by this selective faculty.

It is proposed in the present work to attempt to determine, in so far as these can be examined in the laboratory, some of the factors governing the incidence of superparasitism in a given host: the study of these factors will naturally involve a consideration of the nature of host selection by the parasite. The effect of climatic and geographical factors upon the distribution of the parasite and its progeny will not be discussed.

II. Material and technique

In laboratory experiments on the behaviour of insects and the interactions of host and parasite populations, ease of handling of the host and parasite is the primary consideration. Therefore, the immobile stages of hosts such as the egg or pupa or stationary hosts such as coccids are usually chosen, though it is generally recognized that in behaviour involving any degree of superparasitism the presence or absence of movement on the part of the host may be an important limiting factor.

Excellent experimental material for such studies has been found in the chalcid *Ooencyrtus kuvanae* Howard, and its host, the gipsy moth, *Porthetria dispar* (L.). The only disadvantage with this material is that the host eggs are field collected and as such have to be carefully sifted for parasitization before use. The method of obtaining parasite-free hosts is outlined below.

The gipsy moth lays a compact mass of 300–400 eggs and covers them uniformly with hairs from the abdomen. Laboratory breeding of the parasite *Ooencyrtus kuvanae* Howard, and the identification of parasitized eggs is greatly facilitated if this hair is removed by gently rubbing the eggs over cheese cloth tightly stretched on a wooden frame. This process has been used extensively in routine breeding in the United States, and there are no ill-effects—the very tough chorion prevents any damage to the hibernating larva within (Howard and Fiske 1912).

Hibernating egg-masses received from the United States in the early autumn contain only a small percentage of parasitized material. By the time of despatch from the field the majority of the last generation of *Ooencyrtus* has emerged from the hosts to hibernate as adults: those which still remain as immature stages may be removed by exposing the hosts to a room temperature of 21° C. for a fortnight to 3 weeks, and any which do not emerge in this time can be destroyed by subsequent prolonged confinement in cold storage. The only other parasite of the gipsy moth egg, *Anastatus disparis* Ruschke, hibernates as a fully grown larva within the host egg-shell and is readily recognized by its creamy colour, which contrasts sharply with the normal unparasitized brown gipsy moth larva. This *Anastatus* material is segregated out when the samples of egg-masses are periodically removed from the refrigerator and cleaned for experiment. If these precautions are observed it is reasonably certain that all host eggs used will be unparasitized.

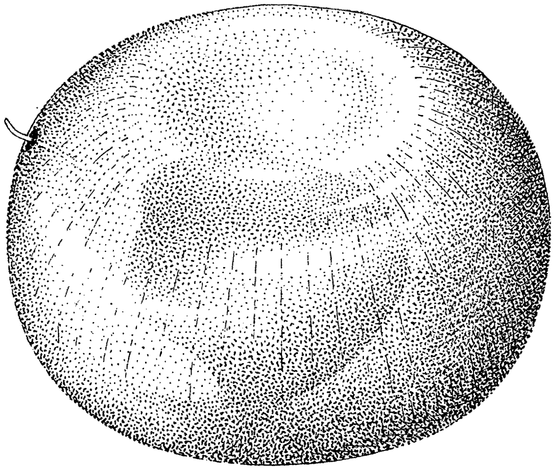
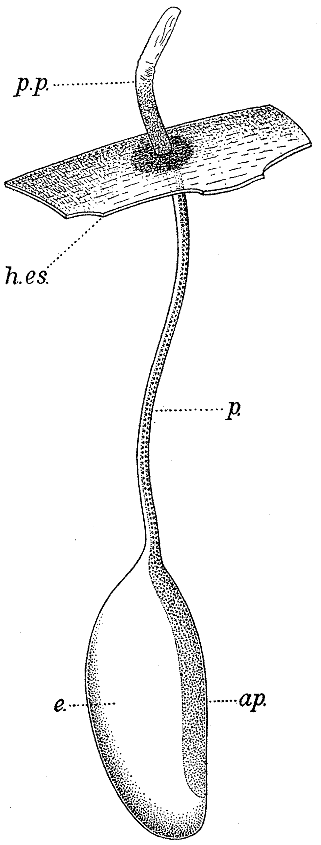


FIG. 1. Gipsy moth egg showing pedicel of *Ooencyrtus* egg. (× 60.)

The egg itself is dark brown in colour, slightly flattened, with the long axis horizontal and the short axis between two depressions which lie on the "upper" and "lower" surfaces; it is quite large for a lepidopterous egg, average figures being 1·2 and 0·90 mm. for the long and short axes respectively. As stated above, the chorion is very tough and resistant and is not sculptured. Within the egg-shell the fully developed larva lies in a semi-spiral so that the dark head capsule is visible externally slightly to one side of one or other of the depressions in the chorion (Fig. 1). It is surrounded by a flocculent white nutrient material, except on the side of the head capsule where there appears to be a small air space. This stage remains in a diapause from July of one year until May of the following year, so that laboratory breeding of the parasite can be continued uninterrupted. In the last three or four months of the diapause, hatching of the gipsy moth larvae occurs on exposure to high temperatures for long periods (more than a week), suggesting that the release from the diapause is a gradual process rather than an abrupt transition. This hatching interferes but little with breeding and experimental work; in any case it can be eliminated by killing the host eggs by immersion in hot water at 60° C. This temperature does not cause a too rapid desiccation of the hibernating larva, and the *Ooencyrtus* readily parasitizes and develops on such hosts (Crossman 1925).

The egg of *Ooencyrtus* has a long pedicel (Figs. 1–3) and is deposited within the body of the host caterpillar with a portion of the egg stalk projecting through the host egg-shell and easily visible under a binocular microscope. The presence of this small portion of the pedicel, external to the host egg-shell, is definite evidence that a parasite egg has been deposited in a particular host; very occasionally a parasite egg is deposited without the pedicel projecting to the exterior, but the percentage of such cases is well within the limits of experimental error.



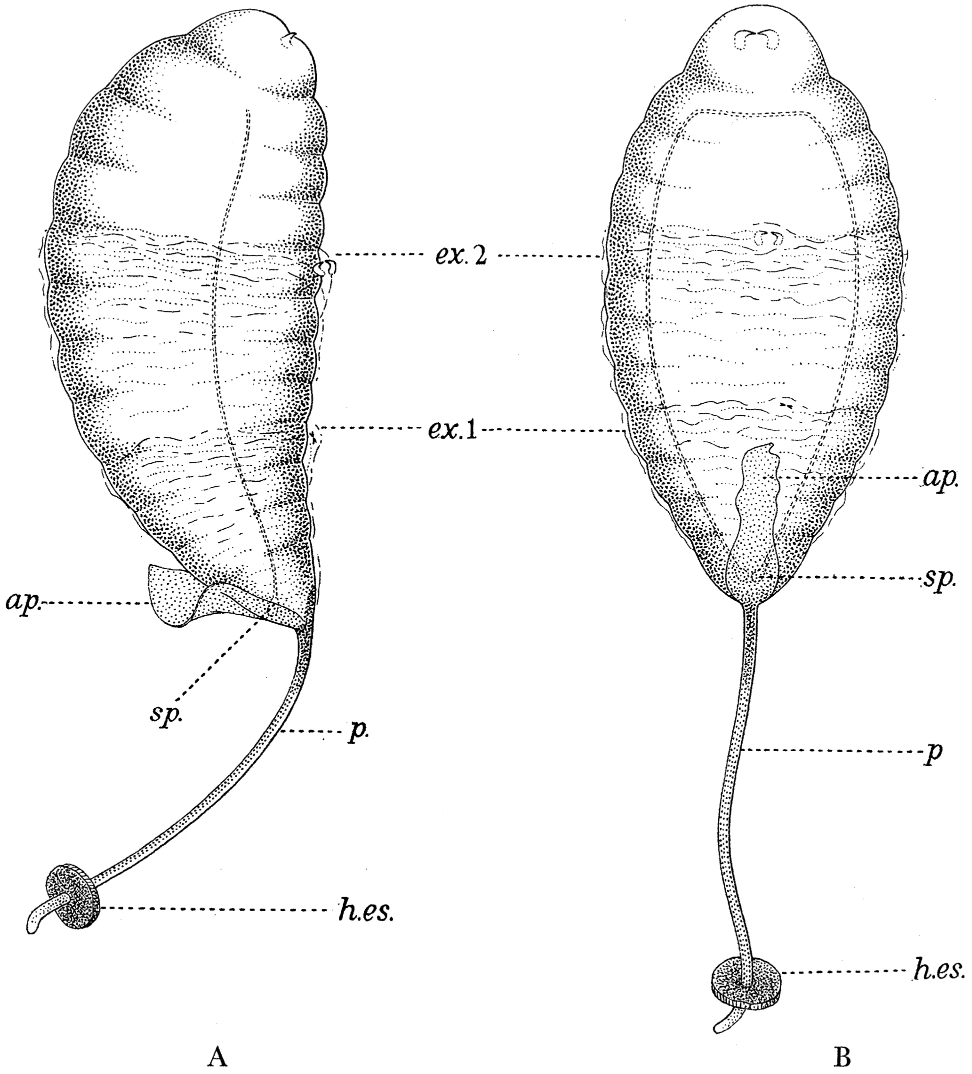


FIG. 2

FIG. 3

FIG. 2. Deposited egg of *Ooencyrtus*. (× 200.) *ap.* aeroscopic membrane (portion of chorion of parasite egg); *e.* egg; *h.es.* host egg-shell; *p.* pedicel; *p.p.* portion of parasite pedicel protruding through host chorion.

FIG. 3. Third instar larva of *Ooencyrtus*. A, lateral view. B, dorsal view. (× 150.) *ex.* 1, 2, exuviae of respective larval instars; *sp.* spiracles; *ap.*, *h.es.*, *p.*, as in fig 2.

All dissection work to determine parasitism can, therefore, be dispensed with, so that the material is admirably suited for any extensive qualitative and quantitative studies. In actual fact, a count of the number of eggs deposited in any given case is more reliable if based on an external examination for pedicels rather than dissections of the host larvae, as the parasite eggs are often laid in organs such as the head capsule, salivary glands, or prolegs, and are easily obscured and overlooked among the numerous long hairs of the gipsy moth larva. Thus in a dissection of a typical sample of 150 hosts, each with a single pedicel projecting through to the exterior, only 141 parasite eggs were found, although the presence of part of the pedicel still adhering to the inside of the egg-shell of the other nine was positive proof that an egg had been deposited.

On hatching, the first instar larva does not become detached from the pedicel but remains partly enveloped posteriorly by the cast egg-shell, part of which has a fine reticulate structure (fig. 2). After each succeeding moult the skin is pushed back and retained and eventually forms a characteristic "anal shield", connexion with which is only broken late in the fifth and final instar; at 25° C. this condition is reached in 8 days, and the fully grown parasite larva becomes clearly visible within the host chorion. This is followed by a brief prepupal period and a pupation of 9 or 10 days, and the entire cycle is completed in approximately 3 weeks. More detailed descriptions of the morphology of the eggs and larvae of related encyrtids have been given by Silvestri (1919) and Maple (1937). Only one parasite is able to develop to maturity in this host. Under laboratory conditions the adult female lives from 4 to 6 weeks.

Maintenance of stock cultures of the parasite presents no difficulties. Several thousands of cleaned hosts are sprinkled on large cardboard disks smeared with gum tragacanth and placed in Petri dishes 6 × 1 in. with the two plates held together with rubber bands. Between thirty and fifty males and females are introduced and left in an incubator at 25° C. for 4 days, when they are removed. At this temperature emergence of the next generation takes place in about 20 days and the first fifty males and females are given a fresh supply of host eggs. Later emergents are segregated in roughly equal numbers of males and females into large tubes and left for 12 hr. for feeding and copulation. If the females are not then used immediately they are set aside at 25° C. until required. Three or four of such stock dishes are kept going so as to ensure an immediate supply of fresh females. A split raisin or a small strip of blotting paper soaked in a solution of one-half honey and one-half water has proved suitable food material.

The method of exposure of the hosts to the action of the female parasites has varied according to the nature of the experiment; for the most part it has been that elaborated by Salt (1934). The essential feature of this technique is the uniform spatial exposure of the hosts under controlled environmental conditions of temperature, humidity and light. This objective is achieved by fastening the host eggs on squared graph paper at chosen intervals—say one-tenth of an inch—with a spot of gum tragacanth. Previous to this the graph paper is cut out in the form of a circle so as to fit conveniently into the larger half of a Petri dish of any required diameter and is held in position by the smaller half and elastic bands. A single fertilized female is then introduced and left for a given time under predetermined conditions of temperature and humidity. All the present work has been done at an arbitrarily selected temperature of 25° C. and

70% relative humidity. At the end of the time period the female is removed and the hosts examined for projecting pedicels to obtain the distribution of the parasite eggs.

III. Factors affecting distribution

(i) *The nature of the hosts available for parasitization*

In the New England States, hibernating females of *Ooencyrtus* commence ovipositing about May in the overwintering eggs of the gipsy moth, and second and subsequent generations are passed in the newly deposited eggs between July and November. These generations so overlap one another that they cannot be sharply separated, but there is time in the prevailing climate for four generations and a partial fifth. The season's gipsy moth eggs are also attacked by *Anastatus disparis* Ruschke, a eupelmid, which has a single generation per annum, closely correlated with that of its host. Hibernation of this parasite is as a fully grown larva within the overwintering gipsy moth eggs, and emergence takes place in the following July when there is a maximum deposition of eggs by the host moth.

It is evident that the occurrence of these overlapping generations, and the presence of two species of parasite attacking the same host at this time, will result in a wide range of media for oviposition for any female *Ooencyrtus*. The hosts available at any time may be unparasitized, though this is only likely soon after deposition, as American records often state that female *Ooencyrtus* and *Anastatus* attack their hosts while the moth is still laying; it is more probable that the hosts encountered by a female will be parasitized to a varying degree by either species of parasite in any of their immature stages. To a limited extent, these conditions can be reproduced in the laboratory and the behaviour of the female studied.

(*a*) *The parasitized or unparasitized condition of the host*

The possibility that the female *Ooencyrtus* is able to discriminate between unparasitized hosts and those parasitized but containing various developmental stages of the parasite, was investigated in the following manner.

Freshly parasitized material was obtained by exposing large numbers of cleaned gipsy moth eggs to a number of fertilized females for a short time, such as 12 hr., after which the females were removed and the hosts examined under the binocular. Hosts parasitized with a single parasite egg were identified by the protruding portion of the pedicel and this was cut off with a sharp scalpel so that the pedicel ended at the point of attachment to the host egg-shell. This removal in no way affects the development of the larva and was necessary for the following reason. With the hosts fixed on squared paper in the manner previously described the pedicels project from any point of the "upper" half, the females apparently having no particular preference for any larval organs for the location of their eggs. In transferring parasitized hosts to fresh dishes, these projecting portions were frequently squashed by the points of the forceps and could not be seen under the binocular. As some of the undamaged pedicels might be confused with those laid later in the experiment, all were removed. These parasitized hosts were then placed in a Wisconsin cabinet at 25° C. and 70 % R.H. and left to develop to the required stage.

