Studies in Insect Parasitism I—III

The effect of different hosts on the physiology, on the development and behaviour and on the sex-ratio of *Microbracon gelechiae Ashmead*

(*Hymenoptera*: *Braconidae*)

By E. S. NARAYANAN & B. R. SUBBA RAO

Division of Entomology
Indian Agricultural Research Institute, New Delhi

(With 7 figures)

Material and Methods

The parasite used in these studies, *Microbracon gelechiae Ashmead*, was imported from Canada in 1944 for the control of the potato tuber moth, *Gnorimoschema opercullela Zeller*, and is now being bred in large numbers on the laboratory host, *Corcyra cephalonica Stainton*, in the parasite laboratory of the Indian Agricultural Research Institute, New Delhi. The hosts which were used in breeding experiments bred in the laboratory for short periods as and when required are the following:

1. *Corcyra cephalonica Stainton*   — The gram flour moth
2. *Prodenia litura Fabricius*   — The tobacco caterpillar
3. *Galleria melonella Linnaeus*   — The wax moth
4. *Pectinophora gossypiella Saunders*   — The pink boll worm
5. *Dichocrosis punctiferalis Guénée*   — The castor seed borer
6. *Scirpophaga nivella Fabricius*   — The sugarcane top borer
7. *Plusia orichalcea Fabricius*   — The gram caterpillar
8. *Chilo zonellus Swinhoe*   — The jowar shoot borer
9. *Gnorimoschema operculella Zeller*   — The potato tuber moth.

The behaviour of parasites bred on different alternate hosts was studied by means of a modified McINDOS olfactometer devised by THORPE & JONES (1937). All breeding experiments and the olfactometer experiments (the latter in a dark chamber) were conducted in a temparature controlled room wherein the temparature was maintained at 25° C and an average relative humidity at 70%. For the study of the spermatheca and associated glands, freshly emerged mated females were dissected in physiological saline solution and carefully studied.

Acknowledgment

I. The effect of different hosts on the physiology of
Microbracon gelechiae Ashmead

Introduction

There is very little published experimental evidence to prove that the effect of a insect host on the fecundity of its parasites that develop on it. But there are a few interesting observations made by some workers on this aspect. ROUBAUD (1924) in studying the chalcid *Pachycrepoideus* has observed 27, 28 or 20 ovarioles according to the size of the parasite, which in turn depends upon the nourishment available to it during its development. PROPER (1931) in another chalcid, *Eupteromalus nidulans* studied and recorded the fecundity of 16 females and found that in general the smallest individuals laid the fewest number of eggs and the largest laid the most. TAYLOR (1937) has observed that the females of *Pleurotropis parvulus* will normally have 6 ovarioles, but small females have as few as 3. In this paper, the effect of different hosts on the fecundity and longevity of *Microbracon gelechiae Ashmead*, a larval parasite of the potato tuber moth, *Gnorimoschema operculella Zell.* have been studied in detail.

Observations

In the course of these investigations it was observed that generally the larger females always laid more eggs than the smaller ones, irrespective of the host on which they were reared. To test this observation, mated females of different sizes, which had not oviposited before, were dissected and the ovaries studied. The females were mostly 2 to 3 days old. Permanent preparations of the ovaries were made and camera lucida drawings made (Fig. 1—4). Table I shows the variation in the number of ovarioles and the number of fully developed eggs in the uteri of the parasites at the time of dissection.

Table I. The variation in the number of ovarioles and the number of fully developed eggs contained in them

Approximate size of the female	No. of ovarioles on each side	No. of eggs in the uteri
1. Large females more than 3.3 mm	Two on each side well developed	12—16
2. Medium sized between 2.5 to 3 mm	Two on each side normal	8—12
3. Small females less than 2.5 mm	Only one on either side. The other pair reduced	Never more than 8
4. Very small under fed runts	Ovaries highly reduced and aborted	Nil

It may be seen from the above table that there is a relation between the size of the adult and the number of ovarioles, as well as the number of

eggs. Thus the fecundity to a certain extent is dependent on the size of the adult female.

It was also observed in the course of these studies that the parasites bred on *S. nivella* and *P. gossypiella*, in addition to their large size, had the ovaries fully developed, whereas the parasites bred on *C. cephalonica*
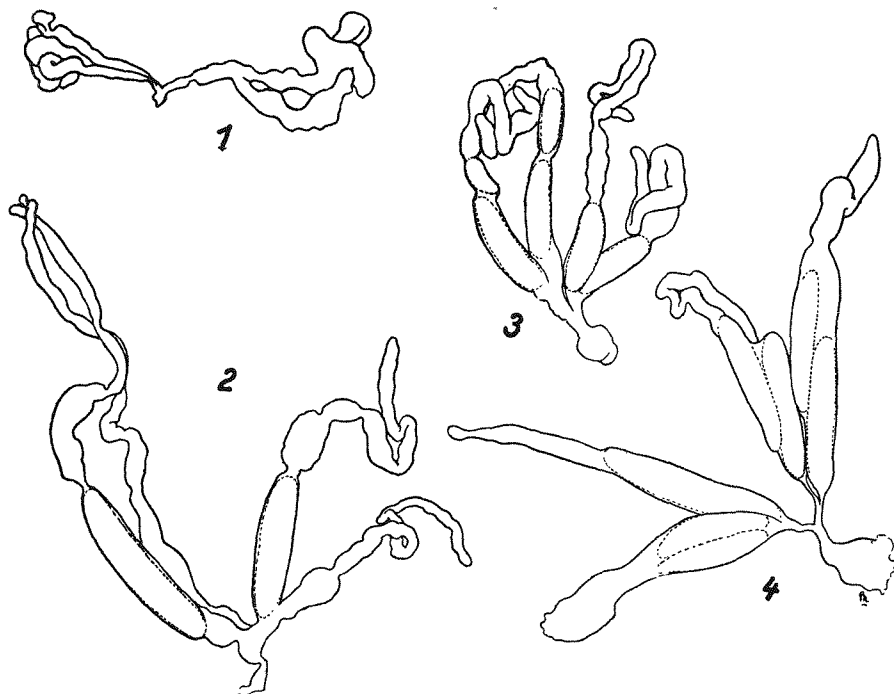

Fig. 1. *Microbracon gelechiae Ashm.* A highly distorted and atrophied ovary of a female 'runt' in which no egg development has taken place. Fig. 2. *Microbracon gelechiae Ashm.* A condition in which the development of the ovariole is not complete and only one side showing the development of eggs. Fig. 3. *Microbracon gelechiae Ashm.* Ovary of a normal female showing normal development. Fig. 4. *Microbracon gelechiae Ashm.* The ovary of a well fed female. The ovary is highly developed and the ovarioles are showing more number of eggs at the time of dissection

and *D. punctiferalis* showed considerable variation in the development of ovarioles. To confirm the above observations the following experiments were conducted.

Newly emerged females of *M. gelechiae* bred on *S. nivella*, *P. gossypiella*, *C. cephalonica* and *D. punctiferalis* were allowed to oviposit in separate oviposition cages. The parasites were provided the same species of hosts for oviposition on which they themselves had developed. After every 24 hours the eggs were counted and removed to separate cavity blocks for further development. This was continued as long as the parasite lived.

Table II. Average fecundity and longevity of *M. gelechiae*
reared on *S. nivella*

No. of generation of parasite	Longevity	No. of eggs laid per female	Remarks
1	25	325	The females laid more than
2	25	260	25 eggs per day for a number
3	35	361	of days and on 4 occasions a
4	20	220	female laid more than 30 eggs
5	39	398	per day
6	38	434	

Average per day   — 10.42 eggs

Average longevity — 30.33 days

Table III. Average fecundity and longevity of *M. gelechiae*
reared on *P. gossypiella*

No. of generation of parasite	Longevity	Fecundity	Remarks
1	16	240	
2	30	400	
3	14	220	The maximum eggs laid by a
4	37	509	female in a day was 26
5	50	539	
6	26	380	

Average per day   — 13.22 eggs

Average longevity — 28.83 days

Table IV. Longevity and fecundity of *M. gelechiae* reared on
C. cephalonica

No. of generation of parasite	Longevity	No. of eggs laid	Remarks
1	31	331	
2	28	225	The maximum number of eggs
3	26	240	laid by a female in a day was
4	28	259	only 23
5	18	210	
6	18	189	