In choosing hosts containing the immature stages of the parasite no attempt was made to limit these stages to definite morphological instars of the parasite: the aim was rather to give the female parasite a choice between hosts containing fairly sharply defined stages of parasite development such as the egg, early larva, late larva and pupa. This was done by exposing the parasitized hosts at 25° C. for 12, 8, 4 and 1 days to obtain parasite pupa, late larva, early larva and egg respectively.

Material so treated was then arranged on squared paper in the requisite combination in the manner outlined above, and with the hosts disposed one-tenth of an inch apart. In each case a total of fifty hosts, consisting of twenty-five of one type of host alternated with twenty-five of another type, was used, the arrangement being *a*, *b*, *a*, *b*, *a*, *b*, *a*, *b*, *a*, *b*, in five rows of ten (see fig. 4). The combinations were:

Series 1: Control (unparasitized).

Series 2: Hosts unparasitized alternating with hosts with one *Ooencyrtus* egg.

Series 3: Hosts with one *Ooencyrtus* egg alternating with hosts with one early *Ooencyrtus* larva.

Series 4: Hosts with one *Ooencyrtus* egg alternating with hosts with one late *Ooencyrtus* larva.

Series 5: Hosts with one *Ooencyrtus* egg alternating with one *Ooencyrtus* pupa.

Series 6: Hosts with one early *Ooencyrtus* larva alternating with hosts with one late *Ooencyrtus* larva.

Series 7: Hosts with one late *Ooencyrtus* larva alternating with hosts with one hibernating larva of *Anastatus*.

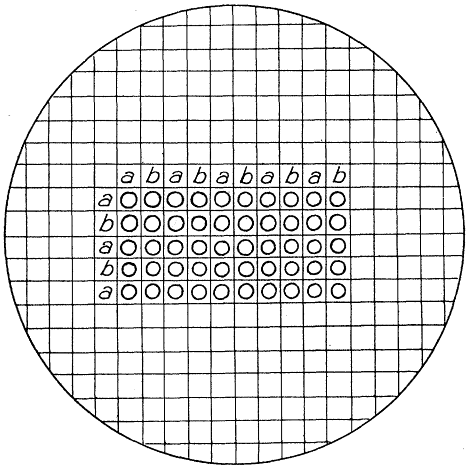


FIG. 4. Method of exposure of hosts for determination of selection by parasite.

A choice between hosts containing late *Ooencyrtus* larvae and hosts containing hibernating *Anastatus* larvae was included so as to obtain some indication of the effect of the type of parasitism on the selective faculty. These hosts containing hibernating *Anastatus* were obtained from the field material. It is to be noted that in Exps. 3–7 parasitized material only was available. A single fertilized female, 2 days old, was introduced into each dish and left for 24 hr. at 25° C. and 70 % R.H. An average of ten females, each with fifty hosts, was taken for each series, so that totals of seventy females and 3500 hosts were used in the whole experiment.

The results are given in Tables I–VII and represented diagramatically in fig. 5.

When a host is said to be superparasitized with one or more eggs it is meant that one or more eggs in addition to the original parasite egg were present. It is evident that the females exercised considerable choice in all conditions, but the selective faculty does not appear to be perfect. When all the hosts were unparasitized, the females laid one egg in each host, there being only one case of superparasitism in a total of 143 eggs laid by the ten females. The superparasitized host occurred in the experiment in which the largest number of eggs was deposited by a female. The spatial distribution of these 143 eggs in the 500 hosts was as would be expected from chance, with seventy-two eggs in "A" type hosts and seventy-one eggs in the alternate "B" type hosts. Where unparasitized hosts were available along with parasitized hosts, the amount of superparasitism was greater than in the control, but the females showed a quite definite general avoidance of hosts already parasitized; when, however, the females were compelled to superparasitize, that is, they were given access only to parasitized hosts, the choice was those hosts which contained the younger parasite stage within them.

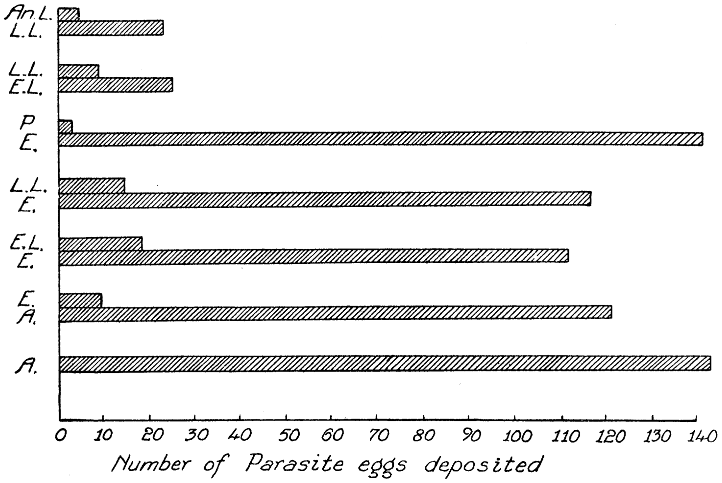


FIG. 5. Selection in hosts containing various developmental stages of the parasites. Females were given a choice of two types of hosts indicated by the couplings. *A*, unparasitized hosts; *E*, hosts with *Ooencyrtus* egg; *EL*, hosts with *Ooencyrtus* early larva; *LL*, hosts with *Ooencyrtus* late larva; *P*, hosts with *Ooencyrtus* pupa; *An.L.* hosts with hibernating larva of *Anastatus disparis*.

The female, therefore, accepted as hosts those which were the nearest approach in general physico-chemical characteristics to the normal unparasitized host, for the development of the parasite larva brings about a progressive disintegration of the hibernating gipsy moth larva.

A striking example of this occurred when the female was confronted with a choice of hosts parasitized by her own species and those parasitized by another species, namely *Anastatus disparis*. The latter species usually parasitizes the host egg before the gipsy moth embryo has developed, so that no trace of a gipsy moth larva is present when a host egg contains a hibernating larva of *Anastatus*. *Ooencyrtus*, on the other hand, attacks the host in any condition and as the gipsy moth egg contains a hibernating

TABLE I

"A" hosts unparasitized. Hosts parasitized with			"B" hosts unparasitized. Hosts parasitized with			Total nos. of eggs laid by females
1 egg	2 eggs	3 eggs	1 egg	2 eggs	3 eggs	
4	—	—	5	—	—	9
8	—	—	10	—	—	18
7	—	—	7	—	—	14
7	—	—	6	—	—	13
11	1	—	13	—	—	26
5	—	—	8	—	—	13
7	—	—	4	—	—	11
5	—	—	4	—	—	9
8	—	—	12	—	—	20
7	—	—	3	—	—	10
69	1	0	72	0	0	
71			72			143

TABLE II

Hosts with one *Ooencyrtus* egg. Hosts superparasitized with			Hosts with no parasite. Hosts parasitized with			Total nos. of eggs laid by females
1 egg	2 eggs	3 eggs	1 egg	2 eggs	3 eggs	
2	—	—	19	—	—	21
1	—	—	6	—	—	7
—	—	—	19	—	—	19
1	—	—	14	—	—	15
2	—	—	15	—	—	17
—	—	—	4	—	—	4
—	—	—	1	—	—	1
—	—	—	12	1	—	14
1	—	—	12	—	—	13
2	—	—	16	—	—	18
9	0	0	118	1	0	
9			120			129

TABLE III

Hosts with one early *Ooencyrtus* larva. Hosts superparasitized with			Hosts with one *Ooencyrtus* egg. Hosts superparasitized with			Total nos. of eggs laid by females
1 egg	2 eggs	3 eggs	1 egg	2 eggs	3 eggs	
2	—	—	8	—	—	10
—	—	—	7	2	—	11
2	—	—	11	—	—	13
—	—	—	—	—	—	0
4	—	—	11	2	—	19
1	—	—	12	3	—	19
2	—	—	9	1	—	13
1	—	—	10	3	—	17
4	—	—	17	1	—	23
1	—	—	3	—	—	4
17	0	0	88	12	0	
17			112			129

TABLE IV

Hosts with one late *Ooencyrtus* larva. Hosts superparasitized with			Hosts with one *Ooencyrtus* egg. Hosts superparasitized with			Total nos. of eggs laid by females
1 egg	2 eggs	3 eggs	1 egg	2 eggs	3 eggs	
2	—	—	15	—	—	17
—	—	—	14	2	1	21
—	—	—	10	1	—	12
4	—	—	11	—	—	15
—	—	—	13	—	—	13
3	1	—	7	—	—	12
1	—	—	8	5	—	19
1	—	—	7	1	—	10
—	—	—	6	1	—	8
1	—	—	2	—	—	3
12	1	0	93	10	1	
	14			116		130

TABLE V

Hosts with one *Ooencyrtus* pupa. Hosts superparasitized with			Hosts with one *Ooencyrtus* egg Hosts superparasitized with			Total nos. of eggs laid by females
1 egg	2 eggs	3 eggs	1 egg	2 eggs	3 eggs	
—	—	—	12	2	—	16
—	—	—	7	1	1	12
—	—	—	9	2	—	13
1	—	—	9	—	1	13
—	—	—	12	4	1	23
—	—	—	7	—	—	7
—	—	—	3	—	—	3
1	—	—	9	5	1	23
—	—	—	12	1	1	17
—	—	—	8	1	2	16
2	0	0	88	16	7	
	2			141		143

TABLE VI

Hosts with one late *Ooencyrtus* larva. Hosts superparasitized with			Hosts with one early *Ooencyrtus* larva. Hosts superparasitized with			Total nos. of eggs laid by females
1 egg	2 eggs	3 eggs	1 egg	2 eggs	3 eggs	
—	—	—	3	—	—	3
1	—	—	2	—	—	3
—	—	—	5	1	—	7
—	—	—	1	—	—	1
4	—	—	3	1	—	9
—	—	—	—	1	—	2
2	—	—	3	—	—	5
—	—	—	2	—	—	2
2	—	—	—	—	—	2
—	—	—	—	—	—	0
9	0	0	19	3	0	
	9			25		34

Table VII

Hosts with one late *Ooencyrtus* larva. Hosts superparasitized with			Hosts with one hibernating *Anastatus* larva. Hosts superparasitized with			Total nos. of eggs laid by females
1 egg	2 eggs	3 eggs	1 egg	2 eggs	3 eggs	
—	—	—	1	—	—	1
4	1	—	—	—	—	6
2	—	—	—	—	—	2
5	—	—	1	—	—	6
1	1	—	—	—	—	3
1	—	—	—	—	—	1
2	—	—	—	—	—	2
2	—	—	2	—	—	4
1	—	—	—	—	—	1
1	—	—	—	—	—	1
19	2	0	4	0	0	
23			4			27

embryo throughout most of the summer, this is the type most frequently attacked. A host egg with an advanced *Ooencyrtus* larva still contains remnants of the host larva, and these seem to be a sufficient inducement for the ovipositing female to lay in such hosts parasitized by a female of the same species in preference to those containing a hibernating *Anastatus* larva. Of course, the female of a given species of parasite may have a greater dislike for hosts parasitized by a different species than for those parasitized by her own species. Unfortunately, it has not been feasible to examine this possibility by allowing the *Ooencyrtus* female a choice between hosts containing *Ooencyrtus* eggs and hosts containing *Anastatus* eggs, because of some laboratory difficulties with the oviposition of the latter species.

In the time allowed for the experiment there was no appreciable reduction in the total number of eggs laid by the females when hosts containing a parasite egg were present. However, when the available parasitized hosts contained any parasite stage after an early larva, the females did not lay to the same extent as the control females, but exercised restraint. This aspect will be dealt with at a later point.

Where superparasitism was enforced—series 3–7—an interesting point arose in regard to the actual distribution of the eggs in the younger parasitized material. In series 2, of the 119 unparasitized hosts accepted, 118 (or 99·2 %) were parasitized with a single egg and one host was superparasitized with one egg. In other series the percentage of hosts superparasitized with one egg was considerably smaller; thus in series 5, of 111 hosts containing a single parasite egg which were accepted, instead of an anticipated 111 hosts superparasitized with one egg, only eighty-eight (or 79·3 %) were superparasitized with a single egg, while sixteen hosts were superparasitized with two eggs and seven hosts with three eggs. It appears, therefore, that the female's selective faculty for choosing the younger parasite stages in such parasitized material as is available is such that the difference between a host parasitized by herself in the course of the experiment and that parasitized say 24 hr. earlier (as would be the case with the hosts containing a single *Ooencyrtus* egg in these experiments) is not of the same order as that between parasitized and unparasitized hosts.

This suggests that there is a limit to the extent to which a female is able to distinguish between the various stages of the parasite development within the host, and that this limit occurs somewhere in the larval development between the first and fourth days after the deposition of the egg, for it will be seen by referring to fig. 5, that the discrimination between hosts containing an early parasite larva and those containing a parasite egg is of much the same order as discrimination between the other alternatives.

The series of experiments was therefore extended to the following types. It should be stated that at 25° C. and 70% R.H. the parasite egg hatches in the third day and that the ages indicated below for the *Ooencyrtus* eggs were those at the commencement of the experimental period.

Series 1: Control: unparasitized.

Series 2: Hosts with one *Ooencyrtus* egg (48 hr. old) alternating with hosts with one *Ooencyrtus* egg (2 hr. old).

Series 3: Hosts with one *Ooencyrtus* egg (24 hr. old) alternating with hosts with one *Ooencyrtus* egg (2 hr. old).

The parasitized hosts were obtained by the method previously outlined, the only modification being an increase in the number of hosts and females used so as to obtain a sufficiently large number of parasitized hosts within two hours. Ten females were used for each series, and each female was given access to fifty hosts for 24 hr. at 25° C. and 70% R.H.

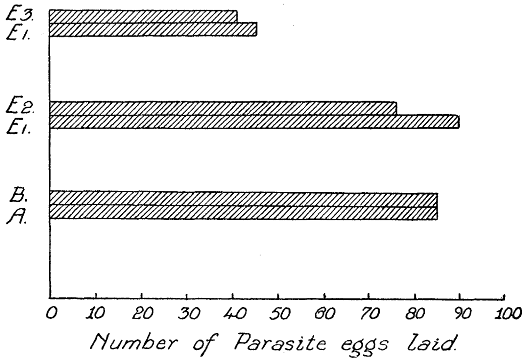


FIG. 6. Selection in hosts containing immature stages of *Ooencyrtus*. *A.B.* unparasitized hosts; E_1, hosts containing *Ooencyrtus* egg 2 hr. old; E_2, hosts containing *Ooencyrtus* egg 48 hr. old; E_3, hosts containing *Ooencyrtus* egg 24 hr. old.

The results are given in Tables VIII–X and represented diagrammatically in fig. 6. The distributions of the eggs laid in these experiments do not suggest that the hatching, which occurs with the 48 hour old parasite egg in the course of the experiment, is of any great significance, but the data, considered in relation to the behaviour in the previous experiments, indicate that the hatching and subsequent changes in the

Table VIII

“A” hosts unparasitized. Hosts parasitized with			“B” hosts unparasitized. Hosts parasitized with			Total nos. of eggs laid by females
1 egg	2 eggs	3 eggs	1 egg	2 eggs	3 eggs	
10	—	—	7	—	—	17
10	—	—	10	—	—	20
12	—	—	9	—	—	21
7	—	—	12	—	—	19
15	—	—	9	—	—	24
6	—	—	4	—	—	10
5	—	—	7	—	—	12
8	—	—	10	—	—	18
3	—	—	6	—	—	9
9	—	—	11	—	—	20
85	0	0	85	0	0	
85			85			170

Table IX

Hosts with one *Ooencyrtus* egg. (48 hr. old) Hosts superparasitized with			Hosts with one *Ooencyrtus* egg. (24 hr. old) Hosts superparasitized with			Total nos. of eggs laid by females
1 egg	2 eggs	3 eggs	1 egg	2 eggs	3 eggs	
9	2	—	8	—	—	21
6	—	—	5	—	—	11
9	1	—	9	1	—	22
4	—	—	9	—	—	13
8	—	—	5	—	—	13
4	—	—	2	—	—	6
6	1	—	11	2	—	23
5	1	—	9	—	—	16
5	—	—	8	—	—	13
8	1	—	7	5	—	27
64	6	0	73	8	0	
76			89			165

Table X

Hosts with one *Ooencyrtus* egg. (24 hr. old) Hosts superparasitized with			Hosts with one *Ooencyrtus* egg. (2 hr. old) Hosts superparasitized with			Total nos. of eggs laid by females
1 egg	2 eggs	3 eggs	1 egg	2 eggs	3 eggs	
3	—	—	3	—	—	6
4	—	—	10	—	—	14
8	—	—	6	—	—	14
1	—	—	4	—	—	5
6	—	—	4	—	—	10
5	—	—	3	—	—	8
6	—	—	7	—	—	13
2	—	—	4	—	—	6
1	—	—	2	—	—	3
5	—	—	2	—	—	7
41	0	0	45	0	0	
41			45			86

host associated with the development of the parasite larva, are responsible for the graded acceptability of such parasitized hosts. These results are opposed to the findings of Salt (1937) with *Trichogramma evanescens* Westwood. This worker found (1) that the female *Trichogramma* recognizes an odour left by previous females on the external surface of the host *Sitotroga cerealella* Olivier and that this odour inhibits attack; and (2) that by insertion of the ovipositor the female detects an internal difference between parasitized and unparasitized hosts, which inhibits oviposition. As far as may be judged from end results, external odour is of no importance with *Ooencyrtus*, for the eggs laid in the experiment are deposited in approximately equal numbers in hosts containing a 2 hr. old parasite egg and in hosts containing a 24 hr. old parasite egg. Any chemical traces left on the external surface of the hosts by the previous female would be of greater intensity on the former hosts. The criticism by Ullyett (1936) of the experimental method of alternation of types of hosts in a confined space as failing to eliminate the odour stimulus is not applicable to the gipsy moth, as these eggs are normally deposited contiguously in masses and we are concerned with the behaviour of the female in such conditions. Furthermore, the criticism is only valid when an experimenter assumes that the acceptability of a host can be gauged from the frequency of attack as indicated by the adoption of the piercing posture by the female. Although this act of piercing has been demonstrated in many insects to be conditional upon the odour stimulus, final acceptance of any host depends upon a combination of factors and can only be assessed by the presence or absence of a parasite egg.

(*b*) *The death of the host and its contained parasite*

After establishing the existence of a selective faculty, attempts were made to ascertain some of the factors upon which this selection is based. A certain proportion of the overwintering *Ooencyrtus* females which survive to the following spring parasitize and the progeny develop in gipsy moth eggs which have failed to hatch. As mentioned in the Introduction, advantage may be taken of this fact to overcome difficulties in the laboratory breeding of this parasite due to the emergence of the host larvae; this is prevented by killing the hosts by immersion in hot water prior to exposure to the female parasites. Reflexion on this technique suggested that an examination of the selection in hosts containing dead or live parasites might reveal some interesting facts bearing on this question. Accordingly, the following series were arranged in the standard manner in Petri dishes, with fifty hosts in each container. With the exception of the control (series 1) the hosts were alternated in the normal way, so that every female had a definite choice of media in which to deposit her eggs.

Series 1: Dead hosts unparasitized.

Series 2: Live hosts unparasitized alternated with dead hosts unparasitized.

Series 3: Dead hosts unparasitized alternated with dead hosts with one live *Ooencyrtus* larva.

Series 4: Dead hosts unparasitized alternated with dead hosts with one dead *Ooencyrtus* larva.

Series 5: Dead hosts with one live *Ooencyrtus* larva alternated with dead hosts with one dead *Ooencyrtus* larva.

Hosts parasitized with a single *Ooencyrtus* egg were obtained by the routine previously outlined and were left for 4 days at 25° C. for the parasite to develop to the early larval stage by the commencement of the experiment. At this age the parasite has made a measurable effect upon the host and is itself capable of some slight movements. The most convenient method of killing the host or the contained parasite was by immersion in hot water at 60° C. for 30 sec. This treatment does not give a 100 % mortality of the gipsy moth larvae and between 12 % and 15 % of the eggs hatch. The percentage emergence of parasites from hosts treated in the above manner was:

Dead hosts containing live parasites	75 %
Dead hosts containing dead parasites	0 %
Normal live hosts containing live parasites ...	92 %

It will be seen, therefore, that the killing of the host by this method may cause a too rapid desiccation and the death of the parasite larva it may later contain. This desiccation is made more pronounced by the second immersion in hot water in order to kill the parasite larva. This feature has to be allowed for in the interpretation of results. Ten females were used in each series so that totals of fifty females and 2500 hosts were used. Each female was left with fifty hosts for 24 hr. at 25° C. and 70 % R.H. The oviposition is given in Tables XI–XV and represented diagrammatically in fig. 7.

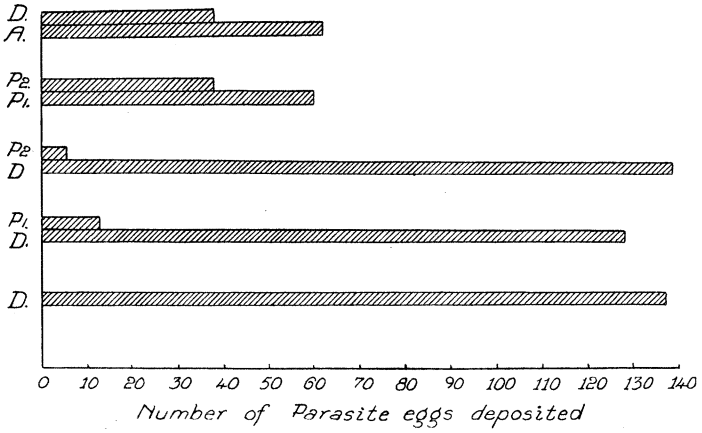


FIG. 7. Selection in hosts containing dead and live parasites. *A*, live hosts unparasitized; *D*, dead hosts unparasitized; P_1, dead hosts containing live early larva of *Ooencyrtus*; P_2, dead hosts containing dead early larva of *Ooencyrtus*.

Although dead hosts are not as acceptable as live hosts, the disparity between the oviposition in these two types is not sufficient to justify the conclusion that dead hosts will usually be avoided in the field. As in the preceding experiments, hosts containing

Table XI

"A" dead hosts unparasitized. Hosts parasitized with			"B" dead hosts unparasitized. Hosts parasitized with			Total nos. of eggs laid by females
1 egg	2 eggs	3 eggs	1 egg	2 eggs	3 eggs	
5	—	—	3	—	—	8
4	—	—	2	—	—	6
10	—	—	12	—	—	22
7	—	—	6	—	—	13
13	—	—	10	—	—	23
6	—	—	7	—	—	13
8	—	—	5	—	—	13
9	—	—	12	—	—	21
3	—	—	1	—	—	4
8	—	—	6	—	—	14
73	0	0	64	0	0	
73			64			137

Table XII

Dead hosts unparasitized. Hosts parasitized with			Live hosts unparasitized. Hosts parasitized with			Total nos. of eggs laid by females
1 egg	2 eggs	3 eggs	1 egg	2 eggs	3 eggs	
5	—	—	4	—	—	9
2	—	—	4	—	—	6
3	—	—	7	—	—	10
9	—	—	5	—	—	14
2	—	—	6	—	—	8
2	—	—	10	—	—	12
5	—	—	9	—	—	14
4	—	—	10	—	—	14
4	—	—	7	—	—	11
2	—	—	—	—	—	2
38	0	0	62	0	0	
38			62			100

Table XIII

Dead hosts with one live *Ooencyrtus* larva. Hosts superparasitized with			Dead hosts unparasitized. Hosts parasitized with			Total nos. of eggs laid by females
1 egg	2 eggs	3 eggs	1 egg	2 eggs	3 eggs	
—	—	—	9	—	—	9
3	—	—	11	—	—	14
1	—	—	17	—	—	18
3	—	—	7	1	—	12
1	—	—	11	—	—	12
3	—	—	11	—	—	14
1	—	—	23	—	—	24
—	—	—	2	—	—	2
—	—	—	14	—	—	14
1	—	—	22	—	—	23
13	0	0	127	1	0	
13			129			142

TABLE XIV

Dead hosts with one dead *Ooencyrtus* larva. Hosts superparasitized with			Dead hosts unparasitized. Hosts parasitized with			Total nos. of eggs laid by females
1 egg	2 eggs	3 eggs	1 egg	2 eggs	3 eggs	
—	—	—	—	—	—	0
—	—	—	5	—	—	5
—	—	—	6	—	—	6
1	—	—	11	—	—	12
1	—	—	10	—	—	11
2	—	—	21	—	—	23
—	—	—	19	3	—	25
1	—	—	20	1	—	23
1	—	—	13	2	—	18
—	—	—	20	1	—	22
6	0	0	125	7	0	
	6			139		145

TABLE XV

Dead hosts with one dead *Ooencyrtus* larva. Hosts superparasitized with			Dead hosts with one live *Ooencyrtus* larva. Hosts superparasitized with			Total nos. of eggs laid by females
1 egg	2 eggs	3 eggs	1 egg	2 eggs	3 eggs	
1	1	—	4	2	—	11
3	—	1	1	—	1	10
3	—	—	4	—	—	7
2	—	—	8	—	—	10
3	1	—	5	1	—	12
5	1	—	4	1	—	13
2	—	—	6	—	—	8
5	—	—	7	—	—	12
2	—	—	4	—	—	6
3	—	—	4	1	—	9
29	3	1	47	5	1	
	38			60		98

a live parasite were practically universally rejected. A similar discrimination occurs when hosts containing a dead parasite were present with unparasitized dead hosts, while if given a choice of hosts containing dead or live parasites, the female *Ooencyrtus* tended to avoid the former.

Several important conclusions are inferred from this experiment. Firstly, the oviposition response is determined, in the main, by conditions *within* the host egg-shell. It has been demonstrated that *Trichogramma evanescens* tends to avoid walking on areas already traversed by females of the same species, because of a repellent odour left by these previous individuals, and detected by later females. Furthermore, this chemical material has been proved to be volatile and water soluble. In the present experiment, an odour left on the external surface of the parasitized hosts might have evaporated as the parasite within was developing to an early larva; it is clear that any traces which might have remained did not influence the distribution of the females' eggs, for the latter actually preferred hosts containing live parasites to hosts containing dead parasites. If water-soluble chemical substances had been present they should have been eliminated by immersion in hot water. Moreover, it cannot be said that other characters of the host type, such as movements of the parasite larvae, outweighed any significance attaching to a possible external odour, for hosts containing a dead parasite were avoided to an extent equal to those containing live parasites. This is the second point emerging from this experiment—the fact that movements of the contained parasite here play a minor part in helping the female to determine the precise nature of the host. One cannot presume this to be general for the entire development of the parasite, as only the limited movements of early larvae are involved here. Thirdly, the experiment raises the problem of the suitability of the hosts chosen by the females for the development of their progeny. It is evident that, even allowing for the appreciable mortality during parasite development because of desiccation of the gipsy moth larvae occurring in hosts which have been immersed twice in hot water, the choice of hosts containing a live parasite rather than hosts containing a dead parasite is an unsuitable one; for with the gipsy moth egg only one *Ooencyrtus* can develop to maturity and the older parasite inhabitant is almost invariably the survivor. This question may be more conveniently considered in conjunction with other data and will be discussed later in the paper.

Finally, attention may be drawn to the action of this selective faculty on the interactions of the host and parasite populations. The data of the preceding experiments indicate that the distribution of the progeny of the parasite is not at random, provided a choice of hosts is available. It is now necessary to examine the response of the parasite when no choice exists.

(ii) *The exercise of restraint*

In a previous section the discriminatory powers of the female *Ooencyrtus* were examined and it was noted that, if only parasitized hosts were accessible and these contained immature stages of the parasite later than the early larvae, the females tended to retain their eggs rather than deposit them in such material. It might be thought that the possession of a selective faculty by the female parasite would result in only a passing interest being evinced in parasitized hosts in the field, but the large amount of superparasitism usually present in all field collected material suggests that some other factor or factors operate in such a way as partially to nullify this capacity for host selection. It seems reasonable to assume that the female parasites do not often traverse completely unparasitized egg-masses of the host under natural conditions, and are repeatedly encountering varying numbers of parasitized hosts which they either accept or reject. It is possible that the behaviour when there is a choice of hosts may be quite different from that when no choice exists, and the acceptance or rejection of a parasitized host may depend, in part, upon the frequency of contact of the female with such parasitized hosts. This latter aspect will be examined later.

The retention of eggs by a female parasite was first demonstrated by Salt (1934) with *Trichogramma evanescens*, and the subject was elaborated by him in a subsequent paper (1936). He concluded that this restraint is related to the number of hosts available and is limited in time, since a partial breakdown occurs if the time of exposure of the female parasite to the hosts is increased. Thus, female *Trichogrammas* given ten hosts for eight hours laid an average of 13·8 eggs, while control females with one hundred hosts deposited an average of 22·5 eggs. In a twenty-four hour exposure the figures were 18·0 and 28·8 for ten and one hundred hosts respectively.

Because of the nature of his experimental material Salt was unable to isolate the two factors involved in such restraint, namely the number of hosts available and the parasitized or unparasitized condition of such hosts. With the present parasite, parasitized hosts can be identified by external examination and the restraint exercised by the female in relation to these two aspects can be determined.

(a) *Its relation to the developmental stage of the parasite in the host*

In investigating the retention of eggs by the female when only parasitized hosts were available, it was decided to include hosts which contained the parasite in various immature stages. This series was arbitrarily selected as hosts containing an *Ooencyrtus* egg, early larva, late larva, pupa, and hibernating larva of *Anastatus disparis*. This latter hibernating larva is also an egg parasite of the gipsy moth, and was chosen from field collected material. The other host types were obtained by the routine process of parasitization and incubation for intervals of 1, 4, 8 and 12 days.

Because of the labour involved in obtaining such material and setting up the experiments in the one day, ten hosts instead of the usual twenty-five were supplied for each female. The eggs were arranged one-tenth of an inch apart in the standard manner in small Petri dishes $2 \times \frac{1}{2}$ in., and a single fertilized female was introduced and left for twenty-four hours in an incubator at 25° C. and 70 % R.H. The hosts were then examined for parasitism under a binocular microscope. The experiments were repeated ten times in each series. The details of oviposition of the females, which had been kept for one day after emergence before being allowed to lay, are given in Table XVI and fig. 8.

Before discussing the results, it is necessary to give a brief description of the development of the parasite and its effect upon the host. At a temperature of 25° C. hatching of the parasite egg occurs in the third day after deposition and the larva passes through the first, second, third and fourth instars in the next 3 or 4 days. During this period growth is very gradual, so that the fourth instar is no bigger than the head capsule of the host. The latter is little affected at this stage, except for disintegration in the immediate vicinity of the parasite larva, and no change in the host can be detected by external examination. On the seventh day the larva enters the fifth and final instar

Table XVI. Oviposition of 1 day old females in hosts containing various developmental stages of the parasite

Parasite stage in host	Eggs laid by female No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	Total nos. of eggs laid by 10 females	Average per female
Nil (control)	18	10	11	0	3	1	9	18	5	3	78	7·8
Egg	19	4	1	8	0	11	9	12	0	1	65	6·5
Early larva	10	10	0	11	1	1	3	7	1	2	46	4·6
Late larva	1	0	6	1	0	0	0	0	2	0	10	1·0
Pupa	0	0	1	0	1	0	0	0	0	0	2	0·2
Anastatus (hibernating larva)	0	0	0	1	0	0	0	0	0	0	1	0·1

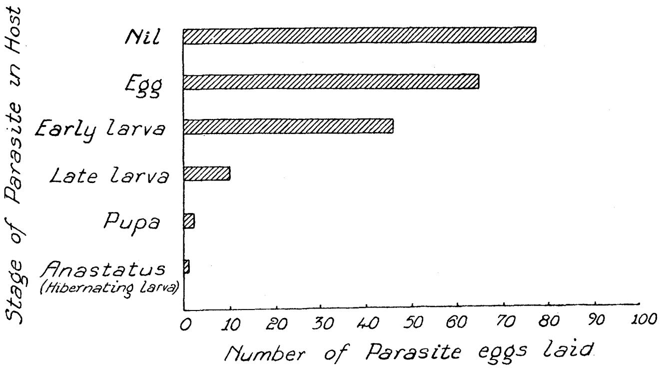


Fig. 8. Oviposition of 1 day old females in hosts containing various developmental stages of the parasite.

and growth is now very rapid. The hibernating gipsy moth larva quickly loses its form and becomes a disintegrated brown mass inside the egg-shell, through which the developing parasite larva is often visible. The entire fluid contents are now devoured and only the chitinized parts and hairs of the host larva are left clinging to the inside of the host egg-shell. These hairs give a characteristic whitish appearance to the hosts which contrasts sharply with the normal brown colour. At this point—8 or 9 days after deposition of the egg—the mature larva occupies the entire space inside the host egg-shell, but with defaecation and the transformation through prepupa to pupa some shrinkage occurs and the latter lies quite free of the host egg-shell. The pupal stage occupies approximately ten days so that the adults emerge about twenty days after the deposition of the egg. The development consists, essentially, of the gradual replacement of the tissues of the gipsy moth larva by the parasite larva, but there is no apparent methodical destruction of these tissues as the parasite egg is deposited in any organ of the host; eventually only the egg-shell remains, lined with chitinized parts of the gipsy moth larva such as hairs and legs.

On examination of Table XVI it will be apparent that the amount of oviposition is intimately related to the state of the parasite within the host. As the contained parasite develops, the host becomes less and less attractive to the ovipositing female parasites and they deposit fewer eggs in such hosts. Thus, ten females laid seventy-eight eggs in a total of one hundred unparasitized hosts, whereas only two eggs were deposited by ten females given access to one hundred parasitized hosts containing parasite pupae. These latter types of host were so infrequently accepted as to justify the conclusion that they are completely ignored under natural conditions, and as the pupal stage occupies roughly half the developmental period passed by the parasite in the host, this inhibition of oviposition is of very real importance to the species. There is, of course, considerable individual variation in the extent of this restraint, particularly in regard to the hosts containing the earlier parasite stages.

The significance of this behaviour will be appreciated when it is realized that the gipsy moth egg contains sufficient food material for only one *Ooencyrtus* to develop to maturity. As a general rule, when a host is superparasitized the older larva proves to be the survivor, but occasionally two small individuals emerge from one host. An exception to this general rule occurs when a host is superparasitized and the last egg is actually deposited within the body of the advanced larva and pupa of its own species lying inside the host egg-shell. Here the egg hatches and the young larva develops as a hyperparasite upon the larger larva or pupa. Sometimes both individuals die. Thus, of thirty-eight advanced *Ooencyrtus* larvae which were selected as having been parasitized with an egg of their own kind, twenty-two gave no emergences and the other sixteen gave eight males and eight females. Similarly twelve *Ooencyrtus* pupae, apparently parasitized with an egg of the same species, gave one non-emergence, nine males and two females. Of course, when the contained parasite stage is very small, it is very improbable that any subsequent eggs will be laid within it; these will usually lie within the body of the gipsy moth larva itself.

The ability of the female parasite to distinguish between parasitized and unparasitized hosts, and to retain eggs when only parasitized hosts are encountered, tends to decrease superparasitism and gives a better distribution of the parasite eggs, for if the female has to retain her eggs she will presumably continue to search for unparasitized hosts. When such hosts are scarce and parasitized hosts are repeatedly encountered, the restraint breaks down, with the result that there is a considerable waste of the progeny of the parasite. However, at this point, another factor assumes importance, and the reproductive activity is regulated to some degree by the precise nature of the parasitized hosts available.

Although the parasite stages were chosen so as to present, to the observer, quite distinct and sharply defined differences, the significant feature of the behaviour of the female parasites is that the response is a graded one. At least two factors, acting independently or in combination, may be responsible for this. The amount of egg laying may be positively correlated with (1) the development of the parasite egg, larval and pupal stages, and their associated movements; or with (2) the quantity of true host material (as opposed to parasite material) present within a host egg-shell at any given time.

As the hibernating gipsy moth larva is in a diapause during which there are no discernible movements, reactions on the part of the host in the form of changes in the intensity or type of movements can be ruled out as the causal agents of this phenomenon.

The suggestion that movements of the immature parasites affect the behaviour of the adult females involves many difficulties. In the development of *Ooencyrtus* from egg to pupa, the intensity of movement rises from zero to a maximum in the fully grown larva and then approaches zero again in the pupal stages. The hibernating larva of *Anastatus* is very much more sluggish in its movements than the corresponding mature *Ooencyrtus* larva, yet the former were almost completely ignored in the experimental period. It is quite evident, therefore, that the determination of the nature of the host and the consequent oviposition response is not correlated with a quantitative factor in the form of amount of movement of the contained parasite stage in the host, since if this were the case one might expect approximately the same number of eggs to be deposited in hosts containing parasite eggs and hosts containing parasite pupae, or hosts containing early larvae of *Ooencyrtus* and hosts containing fully grown hibernating larvae of *Anastatus*.

It has been suggested that with the growth of the parasite larvae, the movements may have a disturbing effect upon the drilling operations of the females. Parker (1933), in an investigation of the competition between the two egg parasites of *Porthetria dispar*, stated that *Ooencyrtus* females were deterred from laying in hosts containing *Anastatus* larvae more than six days old because of the mechanical action of these latter larvae in moving away from the inserted ovipositor. A similar response was noted when *Anastatus* females attempted to oviposit in hosts containing large *Ooencyrtus* larvae, but these were not so successful in moving away because of the attachment to the pedicel and anal shield. It is quite possible that this factor played a part in the above experiments, though it is difficult to see how an early larva of *Ooencyrtus* could have a very disturbing effect on the inserted ovipositor. *Ooencyrtus* pupae, like most other pupae, are capable of very slight independent movement; they lie quite free within the host egg-shell and can be passively moved about by any instrument inserted through it. It might be supposed, therefore, that the pupae are not sufficiently static to permit of an ovipositor being inserted into them. This explanation, however, presupposes the insertion of the ovipositor into the host in order to determine its nature. This does not appear to occur.

It is evident that if movements are responsible for this gradation in egg laying, they must be recognized by sense organs, which are able to identify various degrees and kinds of movements. But differentiation between *kinds* of movements implies that other factors also may be partly responsible for the rejection. To attribute the complete avoidance of hosts containing, say, sluggish hibernating *Anastatus* larvae to a type of movement is unjustified, for the differences between fully grown *Anastatus* and *Ooencyrtus* are quite as pronounced from the chemical and other aspects.

In an attempt to ascertain the part played by movement of the parasite larvae in the rejection of hosts containing them, parasitized hosts were killed by immersion in hot water at 60° C. for thirty seconds. Three hundred unparasitized hosts, three hundred hosts parasitized with an early larva and three hundred with a late parasite larva, were so treated, and divided into groups of tens to be set up in the standard manner in small Petri dishes $2 \times \frac{1}{2}$ in. A similar number of experiments was set up with hosts which had not been inserted in hot water. A single fertilized female was placed in each dish and left for 24 hr. in an incubator at 25° C. The entire experiment involved a total of 180 females, each of which was given ten hosts. It will be noticed from the results in Table XVII that some of the parasites had been denied opportunity for ovipositing for 4 and 10 days. The change of behaviour following this enforced restriction will be discussed at a later point; for the present purpose the important figures are those of the total eggs laid by thirty females in each of the different host types.

TABLE XVII. THE EFFECT OF DEATH OF THE PARASITE STAGE ON ACCEPTABILITY OF HOST BY FEMALE PARASITE

		State of host					
		Control		Containing early larva of parasite		Containing late larva of parasite	
Hosts available for each female	Age of female before being given access to hosts	Un-parasitized alive	Un-parasitized dead	Parasite alive	Parasite dead	Parasite alive	Parasite dead
10	1 day	78	41	46	40	10	0
10	4 days	146	103	147	76	112	21
10	10 days	100	72	27	24	13	4
Total	30 females	324	216	220	140	135	25

On the death of the normal unparasitized host, the females lay fewer eggs, probably because of chemical changes which accompany and follow death. The attraction of such hosts, as gauged by the number of eggs deposited in them by the parasite, is roughly two-thirds that of the normal live host. A similar relation obtains between the hosts with a live early larva of the parasite and those dead hosts containing a dead early larva. This suggests that the reactions in the latter cases are conditioned by factors similar to those which distinguish dead and live unparasitized hosts, that is, the factors are chemical in nature. Is it mere coincidence that the numbers of eggs laid in dead unparasitized hosts and live hosts parasitized with an early larva are practically identical and approximately two-thirds the number laid in normal live unparasitized hosts? Or does it imply an underlying similarity in the nature of these two types of hosts as opposed to the normal host? In hosts containing dead early larvae the females laid 140 eggs—just two-thirds of the number laid in dead unparasitized hosts and parasitized hosts with a live early larva. One hundred and thirty-five eggs were deposited in hosts containing an advanced parasite larva; for practical purposes this is the same as that laid in dead hosts with a dead early larva.

It is probable that the most profound change following the killing of a host and/or its contained parasite stage is chemical, and the figures given in Table XVII seem to indicate that the usual reaction of the parasite females to hosts containing a given stage of the parasite can be duplicated by killing hosts containing an earlier stage of the parasite. Thus hosts with early larvae, if killed by immersion in hot water, have an attraction similar to that of normal live hosts containing advanced parasite larvae. Whether one can infer a similarity in the nature of the hosts from such figures is very doubtful. This is indeed highly improbable, for it is very difficult to imagine that a host containing a dead advanced parasite larva is of similar chemical composition to a host containing a live parasite pupa. But such hosts have in common the fact that they are very unsuitable for the development of the parasite's progeny. It is clear from the present results that any possible advantage accruing from the absence of disturbing movements on the part of the parasite larvae is wholly counteracted by other more repellent characters possessed by the dead larvae. It seems impossible to assess the importance of movements of the parasite larvae by the present methods; any effect they may have is obscured by the change in the chemical composition of the host after death.

This leads to the second suggestion that the quantity of host material is positively correlated with the gradation in the oviposition response. The first point to be decided is the exact nature of the so-called "host" material. It has been shown that until the onset of the fifth and final instar of the parasite the hibernating gipsy moth larva can be quite clearly recognized as such within the egg-shell. But it is evident that, even if it be established that the oviposition response is related to the quantity of host material present, this activity is the result of a sum of physico-chemical factors, which, taken as a whole, represent a hibernating gipsy moth larva. Whether this latter be present in whole or in part does not simplify to any appreciable extent the problem of how and why the *Ooencyrtus* chooses the gipsy moth as a host. The problem is merely pushed one stage further back. Actually, parasitization of the host must involve a very complex physico-chemical process, which commences as soon as the parasite egg is laid. Casual observations in the course of the present work suggest that if hosts are about to break the diapause and are parasitized in this condition, the moth larva will not survive unless it emerges within the next 24 hr. Howard and Fiske (1912) also state that oviposition at any time within a few hours of the time when the (host) eggs would otherwise hatch was generally successful. It seems that although the parasite egg is laid in any organ of the host, its presence in some way inhibits the normal development of the host larva and this effect may be produced even before the parasite egg hatches. Possibly the effect is brought about by the ovipositing female. Presumably a similar condition is produced in hosts in a diapause and it is quite likely that a gipsy moth larva containing any parasite stage differs qualitatively and quantitatively from one which is unparasitized.

However, in a general way, it may be said that the growth of the parasite brings about a gradual replacement of the host tissues by another set of tissues. At the time of pupation of the parasite larva, none of the normal host's characteristics remains except, perhaps, some vague odour and the purely physical dimensions; and the attraction of such characters of the host is very slight. Oviposition is restricted, because, as far as the female parasite is concerned, there is no host there. The gradation in egg laying proves conclusively that the behaviour of the *Ooencyrtus* does not depend upon the precise physical characters of the egg-shell, but on what lies inside it. Now *Anastatus disparis* usually parasitizes the gipsy moth egg before the embryo has developed so that a host containing a fully grown hibernating larva of *Anastatus* contains no trace of gipsy moth larval material as would a corresponding stage with *Ooencyrtus*. The "host" here is simply a gipsy moth egg-shell containing an *Anastatus* larva and possibly a little nutrient material from the gipsy moth egg, and as such it is completely rejected by *Ooencyrtus* females. Where the amount of host material present within the chorion is quite appreciable the oviposition response is positive. Thus, we may say that a gipsy moth egg containing an early larva of the parasite is 80% "host" and the female parasite oviposits accordingly.

The extraordinary sensitiveness of the female in gauging the amount of host material present at any time implied by this conception of the oviposition response is such that one is inclined to doubt the existence of such a faculty. The difference in bulk between a parasite egg and early larva, considered in relation to the entire mass of the host, is very small; even the fourth instar of the parasite is only equal in size to the head capsule of its host, and yet the ovipositing female shows by her behaviour a quite definite appreciation of its significance for her offspring. In this connexion some recent work of Maple (1937) with a related species, *Ooencyrtus johnsoni* Howard is of interest. This worker found that the female parasite frequently lays two or three eggs in the host, *Murgantia histrionica* Hahn., which is capable of supporting this number of parasites to maturity. After one egg is deposited usually a second and sometimes a third is laid immediately through the same puncture in the host chorion without the ovipositor being sheathed. In laboratory experiments hosts containing two parasite eggs were more common than those containing one or three. Although superparasitism with a partial survival may occur, the facts indicate that the oviposition response is in some way related to the quantity of host material present, and accords with the above theory.

This interpretation of the behaviour is a special application of the general principle noted by Thompson and Parker (1927) to the effect that the number of eggs deposited by a parasite in a host is roughly proportional to the amount of food material available. In the present instance, however, it seems essential to introduce some conception of the quality of the food material available, for the differences in quantity of host material or movements of parasite stages between host types which are unparasitized, parasitized with a parasite egg, and parasitized with an early larva, are so small that, taken singly or together, one cannot see how they are perceptible, but considered as parts related to a whole they function in helping the female to determine the nature of the host.

These aspects will be discussed more fully at a later point in connexion with the problem of host selection. Here it will suffice to point out that with the parasitization of this host and the subsequent qualitative and quantitative changes as the parasite larvae, etc., develop, the conditions differ only in degree from those obtaining with a polyphagous parasite, which chooses one of several hosts at a given time. The most recent workers on host selection, Salt (1935) and Ullyett (1936), have both attempted to resolve the host into characters such as odour, shape, colour, size, texture, movement, etc., and assess the attraction of such factors by the use of various objects, as false hosts. By the utilization of selected parasitized hosts such an analysis may be possible with perfectly natural material which the parasite is liable to meet in the field, and the relative importance of the various characters can be gauged by the actual number of eggs laid rather than by the number of "attacks" made when the female adopts a piercing posture. This method has the great merit of focusing attention upon an aspect which is often conveniently ignored—the physico-chemical nature of the *living* host—instead of upon abstractions such as movements, smell, etc. The problem is greatly simplified in such a case, and until the behaviour in these virtually monophagous types is understood, it seems futile to attempt an analysis of that of polyphagous species.

(*b*) *Its relation to the age and condition of the ovary of the female*

In the field it is very unlikely that any parasite is always in the immediate contact with its host that obtains when it is confined in a Petri dish in the laboratory. Indeed, from what little is known, it appears that parasites spend varying periods of their lives in activities other than those of oviposition. These pauses between the actual oviposition periods, whether they be caused by inclement weather or other factors, may have a great effect on the subsequent actions, particularly when the pauses are spent in searching for hosts which are very scarce; for the delayed facilities for oviposition may result in an accumulation of mature eggs in the ovary and a consequent change in the behaviour of the female. The physiological urge to oviposit is very powerful, and, irrespective of the mechanism which arouses the desire to lay, it is essential to recognize this irregularity in the oviposition facilities of the female parasite under natural conditions and to attempt to assess its significance.

The method adopted was the following. Immediately on emerging from a culture, one hundred males and one hundred females were placed in a tube $3\frac{1}{2}\times 1$ in. and supplied with honey water and a split raisin. They were kept at 25° C. for 2 days and then used in experiments in the normal manner. The females were, therefore, 2 days old at the commencement of the experiments. Each female was given ten hosts of a given type set out one-tenth of an inch apart in a small Petri dish $2\times\frac{1}{2}$ in. and was then left for 24 hr. at 25° C. The hosts were then examined for parasitism under a binocular microscope. The types of hosts selected were those of the previous experiments, namely, normal unparasitized, parasitized with an *Ooencyrtus* egg, parasitized with an *Ooencyrtus* early larva, parasitized with an *Ooencyrtus* late larva, parasitized with an *Ooencyrtus* pupa, and these were obtained by the standard routine described above. Ten females, each of which was given ten hosts, were used for each type of host, and it must be emphasized that different sets of females were utilized for each type of host and time period. This procedure was repeated with females which had been kept for 4, 6 8 and 10 days before being provided with hosts, but because of the numbers of types of hosts which had to be prepared and later examined, it was not possible to complete the entire series with a single generation. The work was spread over three or four generations and the experiments with females of given ages finished within one particular generation. This feature may, or may not, have introduced complications.

The oviposition of the females is shown in Tables XVIII–XXIII inclusive, summarized in Table XXIV, and represented diagrammatically in fig. 9. Some of the parasitized hosts could not be obtained at the required time, and had to be excluded from the experiment; these are indicated by blank spaces. The experiment with 10 day old females was repeated. One unavoidable complication has been introduced into the experiments, since the females often laid more eggs than there were hosts available. Thus, when ten females deposited more than one hundred eggs in, for example, one hundred unparasitized hosts supplied, there was some overlapping with the conditions when only hosts containing parasite eggs were supplied at the commencement of the experiment.

Table XVIII. Oviposition of 2 day old females in hosts containing various developmental stages of the parasite

Parasite stage in host	Eggs laid by female										Total nos. of eggs laid by 10 females	Average per female
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10		
Nil (control)	24	15	13	9	0	21	17	19	12	22	152	15·2
Egg	10	19	0	15	15	11	13	16	13	18	130	13·0
Early larva	13	4	16	16	10	8	9	8	10	8	102	10·2
Late larva	9	5	4	6	1	1	4	5	6	2	43	4·3
Early pupa	0	1	0	0	1	0	0	0	0	0	2	0·2
Late pupa	0	0	0	0	1	0	0	0	0	0	1	0·1

Table XIX. Oviposition of 4 day old females in hosts containing various developmental stages of the parasite

Parasite stage in host	Eggs laid by female										Total nos. of eggs laid by 10 females	Average per female
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10		
Nil (control)	1	11	14	19	17	20	11	16	19	16	144	14·4
Egg	11	22	18	25	21	27	7	23	20	15	189	18·9
Early larva	13	0	17	16	19	21	17	11	17	16	147	14·7
Late larva	21	15	8	16	0	4	17	8	16	7	112	11·2
Pupa	—	—	—	—	—	—	—	—	—	—	—	—

TABLE XX. OVIPOSITION OF 6 DAY OLD FEMALES IN HOSTS CONTAINING VARIOUS DEVELOPMENTAL STAGES OF PARASITE

Parasite stage in host	Eggs laid by female										Total nos. of eggs laid by 10 females	Average per female
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10		
Nil (control)	22	7	15	0	20	22	0	0	24	26	136	13·6
Egg	5	13	14	20	0	1	15	10	9	9	96	9·6
Early larva	12	2	22	13	14	8	5	2	8	9	95	9·5
Late larva	5	0	6	0	1	1	0	2	1	1	17	1·7
Pupa	3	0	3	0	1	0	2	0	1	1	11	1·1
Anastatus (hibernating larva)	3	0	3	0	1	5	0	0	0	3	15	1·5

TABLE XXI. OVIPOSITION OF 8 DAY OLD FEMALES IN HOSTS CONTAINING VARIOUS DEVELOPMENTAL STAGES OF PARASITE

Parasite stage in host	Eggs laid by female										Total nos. of eggs laid by 10 females	Average per female
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10		
Nil (control)	21	16	9	14	0	14	21	18	0	10	123	12·3
Egg	18	0	12	8	15	21	4	17	17	1	113	11·3
Early larva	16	4	1	12	18	0	18	14	0	10	93	9·3
Late larva	1	8	3	8	2	0	0	5	6	3	36	3·6
Pupa	0	1	1	0	0	0	0	0	0	0	2	0·2
Anastatus (hibernating larva)	0	10	4	0	0	2	1	1	4	7	29	2·9

TABLE XXII. OVIPOSITION OF 10 DAY OLD FEMALES IN HOSTS CONTAINING VARIOUS DEVELOPMENTAL STAGES OF PARASITE

Parasite stage in host	Eggs laid by female										Total nos. of eggs laid by 10 females	Average per female
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10		
Nil (control)	13	13	10	14	3	10	16	16	12	10	117	11·7
Egg	12	6	0	14	12	9	0	8	9	0	70	7·0
Early larva	0	0	3	6	4	6	0	1	2	0	22	2·2
Late larva	1	0	3	3	5	0	0	2	1	0	15	1·5
Pupa	0	0	0	0	0	0	1	0	0	0	1	0·1
Anastatus (hibernating larva)	0	0	0	0	0	0	0	0	0	0	0	0·0

TABLE XXIII. OVIPOSITION OF 10 DAY OLD FEMALES IN HOSTS CONTAINING VARIOUS DEVELOPMENTAL STAGES OF PARASITE

Parasite stages in host	Eggs laid by female										Total nos. of eggs laid by 10 females	Average per female
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10		
Nil (control)	12	12	10	4	14	14	19	2	11	2	100	10·0
Egg	Not determined										—	—
Early larva	1	0	8	6	0	0	1	0	1	10	27	2·7
Medium larva	2	0	2	4	6	0	3	0	2	1	20	2·0
Late larva	3	1	1	0	5	1	0	1	0	1	13	1·3
Pupa	0	0	0	1	0	0	0	0	0	0	1	0·1

TABLE XXIV. RESTRAINT: ITS RELATION TO THE DEVELOPMENTAL STAGE OF THE PARASITE IN THE HOST AND THE CONDITION OF THE OVARY OF THE FEMALE

Age of females before being given access to hosts	Parasite stage in host					
	Nil (control)	egg	Early larva	Late larva	Pupa	Hibernating larva of *Anastatus*
1 day	78	65	46	10	2	1
2 days	152	130	102	43	1	—
4 days	144	189	147	112	—	—
6 days	136	96	95	17	11	15
8 days	123	113	93	36	2	29
10 days	100	—	27	13	1	—
10 days	117	70	22	15	1	0

(— not determined).

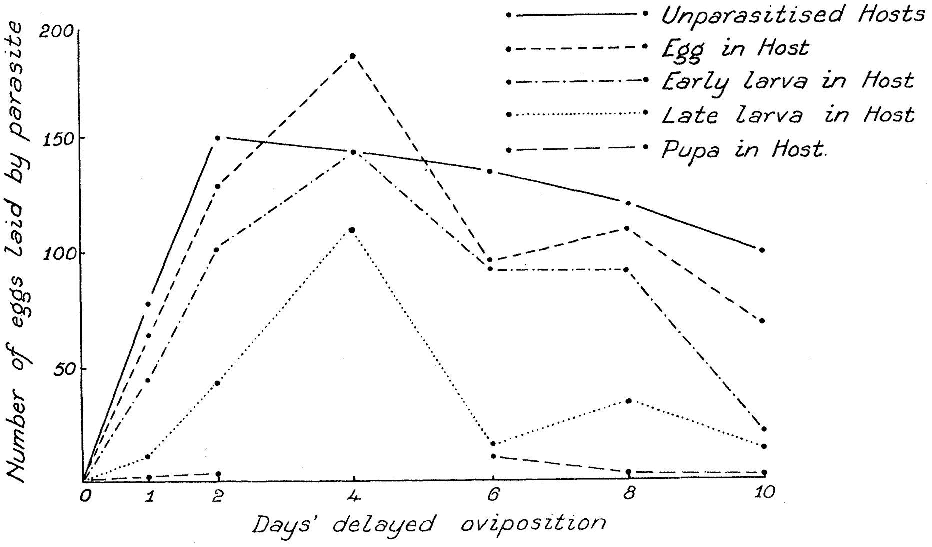


FIG. 9. Restraint in relation to the developmental stage of the parasite in the host and the age and condition of the ovary of the female.

For reasons already stated, it was not possible to allow each female more than ten hosts of a given type. However, the presence of this complication does not seem to invalidate the general conclusions.

Several striking points emerge from this series of experiments. With one significant exception, namely the 4 day old females, all the ovipositing parasites showed the differential reaction towards hosts containing various instars of the parasite, and this type of response can therefore be regarded as an established fact. The oviposition of the 4 day old females, however, indicates that this behaviour may be dependent upon the condition of the ovary, and the factors normally responsible for the exercise of restraint were so completely suppressed that hosts were oviposited in without any apparent reference to the nature of the parasite stage within them; hosts parasitized with *Ooencyrtus* eggs or early larvae at the commencement of the experiment were attacked to an equal or even greater extent than the unparasitized control.

Surprisingly, when the inhibition of oviposition was extended beyond the first four days of life, restraint again became evident so that the females which had been kept ten days before being given hosts showed greater restraint that the younger females. Although the number of eggs laid in the unparasitized controls remained fairly uniform throughout (see fig. 9), the oviposition in the parasitized hosts is much more erratic and suggests that the parasitized hosts exert a decreasing attraction on the females as the latter age. It is possible, however, that delaying facilities for oviposition for the first 10 days of a female's life—a period approximately one-quarter that of the normal life of a female at 25° C. and 70% R.H.—results in a physiological upset of the normal ovariole development. Flanders (1935) has shown that with the pteromalids, *Dibrachoides*, *Peridesmia*, *Spintherus* and *Eutelus*, if environmental conditions prevent oviposition the ovarian follicles and the eggs apparently disintegrate and are absorbed, though this may be due to the fact that at the same time the female parasites are forced to change from a mixed diet of host fluids and carbohydrates to a purely carbohydrate diet. This period of "phasic castration" may last nine months, and ends with the appearance in the field of the susceptible stage of the host.

The possibility that this factor of reabsorption of the eggs might be involved in the above behaviour was investigated by the method outlined below. The fact that appreciable numbers of eggs were laid by the older females indicated that the reabsorption, if any, was only partial, and this could, therefore, be most conveniently gauged by supplying females, which had been kept for various time periods without hosts, with large numbers of unparasitized gipsy moth eggs. Partial reabsorption of the mature eggs of the ovaries should affect the amount of parasitism in these higher host densities. By varying the host densities it was possible to extend the scope of the experiment to give some indication of the effect of density of the host upon the exercise of restraint.

Densities of ten, twenty-five and fifty hosts were selected and these were arranged in the standard manner one-tenth of an inch apart in small Petri dishes $2 \times \frac{1}{2}$ in. Twenty dishes were set up for each density. Immediately on emergence from a stock culture, groups of approximately one hundred females were placed in tubes containing food and males, and left in an incubator at 25° C. and 70% R.H. until required. On the second, fourth, sixth, eighth and tenth days after emergence, sixty females were removed from the tubes and one female placed in each dish. The dishes were placed in an incubator at 25° C. and 70% R.H., left for 24 hr., and then removed for examination for parasitism. Thus on each of the above days the following experiments were in progress:

(1) Twenty females, each with ten hosts.
(2) Twenty females, each with twenty-five hosts.
(3) Twenty females, each with fifty hosts.

At a density of twenty-five hosts the series was continued until the fourteenth day after emergence. The results are given in Table XXV.

TABLE XXV. NUMBER OF EGGS DEPOSITED BY A FEMALE IN 24 HR. AFTER BEING KEPT FROM HOSTS FOR VARYING PERIODS. EACH READING REPRESENTS AN AVERAGE OF TWENTY FEMALES

Age of female before being given access to hosts	Number of hosts available		
	50	25	10
2 days	12·2	14·1	9·4
4 days	23·0	15·5	15·7
6 days	23·8	19·2	11·9
8 days	21·1	18·8	12·4
10 days	21·7	17·6	10·1
12 days	—	18·5	—
14 days	—	20·7	—

It is apparent that prevention of oviposition for a period up to fourteen days does not affect the ultimate output of mature eggs provided sufficient hosts are available, and absorption is therefore non-existent. There is a maximum number of mature eggs which the ovaries are capable of containing, and this maximum is fairly quickly reached—usually after 4–6 days' delayed oviposition. No further increase occurs even if the female parasite is kept for 14 days. This finding somewhat clarifies the behaviour of the female towards parasitized hosts in the previous experiments. The failure of restraint apparently coincides with the attainment of the peak point of the ovariole output of mature eggs; it may also coincide with the maximum general body activity of the females. The later restraint, which will have to be confirmed by further work, can only be attributed to some ageing factor on whose nature it is only possible to speculate.

That the behaviour of female parasites towards parasitized hosts may be one of great complexity has been illustrated by some recent work of Flanders (1936). This worker demonstrated that in several species of *Coccophagus* the male sex is produced only hyperparasitically on individuals of its own or similar parasitic species. The striking feature was the change in oviposition response of species, such as *C. scutellaris*, following the stimulus of fertilization. This species, when mated, oviposits normally in the body of a lecaniine scale; before mating, it is attracted to parasitized scales only and deposits its eggs in the first and second instars of the primary parasite. Williams (in Flanders, *l.c.*) showed that a similar phenomenon occurs with the eulophid, *Tetrastichus* sp. in *Malacosoma americana*. This behaviour is remarkable when considered in relation to the preceding discussion of the nature of the oviposition response of a female to unparasitized and parasitized hosts. It means a complete reversal of the "normal" reactions, and it is very difficult to understand by what mechanism fertilization is able to effect this change.

(*c*) *Its relation to the number and nature of the hosts available*

In the preceding section the table, giving details of the accumulation of eggs in the ovaries of females which have been denied opportunity for ovipositing, shows clearly that the number of eggs deposited by this parasite in any given host population is related, in some way, to the number of hosts available. In the forementioned experiment the maximum number of mature eggs which the ovaries were capable of containing was 23 or 24, and it is surprising to find that the release of this number was dependent upon the number of hosts available being considerably in excess of the number of hosts that were actually utilized for parasitism. Thus, although this maximum number of parasite eggs could have been deposited when 25 hosts were accessible to the females, it seems likely that the presence of parasitized hosts and the contact with these had a depressing effect upon the ovipositing females, and the number of eggs deposited never rose above an average of 19·2 per female. A similar phenomenon has been noted by MacLagan (1935) with *Sitophilus oryzae* and its host, the wheat grain, where the highest average number of eggs per female weevil was obtained when the number of grains available was at least eleven times that actually used for oviposition. Any reduction in this number of grains was accompanied by a reduction in the number of eggs laid per female.

The occurrence of this increase in egg deposition, with a rising host density, suggested that a large scale experiment extending over the first fortnight of a female's life might be of interest. The following experiment was therefore performed. Immediately on emergence from a stock culture approximately 100 males and females were placed in tubes and left for 12 hr. for mating and feeding. Fifty of the normal Petri dishes $2 \times \frac{1}{2}$ in. were set up in the standard manner and contained densities of twenty-five, ten and five, of unparasitized hosts and twenty-five and ten of hosts parasitized with an *Ooencyrtus* egg, there being ten dishes for each density. The protruding portions of pedicels were removed from these parasitized hosts as in previous experiments. A single fertilized female was placed in each, supplied with food in the form of a piece of blotting paper soaked in honey water, and the entire series was placed in an incubator at 25° C. and 70% R.H. Within the next 24 hr. a duplicate series of dishes was arranged so that each female could be given a fresh supply of hosts. The changing of the fifty females and supplying with new hosts and food usually took 30–45 min. and was carried out at intervals of 24 hr. The 725 hosts were examined each day for parasitism. The female parasites were numbered so that a record of their daily oviposition could be obtained. Details of the oviposition are given in Table XXVI. In all cases, the figure for each day is an average of the oviposition of ten females. These results have not been represented graphically, because the considerable overlapping of the five curves obscured their significance, but a similar experiment extending over the first 3 weeks of life and involving ninety females divided among host densities of twenty-five and ten (unparasitized) and ten (parasitized with an *Ooencyrtus* egg) is represented in fig. 10, and shows more clearly the reduced rates of laying with the lower host densities.

Table XXVI. Daily oviposition of *Ooencyrtus* females in various densities and types of hosts for first 14 days of life. Each column represents an average of oviposition of ten females

Age of females in days	Number and nature of the hosts supplied to females each day				
	25 unparasitized	10 unparasitized	5 unparasitized	25 parasitized with *Ooencyrtus* egg	10 parasitized with *Ooencyrtus* egg
1	13·4	11·6	11·1	11·5	8·6
2	10·3	7·2	6·5	9·7	8·0
3	11·0	9·7	7·5	9·0	8·3
4	10·1	9·7	8·6	9·2	8·3
5	9·7	8·1	7·4	6·3	4·9
6	5·7	4·2	4·7	4·6	3·8
7	5·6	3·3	4·7	6·2	4·9
8	6·2	2·6	3·8	4·0	1·4
9	4·1	1·9	3·2	4·2	2·0
10	3·8	1·5	2·7	4·5	2·8
11	3·5	1·4	2·2	4·0	2·8
12	3·4	1·8	2·9	3·9	1·6
13	2·7	1·6	2·7	3·3	2·2
14	3·4	1·5	2·2	3·2	2·3
Total laid per female	92·9	66·1	70·2	83·6	61·9

It is evident from an examination of the table that the influence of the number and nature of the hosts available for parasitism affects the rate of oviposition throughout the experimental period, and it may be presumed to extend over the entire lifetime of the parasite. The tendency to retain eggs rather than deposit them on meeting with parasitized hosts is clearly indicated. It must be remembered that the parasitized hosts used in these experiments contained *Ooencyrtus* eggs. It has been demonstrated that this is the most acceptable of parasitized material, and it is probable that this restraint would have been more pronounced if more advanced immature stages of the parasite had been used. It is remarkable to find that the female parasites continue to exercise this restraint although they are repeatedly confronted with these parasitized hosts throughout the first 2 or 3 weeks of their lives, and have had no actual experience of an unparasitized host.

Another striking feature which will be observed in the table and graph is the fact that although the number of eggs laid per day after the fifth day of the parasite's life is considerably less than ten when twenty-five unparasitized hosts are supplied daily to the female, this average is still further reduced when only ten unparasitized hosts are available daily. The importance of this frequency of contact with hosts as a stimulus for oviposition is apparent, too, when the female is given parasitized hosts, for there is an appreciable difference between the average number of eggs laid by the females supplied with twenty-five or ten parasitized hosts.

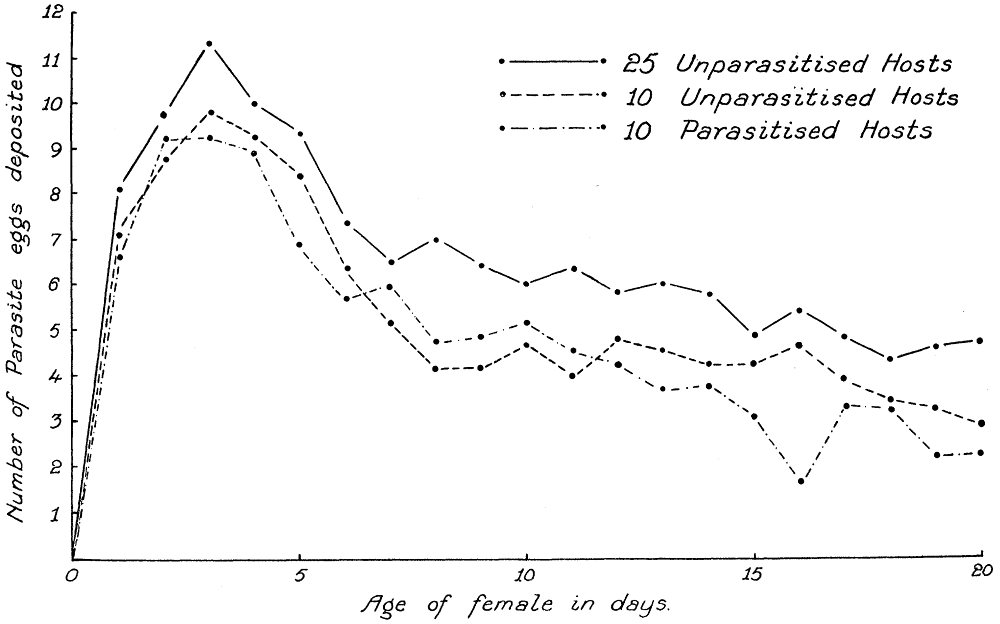


FIG. 10. Daily oviposition of *Ooencyrtus* in relation to the number and nature of the hosts available.

The fundamental importance of this frequency of contact with hosts in the host-parasite economy will be apparent from the following experiment.

Sets of twenty-five unparasitized hosts were arranged in the standard manner one-tenth of an inch apart and exposed to the action of a single *Ooencyrtus* female for 24 hr. at 25° C. The females were kept for periods of 2, 4, 6, 8, 10, 12 and 14 days before being given access to the hosts, and the accumulation of eggs in the ovaries resulted in the number of eggs laid by any one female varying from one to thirty-two. The oviposition of, and the number of hosts attacked by, 156 females, each of which was given twenty-five hosts, are shown in figs. 11 and 12; the curve in the latter is obtained by successive smoothing. A high discrimination existed and this is shown, in Table XXVII, to persist for at least the first 14 days of a female's life. But the striking feature of these results is the apparent failure of the females to find unparasitized hosts when the

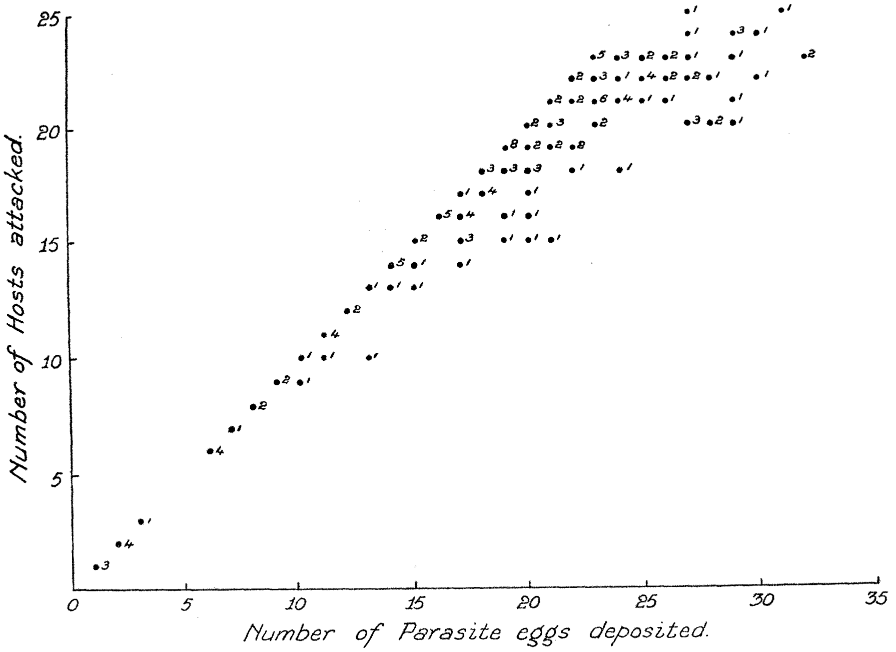


FIG. 11. The effect of frequency of contact with the parasitized hosts on the parasitism of a given host population.

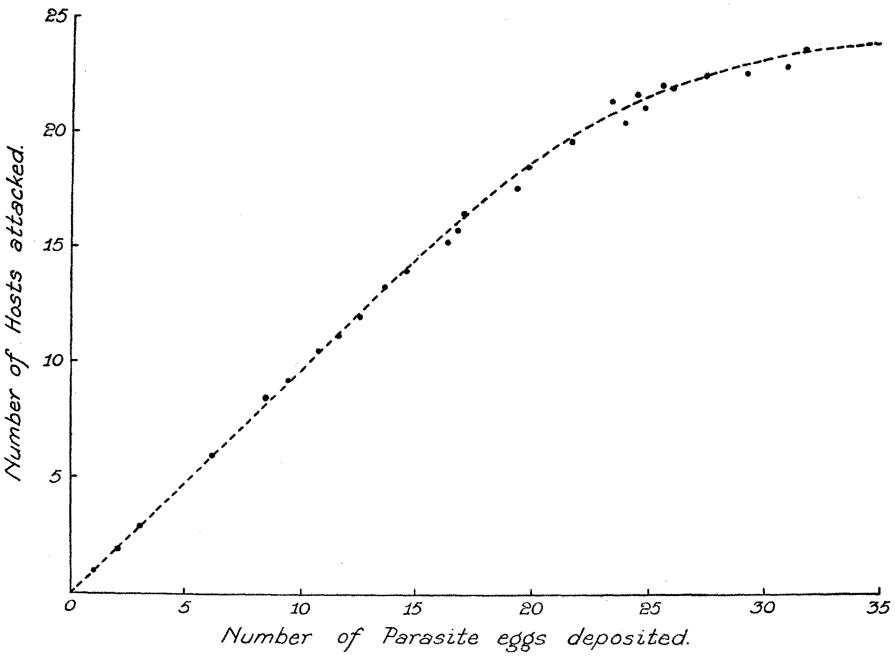


FIG. 12. The effect of frequency of contact with the parasitized hosts on the parasitism of a given host population.

parasitism in the given population is between 80 and 95%. The cause of this failure cannot be the fact that the females were deterred from attacking these unparasitized hosts, because of some inhibitory odour left on them by other females (as occurs in *Trichogramma evanescens*) for only one female was confined with every twenty-five hosts. The reason appears to lie in the frequency of contact with the hosts which had been parasitized by the female in the 24 hr. of the experiment. Naturally, these hosts contain eggs of *Ooencyrtus*, and it has been conclusively demonstrated above that the female needs but slight provocation to superparasitize them. This intrinsic weakness in the oviposition response of the parasite ensures that, even if conditions favourable to a large increase in the parasite population do arise, it will prove very difficult to eliminate the host completely and hence its parasite. Of course, it is possible that the intensity of searching by a female in these heavily parasitized host populations will depend, partly, upon the precise nature of the parasitized hosts. Further experiments are necessary to decide this point.

TABLE XXVII. DISCRIMINATION—ITS RELATION TO THE AGE OF THE FEMALE AND ACTIVITY OF THE OVARY

Age of female before being given access to hosts	No. of hosts available per female	Average no. of eggs laid	Average no. of hosts parasitized	Percentage of hosts not superparasitized
2 days	25	14·1	13·0	92·2
4 days	25	15·5	14·8	95·4
6 days	25	19·2	17·7	92·2
8 days	25	18·8	17·6	93·6
10 days	25	17·6	16·3	92·6
12 days	25	18·5	16·6	89·7
14 days	25	20·7	19·1	92·2

One further aspect of the behaviour of this parasite may be conveniently treated here. This is the effect of the cleaning of the moth's hairs from the eggs on the selective faculty of the female. Two sets of experiments were therefore set out:

Series 1: Normal hairy eggs arranged one-tenth of an inch apart.

Series 2: Normal hairy eggs arranged contiguously to form a square.

The uniform surface of the natural field mass is invariably broken when attempts are made to obtain a constant number of eggs in a given area, so that Series 2 above is the best approximation to the field mass which can be obtained in the laboratory. Ten fertilized females were each given 25 hosts in both the above Series and left for 24 hr. at 25° C. The hosts were then dissected to determine the parasitism, as the presence of a pedicel cannot be observed through the hairs. The average number of eggs laid and the number of hosts attacked is given in the following table:

Series no.	Average number of eggs laid per female	Average number of hosts attacked
1	20·7	18·4
2	17·0	14·8

If these figures are compared with those obtained by the standard method, and represented in fig. 12, it will be seen that the degree of discrimination is only slightly reduced. This fact has to be allowed for in all the preceding experiments.

(*d*) *General discussion of restraint*

Ullyett (1936) has maintained that the application to field conditions of results involving the exercise of restraint by a parasite demands considerable caution. This worker finds it difficult to believe that after an exhaustive search for hosts which may be widely distributed over many host plants, a female parasite will leave a host without ovipositing because it happens to be parasitized. But in the few cases of field parasitism which have been examined in detail, the amount of superparasitism does suggest the existence of a selective faculty, and a selective faculty of any kind implies an ability to retain eggs rather than deposit them in unsuitable media. The one faculty cannot exist without the other. The preceding studies of restraint indicate that it is a very real phenomenon, and the results emphasize the importance of frequency of contact in deciding the acceptance or rejection of a particular type of host. The discriminating powers of the *Ooencyrtus* female may be perfect, but they are so interrelated with other physiological processes which underlie and determine the oviposition response, that their effect is often nullified. Thus, suppose a female encounters a mass of gipsy moth eggs, 75 % of which are already parasitized and contain *Ooencyrtus* eggs or early larvae. It is probable that her initial actions will be the rejection of parasitized hosts, but if during the traverse of the host mass the parasite repeatedly meets with these parasitized hosts, she will eventually accept some of them. On the other hand, if the hosts are parasitized with fully grown *Ooencyrtus* larvae or pupae, these would only be accepted after intensive searching had failed to reveal any more suitable hosts. The inference from the foregoing experiments is that the intensity of the searching by the parasite may be a function of the nature of the hosts available.

The limitation of the present laboratory technique is the failure to engender conditions wherein the female parasites may deliberately search for hosts and spend varying periods before finding them. Enclosure in a Petri dish or other container means immediate contact with hosts when no contact may be desired, and this has an important bearing on restraint. It can be argued that easy accessibility of hosts in a Petri dish will tend to exaggerate any inclination of the female to release her eggs, and it has been proved that the variations in the time between contacts with host do not wholly destroy this faculty. The desire of the female to oviposit and her consequent search for hosts must be correlated with developmental cycles in the gonads, and these have been shown to have a pronounced effect upon the subsequent behaviour of the females. This suggests that any environmental conditions, such as temperature, humidity, scarcity of hosts, etc., which may affect the rate of oviposition, will increase the incidence of superparasitism. This point is illustrated in the curves of the daily oviposition of this parasite. However, it cannot be assumed that any combination of factors which leads to a frustration of oviposition will automatically result in a failure of restraint at a later point of a female's life, because of a possible accumulation of mature eggs in the gonads. Age may play a big part in the oviposition response of this parasite. Moreover, the *Ooencyrtus* females used in all the previous experiments had had no actual individual experience of a normal unparasitized gipsy moth egg. Now Marchal (1936) has shown that *Trichogramma evanescens*, immediately on emergence from its host, seems to have a very generalized conception of a host and attacks a large variety of true and false hosts, but the female's interest is much more restricted after she meets a normal host similar to that from which it has itself emerged. This is an aspect of behaviour which may have an appreciable effect upon the exercise of restraint.

IV. Conclusions on the nature of host selection by this parasite

The results obtained in the present investigations are of particular interest because of the light they throw on the nature of perception in the so-called "instinctive" behaviour of this insect. The main object of this work has been to answer the question: how does the insect choose its host? Or, to what does the insect respond?

It has been shown that by confronting the female parasite with hosts already parasitized and with the contained parasite in various developmental stages, and giving the females a choice between such hosts for egg deposition, the problem presented is not essentially different from that of a polyphagous species, such as *Trichogramma*. The problem is simplified in that characters such as shape, size, texture, etc., are standardized and the gross chemical nature only is varied. As both *Ooencyrtus* and *Anastatus* may be used in any of their developmental stages inside the gipsy moth egg, the host varies quantitatively and qualitatively.

In the present instance the graded oviposition response of the female to hosts of similar nature save for what may be called quantity or gross bulk of parasitism, may be interpreted in two ways. First, it may be said that the acceptance of a normal host is a mechanical response to a summation of stimuli of shape, texture, chemical odour, etc. The deposition of an egg in the host follows the reception of a series of such stimuli by the sense organs, and the successive stimuli initiate appropriate reflexes, which are inflexible. It is evident that with *Ooencyrtus* no one stimulus pertaining to the host predominates in determining this acceptance. Secondly, it may be said that the acceptance of a normal host is essentially a response to an organized whole of which the parts or elements cannot properly be separated; and that this whole is more than a summation of discrete responses to discrete stimuli.

The distinction between these points of view will be clear if the exercise of choice between hosts parasitized and unparasitized, or hosts parasitized with various developmental stages of the parasite, is considered. It will be recalled that females were given a choice of the types in the following series:

Series 1: Hosts unparasitized alternating with hosts with one *Ooencyrtus* egg.

Series 2: Hosts with one *Ooencyrtus* egg alternating with hosts with one early *Ooencyrtus* larva.

Series 3: Hosts with one *Ooencyrtus* egg alternating with hosts with one late *Ooencyrtus* larva.

Series 4: Hosts with one *Ooencyrtus* egg alternating with hosts with one *Ooencyrtus* pupa.

Series 5: Hosts with one early *Ooencyrtus* larva alternating with hosts with one late *Ooencyrtus* larva.

It was found that with few exceptions the parasites always chose those hosts which had the most complete set of characters of the normal host, namely the first type in each of the series above. It might be claimed with Salt (1937) that the rejection of a parasitized host and the acceptance of an unparasitized one can be referred to a simple stimulus in the form of an odour left on the external surface of the parasitized hosts, or to an internal chemical difference recognized on insertion of the ovipositor into the host. The above evidence indicates that such a simple explanation is untenable in the present case, and it now becomes necessary to postulate a large variety of chemical and other stimuli by which the female's acceptance of a parasitized host is regulated.

At this point it is as well to examine in more detail the question of the characters or parts of an object. Woodger (1929) has pointed out that the characters of a perceptual object may be classed as separables or distinguishables. Thus the top, bottom and sides of a wooden box may be distinguished mentally and separated physically, but colours, odours, textures and other sensible qualities are dissociated as the result of a mental process and cannot usually be separated physically. No object is perceived except in relation to some sense quality. The author suggests that what is perceived as an object is a region or volume in which various qualities are related in a complex way, not only to the volume known as the object, but also to the volume known as the body of the percipient and to the intervening medium. Volumes have spatial relations and can be related in a definite way to volumes by yet other volumes, whereas the location or "thereness" of the sense qualities is only vaguely related to the object volume. Colour appears to be in the object though two observers may see different colours; smells and sounds seem to come from an object, and tastes and tangible attributes are perceived upon contact of the object with the sentient body. The physical sciences have always ignored the sense qualities as being non-metrical, non-essential elements of reality and located them in the sentient body, but as Woodger remarks—"putting them in people's heads neither banishes them from the world of being, nor makes their coming and going any more intelligible".

These remarks are of interest in an examination of host selection where the reactions of one sensory organism to another are being examined; they emphasize both the abstract nature of the physical sciences and the complex inter-relatedness of the various qualities "residing in" an actual object. In analysing the selection by an insect of a medium for oviposition, workers proceed on the assumption that the medium can be resolved into a given group of parts without pausing to consider whether such parts have any actual independent existence "in nature". Thus houseflies are known to breed in manure, and it has been demonstrated that they are attracted to this manure by an odour of ammonia. It has even been shown that the flies will oviposit on cotton wool soaked in ammonium carbonate, and Loeb (1918) has asserted that the fly's oviposition response is a simple mechanical chemotropism. It is, however, very difficult to assess the significance of such data. It cannot mean that the fly simply responds to ammonium carbonate as such, because what actually exists in nature is ammonium carbonate emanating from nitrogenous animal or vegetable matter and the relation of the other constituents of manure other than the carbonate with this compound is essential for the continued existence of the fly larvae. To say that the medium appears to the fly to be mainly the carbonate does not alter the fact that throughout its evolution the fly has required a certain minimum amount of organic material for its existence and presumably the race as a whole has never responded to anything else. Crystals of the carbonate and an equivalent mass of nitrogenous matter have in common the same odour, but in no other sense are qualities given in bare perception the same. That these other sense qualities are of importance to the fly has been shown by later workers, and the carbonate soaked in pine sawdust was found to give better responses than cotton, while chaff or acidulated horse manure was superior to the sawdust.

Such considerations are of even greater importance in the host-parasite relationship. Thus suppose an organic compound A was isolated from the gipsy moth egg and *Ooencyrtus* females were attracted to the compound by its smell. As A is a material body it will be characterized by other qualities which will be given in perception and, in so far as these are important to the female, the compound will be rejected. What the female seems to become aware of is an odour emanating from something, that is, that the smell is a part of, or related to, some concrete object. She then moves towards this source and ascertains by further sense perception the volume, texture, sound, etc. of the body. McDougall (1936) expresses this by saying that an animal responds to a sequence of sense impressions with awareness of a unitary object. Now if, under natural conditions, A only occurs in association with other constituents which together constitute an object known as a gipsy moth egg, it cannot be asserted that the female normally responds to anything less than an egg, and the notion that a parasite's host can be resolved into anything less than a complete host (or its normal variations) is illusory. In previous sections it has been suggested that the female *Ooencyrtus* chooses a gipsy moth egg containing an advanced larva of *Ooencyrtus* in preference to one containing a hibernating larva of *Anastatus*, because in the former slight traces of the gipsy moth larva remain within the host egg-shell. Clearly the female is aware of a difference, but the remains of the gipsy moth larva appear together with a fully grown *Ooencyrtus* larva only, and the latter is recognized as something quite distinct from the *Anastatus* larva. The possibility that any of the other differences between these forms might be responsible for the differential action, should, therefore, be accepted. But none of these differences is capable of independent existence apart from all the other characters of the host condition, for apparently a complex such as that of the remains of the gipsy moth larva remains is what it is in virtue of its relations with the advanced *Ooencyrtus* larva. Thus it seems that one should recognize that the various host conditions are self-contained units and that the *Ooencyrtus* female reacts to complexes such as the gipsy moth egg, gipsy moth egg containing a parasite larva, etc. To relate the behaviour to anything less is to relate it to abstractions which do not exist, as such, in nature. Thompson (1937) states that in the case of the Tachinid *Compsilura concinnata* and its hosts, the "latter have in common practically nothing but the fact that they are attacked by *Compsilura* and that they are all 'caterpillars'—a term to which, however, no definition can be attached that is not applicable to the forms not attacked by the parasite". However, the relationship *Compsilura*—caterpillar is a perfectly valid biological concept of a general tendency in host selection by this parasite, much as the statement that the passage of steam over heated metals will give metallic oxides expresses a general chemical tendency. As both hosts and parasite are restricted in distribution by climatic and other factors, the behaviour of *Compsilura* and host A, host B (not *any* caterpillar) is tolerably determinate. In this respect it is interesting to note that Flanders (1937) has found that with the polyphagous egg parasite, *Trichogramma*, the natural hosts of the different species were fewer than the suitable hosts (determined in artificial conditions) and that the groupings of hosts found in different habitats—arboreal, field and marsh—were usually parasitized by only one species of *Trichogramma*; if a species was transferred to a habitat to which it was not adapted it soon disappeared even when suitable hosts were present.

A further difficulty in a mechanical interpretation concerns the fact that in the preceding experiments one host was chosen in contrast to another. In the selection of hosts parasitized with an *Ooencyrtus* egg rather than those parasitized with early larvae as a deciding factor in the egg laying response, the relation between the hosts is as significant as the conditions between which the relation holds. To say that the oviposition of the female is attributable to a chain of reflexes and that hosts containing, say, a parasite egg are attacked because they possess one more stimulus than the alternate host, seems to be an incomplete description of the behaviour. In selecting one of the hosts in which to deposit an egg, the female is not reacting to the difference between hosts containing a parasite egg or early larva, but to this difference as a part of all details of the situation, that is, to the organized whole or total situation. It is organized in the sense that each element has perfectly determinate relationships with all the other elements characterizing the situation, and the physical existence of the wholes (hosts) is dependent upon the maintenance of those relations. The female cannot, from the nature of things, react solely to differences in movements between the various parasite stages contained within the hosts, because these movements, as such, do not exist apart from all the other features which characterize these hosts. The parasite examines hosts A and B and relates their nature to her need for ovipositing.

The *Ooencyrtus* female, like all other parasites, has an innate tendency to seek out objects—here gipsy moth eggs—as hosts, and all hosts encountered are, as it were, related to knowledge or experience of a normal unparasitized gipsy moth egg. The essential feature of the preceding experiments is the *synthetic relating activity* of the female parasite; this activity integrates the sense data so that the objects are perceived as wholes and at the same time has reference to future conditions such as the development of the parasite in the host. In the series above, the selection by the ovipositing female of those hosts containing the younger parasite stages becomes understandable when we find that she usually chooses unparasitized hosts in preference to parasitized ones, and that these gipsy moth eggs only contain sufficient food material for one *Ooencyrtus* to develop to maturity. The female's activity now acquires a meaning—she is selecting as her hosts those in which her progeny are most likely to develop, though she herself has no knowledge of the outcome of this action.

The fact that animals may respond, not to simple physico-chemical stimuli, but to perceptual complexes, that is, to things perceived in their relation to one another, is now widely recognized by a large school of workers on animal behaviour (Bierens de Haan 1929; Russell 1932, 1934). Köhler (1915), one of many workers on "relative choice", has shown that hens can be trained to pick corn from one of two grey papers of different tints, for instance, the lighter of the two. If the papers be replaced by another pair, of which the one to which the hen had been trained is now the darker of the two, the hen still chooses the lighter of the pair; that is, the response is to *a relation*. It has been maintained (Hempelmann 1929) that instinct is merely a concatenation of reflexes, but that the grasping of the objective inner relationship between two things such as occurs with the hen is a performance superior to instinctive action. And yet from one point of view the behaviour in both hen and parasite is essentially similar. If a female is confronted with a choice of hosts parasitized with a parasite egg or early larva (corresponding to the lighter or darker tints above) she chooses the host containing the parasite egg; given a choice between a host containing a parasite egg and a host with no parasite, she chooses the latter (corresponding to the lighter of the second pair of tints). The parasite is quite clearly guided by relations and these relations in the preceding experiments lead to the selection of hosts with the most complete set of characters of the normal host. The hen's behaviour is understandable, because it has been taught to pick up corn from the lighter of the tints; she picks from the lighter tint as it is associated with food she desires to eat. With *Ooencyrtus* the problem is more complicated. From a complex of stimuli the female responds to the pattern which gives the closest approximation to the normal host, and she is apparently doing this because only one parasite can develop in a gipsy moth egg. The reactions of the *Ooencyrtus* thus accord with the Köhler-Wertheim theory of Gestalt, which maintains that response is primarily to the perceptual field as an organized whole and that the animal reacts, not to elements in the perceptual field, but to relations or patterns within it.

This conclusion applies also to the findings of Ullyett (1936) with *Microplectron fuscipennis*, in which it was claimed that a pattern of sense pictures or inherent abstractions in the mind of the insect was correlated with the physico-chemical attributes of the hosts encountered. It is suggested that with *Ooencyrtus*, too, the action of the female in rejecting parasitized hosts is only comprehensible if such a host is related to a knowledge or experience of a normal unparasitized host. However, such a psychological interpretation of the behaviour involves many difficulties. Apart from the question of the possession of a pattern of sense pictures or "image" of a normal host before the adult female has even encountered one, there are the further difficulties concerning the location of the sense qualities. A man perceiving a lemon becomes aware of a yellow body of certain size with a particular smell, texture, etc. In imagination all that seems to be given is the notion of shape predicated by colour; it is much more difficult to recapture the smell, feel, etc. This emphasis of spatial extension seems to be related to the importance of visual perception in man and there is no justification for supposing that any such condition obtains in an insect, particularly since the senses seem to have a reversed order of importance. Perhaps the most that can be said is that a female parasite has the potentiality of becoming aware of certain hosts. Some of these difficulties may be due to a failure to recognize that a living organism requires a certain minimum time and space in which to manifest itself. Thus C. D. Broad (1923) states: "In ordinary life we distinguish between an object and its history, and we are inclined to think that the former is logically prior to the latter.... You might, we think, have an object without a history, but you could not have a history without an object. I believe this to be a profound mistake, which arises from taking 'history' in too narrow a sense. An object separated from its history is clearly not the kind of thing which could possibly exist. Every object that is not merely momentary has a history of some kind and no merely momentary object could possibly exist. 'Object' apart from 'history' is, therefore, as much an abstraction as 'history' apart from 'object'." In regard to host selection what cannot be done in reality is to separate an adult parasite from its history or temporal extension. The adult stands in the relation of having been elaborated from its host and cannot be supposed to have had no experience of a host, although it may be an experience on which little further can be said.

It has been maintained by Thompson and Parker (1927) that the selection of hosts by parasites is a typical example of an instinctive action on the part of the insect, the hosts chosen being those which are suitable for the development of the progeny of the parasite. Reference of this behaviour to an "instinct" is merely a description of the observable fact that under natural conditions the reproductive activities of the female parasites are so integrated that, among many suitable and unsuitable hosts recognized by the observer in any given ecological station, the females usually find and parasitize those from which their progeny are able to emerge. If this were not a general rule, parasitic species would soon be non-existent. The behaviour is described as instinctive because the female's actions are such that they are preadapted to situations which are likely to occur; for instance, the female searches for hosts which she may never have encountered as an adult.

Salt (1937) states that the fact that *Trichogramma* will reject true but unparasitized hosts provided they are contaminated with the smell of a female, indicates that this parasite selects its hosts by criteria which are not necessarily criteria of suitability. Now it will be generally conceded that the seeking out of hosts by parasites is done with the object of either depositing an egg, or possibly feeding on the host contents; unless considered in relation to these ends, the activity is meaningless to the observer. As apparently *Trichogramma* does not feed on its hosts, one may justifiably claim that unparasitized hosts which are examined, will, in the natural course of events, be parasitized by the examining female. The criteria used are liable, but not certain, to be criteria of suitability, for a host may be rejected for no apparent reason. But why should a female, having sought for a host and found an unparasitized one, leave it without depositing an egg? In the laboratory, the significance of this odour to the female is obscured because in confining females in a Petri dish they may wander aimlessly over hosts and show no apparent desire to attack them, whereas normally a female parasite would seek hosts only if she desired to oviposit in them. Clearly, the presence of an external odour cannot be considered apart from its usual concomitant, the deposition of an egg; and in tending to avoid hosts with this external odour the female parasite is exhibiting a diagnostic feature of "instinctive" behaviour —anticipation of a situation which is likely to occur.

The method of detection of parasitized hosts by an external odour is conceded to be imperfect, because of the transitory nature of the odour; and it certainly is not the major criterion. With *Ooencyrtus*, although the initial determination of the nature of a host may be by some external odour, it is evident that as far as actual deposition of parasite eggs is concerned, this is completely subordinated to other features associated with the stage of the parasite within the host. In other words, the major criteria used in determining the nature of a host are quite definitely criteria of suitability. It seems likely that this is generally true with other parasites and their hosts.

In connexion with this question of suitability, one may recall the oviposition response of *Ooencyrtus* towards host types which had been killed by immersion in hot water and in which this parasite is able to develop. It was found that the females avoided depositing eggs in dead hosts containing a live or dead parasite and further, that, if given a choice between dead hosts containing live early parasite larvae and dead hosts containing dead early parasite larvae, the former proved more attractive. For purposes of survival of progeny, hosts containing a dead young parasite are definitely preferable to those containing a live young parasite, and it may be said that the females did not make the most suitable choice of the available host supply.

In assessing the significance of these results, it is essential to appreciate conditions in the field where gipsy moth eggs containing dead parasites only occur in peculiar circumstances. Thus a part of the immature parasites of the fifth summer generation may be killed by sudden inclement weather, but the death of any parasites in this host at any other time can be referred only to unsuitable conditions such as heavy multiparasitism or possible immunity factors of the host. Now it is probable that some of the hosts containing *young* parasites killed by inclement weather are suitable for further parasite development in the succeeding spring, and in the field these are the only hosts which the parasite is likely to encounter, which are perfectly good for development of progeny, and yet contain a dead parasite. The laboratory experiments show the limitations of the adaptive behaviour of the female. It remains a fact that the overwhelming percentage of the hosts available at any given time are live hosts, some perhaps containing live parasites, some perhaps dead parasites, and the oviposition response of the *Ooencyrtus* is such that she generally chooses the best of those hosts. Again, the criteria used are not certain to be those of suitability, but, for the usual or most probable course of events, they are.

A similar interpretation can be applied to the behaviour of the polyphagous *Trichogramma evanescens*, which is so occupied with size or quantity in selecting hosts, that in the laboratory the females choose large hosts unsuitable for the development of their progeny (or even large false hosts in which no progeny can be deposited) although the normal small host, *Sitotroga*, is present. Marchal (1936) showed that *Trichogramma* sp. bred from eggs of *Mamestra brassicae* paid little attention to suitable hosts such as *Sitotroga*, *Ephestia* and *Pieris*, which were smaller than their normal host. It seems, therefore, that this chalcid has a natural bias for large hosts, and in giving this parasite a choice between the small *Sitotroga* and a large host unsuitable for the development of its progeny a complication is introduced. In the laboratory the oviposition response of the *Trichogramma* is to any object of a size sufficient to contain an adult parasite—an observation of considerable importance because it indicates that the parasite has some appreciation of volume. But the fact that the chalcid exists in nature indicates that the factors recognized by analysis as leading a female parasite to a given ecological station must be such that, as *a general rule*, these objects must also be characterized by other features, which enable the progeny of the parasite to develop. Thus, in reality, "object of a given size" becomes synonymous with a normal host, for the most common objects of this size occurring in the particular environment of *Trichogramma* are eggs of various insects, which from their nature contain other characters co-existent with this size factor. The stupidity of the actions of the female is more apparent than real, for in recognizing the so-called ecological and psychological selection, the investigator is confusing isolation in thought with isolation in nature.

It seems, therefore, that the contention that a parasite *generally* "chooses" as its hosts those which are suitable for the development of its progeny must be accepted as an observable fact; the primary tendency of *Ooencyrtus* and *Trichogramma* to avoid ovipositing in parasitized hosts in which only one parasite can develop is a particularly interesting illustration of the degree to which this obtains in nature. The action of the female *Ooencyrtus* in rejecting a parasitized gipsy moth egg can only be understood by reference to the aim or purpose which it serves, namely the maintenance of the species; and the behaviour of the female, confronted with a choice of hosts, some of which contain parasites in various stages of development, is such that one is justified in inferring that the synthetic relating activity involved in the method of selection is analogous to a psychological process in man. The graded oviposition response of the parasite to a decreasing quantity of host material indicates vividly the complexity of the "physico-chemical" factors regulating the deposition of an egg by this species. In the laboratory the *Ooencyrtus* also parasitizes the eggs of eight other species of Lepidoptera, including the brown tail moth. The results of the present investigation suggest that the attempt to find by analysis some group of factors common to those hosts by which the oviposition response is governed, would prove to be useless. Any character or group of characters, that may be abstracted from these, do not, in reality, exist apart from the remainder of the characters which constitute any of the forementioned eggs in which development of the parasite is possible; if such a group be isolated and found to occur in hosts in which *Ooencyrtus* is unable to develop, one is forced to conclude that such hosts are not usually encountered under natural conditions. It is this integration of the active relations of the various parts and functions of the living organism with its environment so that the persistence of the whole depends upon the maintenance of those relations which analysis destroys by the nature of its method.

V. Acknowledgements

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VI. Summary

The present work attempts to determine the factors involved in the selection of hosts by the chalcid *Ooencyrtus kuvanae* Howard, and examines the distribution of the eggs of this parasite in a restricted environment such as obtains when the females are kept in immediate contact with the host eggs (the gipsy moth, *Porthetria dispar* L.) in Petri dishes.

It is shown that the distribution of the eggs by the parasite is not at random, but has reference to the nature of the hosts available at the time. The female usually selects unparasitized hosts, and, if forced to superparasitize, chooses those hosts containing

the youngest parasite stage. It is suggested that the graded oviposition response of the females towards hosts containing advancing developmental stages of the parasite may be conveniently correlated with the decreasing quantity of actual host material present within the host egg-shell rather than with a quantitative increase in intensity of movements of parasite larvae, etc. This is supported by the fact that hosts containing dead second and third instars of the larvae are rejected to an extent comparable with those hosts containing similar live larval instars. However, as a decreasing host quantity is accompanied by corresponding maturing parasite stages, the characters of these latter are also of significance.

If only parasitized hosts are accessible the female tends to retain her eggs rather than deposit them. The exercise of this restraint is shown to be related to:

(1) the developmental stage of the parasite in the parasitized host;
(2) the age and condition of the ovary of the female;
(3) the number and nature of the hosts available.

It is suggested that the incidence of superparasitism in the field cannot be referred to any single constant cause, but will depend, among other things, upon the frequency of contact with the particular type of hosts.

It is concluded that the oviposition response is to a perceptual complex of stimuli which is such that the female tends to choose those hosts in which its progeny are able to develop. This accords with the classical view of the instinctive nature of animal behaviour and with the Gestalt thesis of reactions to relations in the perceptual field. These perceptual complexes take the form of complete wholes such as an unparasitized gipsy moth egg, a gipsy moth egg containing a parasite larva, etc., and when a female is confronted with a choice of such host types one host is selected in contrast to another, the selection being that with the most complete set of characters of a normal unparasitized host. This apparent striving for the ideal unparasitized host seems to be a dominant factor also in the exercise of restraint when parasitized hosts only are available. It is inferred that the synthetic relating activity involved in such behaviour is analogous to a psychological phenomenon in man.

REFERENCES

Bierens de Haan 1929 "Introduction to Animal Psychology." London.

Broad, C. D. 1923 "Scientific Thought." London.

Crossman, S. S. 1925 Two imported egg parasites of the gipsy moth, *Anastatus bifasciatus* Fonse, and *Schedius kuvanae* How. *J. Agric. Res.* **30**, 643–75.

Flanders, S. E. 1935 An apparent correlation between the feeding habits of certain Pteromalids and the condition of the ovarian follicles. *Ann. Ent. Soc. Amer.* **28**, 438–44.

— 1936 A biological phenomenon affecting the establishment of Aphelinidae as parasites. *Ann. Ent. Soc. Amer.* **29**, 251–55.

— 1937 Habitat selection by *Trichogramma*. *Ann. Ent. Soc. Amer.* **30**, 208.

Hempelmann, F. 1929 "Comparative psychology." *Encyc. Brit.* 14th ed.

Howard, L. O. and Fiske, W. F. 1912 The importation into the United States of the parasites of the gipsy moth and the brown tail moth. *Bull. U.S. Dep. Ent.* No. 91, p. 312.

Köhler, W. 1915 In Russell, E. S. (1934).

Loeb, J. 1918 "Forced Movements, Tropisms and Animal Conduct." Philadelphia and London.

McDougall, W. 1936 "An Outline of Psychology." London.

MacLagan, D. S. and Dunn, E. 1935 The experimental analysis of the growth of an insect population. *Proc. Roy. Soc. Edinb.* **55**, 126–39.

Maple, J. D. 1937 The biology of *Ooencyrtus johnsoni* How., and the role of the egg-shell in the respiration of certain Encyrtid larvae. *Ann. Ent. Soc. Amer.* **30**, 123–55.

Marchal, Paul 1936 Les Trichogrammes. *Extrait des Annales des Epiphyties et de Phytogénétique.* Paris.

Parker, D. L. 1933 The interrelations of two hymenopterous egg parasites of the gipsy moth, with notes on the larval instars of each. *J. Agric. Res.* **46**, 23–34.

Russell, E. S. 1932 The limitations of analysis in biology. *Proc. Aris. Soc. Lond.* p. 147.

— 1934 "The Behaviour of Animals." London.

Salt, G. 1934 Experimental studies in insect parasitism. *Proc. Roy. Soc.* B, **114**, 455–77.

— 1935 Experimental studies in insect parasitism. III. Host Selection. *Proc. Roy. Soc.* B, **117**, 413–36.

— 1936 Experimental studies in insect parasitism. The effect of superparasitism on populations of *Trichogramma evanescens*. *J. Exp. Biol.* **13**, 352–63.

— 1937 The sense used by *Trichogramma* to distinguish between parasitized and unparasitized hosts. *Proc. Roy. Soc.* B, **122**, 57–76.

Silvestri, F. 1919 Contribuzioni alla conoscenza degli insetti dannosi e dei loro simbionti. IV. La cocciniglia de prugno (*Sphaerolecanium prunastri* Fonsc.). V. La cocciniglia del nocciuola (*Eulecanium coryli* L.). *Boll. Lab. Zool. Portici*, **13**, 70–192.

Thompson, W. R. 1937 "Science and Common Sense." London.

Thompson, W. R. and Parker, H. L. 1927 The problem of host relations with special reference to entomophagous parasites. *Parasitology*, **19**, 1–34.

Ullyett, G. C. 1936 Host selection by *Microplectron fuscipennis*, Zett. *Proc. Roy. Soc.* B, **120**, 253–91.

Woodger, J. H. 1929 "Biological Principles." London.