Average per day   — 9.75 eggs

Average longevity — 24.83 days

Table V. Longevity and fecundity of *M. gelechiae* reared on
D. punctiferalis

No. of generation of parasite	Longevity	No. of eggs laid	Remarks
1	18	24	The maximum number of eggs
2	20	54	laid in a day was only 10. In
3	21	106	the 3rd generation Corcyra hosts were supplied and the parasite laid 106 eggs

Average per day   —   3.11 eggs
Average longevity — 19.66 days

During this period the parasites were fed with 10% sugar solution. The fecundity and longevity of the parasites after each generation was studied carefully. The following table shows the average longevity and fecundity of the parasite in that particular generation.

The data obtained from these experiments show that the fecundity of adult parasites bred on *P. gossypiella* was very high and that of parasites bred on *D. punctiferalis* was very low. But the longevity of parasites bred on *S. nivella* was high and was very closely followed by the parasites bred on *P. gossypiella*. It was interesting to see that the parasites bred on *D. punctiferalis* laid on an average only 25 eggs per female on *Dichocrosis*. In the second generation they laid on an average 54 eggs per female. But in the third generation when the parasites were given *Corcyra* hosts they laid 106 eggs per female. In one of the experiments a female parasite reared on *P. gossypiella* was not provided with any food. It was observed that the parasite regularly fed on the host body fluid that oozed out as a result of stabbing by the female prior to oviposition. A few parasitised larvae on examination revealed that the female parasites had constructed some feeding tubes.

Effect of host colouration on the pigmentation of the eggs of the progeny

Certain interesting observations with regard to the pigmentation of eggs laid by adults bred on different hosts were made.

The egg laid by a female bred on its natural host, *Gnorimoschema operculella* is creamy yellow in colour while adults reared on the laboratory host *Corcyra cephalonica* usually lay glistening white eggs.

The parasites were continuously reared on the alternate hosts, *P. gossypiella, Plusia orichalcea, S. nivella, D. punctiferalis* and *Agrotis ypsilon* and the changes in colouration of eggs in the succeeding generation were studied. The parasites bred on different hosts were kept separate. At the

end of two or three generations it was observed that the colouration of the eggs laid by the parasites, gradually started changing and more or less took after the colouration of their respective hosts. The abdomens of the gravid females bred on various hosts were also prominantly pigmented and varied from yellowish to greenish. It was also noticed that the grubs fed on *S. nivella* spun deep yellow silken cocoons instead of the normal glistening white cocoon. The following tabular statement gives the host colour, the colouration of the egg of the progeny, and the number of generations taken for the transference of the host pigmentation to the offspring.

Table VI.

Name of the host	General colour of host	Colour of egg	No. of generation taken for the transference of the pigments
1. *Corcyra cephalonica*	Pale yellow	Glistening white	—
2. *Scirpophaga nivella*	Greenish yellow	Light yellow	4 generations
3. *Pectinophora gossypiella*	Pinkish	Pinkish yellow	4 generations
4. *Dichocrosis punctiferalis*	Brownish with yellow and black patches	Yellowish green	2 generations
5. *Plusia orichalcea*	Green	Light green	2 generations
6. *Agrotis ypsilon*	Green	Light green	2 generations

When the females which were bred on the above alternate hosts were again reared back on *Corcyra* host, the eggs gradually lost their pigmentation, becoming glistening white once again.

From the above experiment, it can be safely said that it is a case of direct transference of some pigmented material from the host to the yolk of the eggs. The change in the colouration of the abdomen of the females indicated the gradual absorption of the pigmented material by the eggs stored in the abdomen.

Discussion

Though there are many contributions here and there on this aspect of the problem, the effect of the physiological processes in the body of the host on the parasite as a whole has not been studied in detail by earlier workers. The works of ROUBAUD (1924) on *Pachycrepoideus*; PROPER (1931) on *Eupteromalus*; TAYLOR (1937) on *Pleurotropis*; DOWDEN (1934) on *Zenilla*, deal only with the number of ovarioles and their development. But the other aspects of the problem have not been tackled by them. As SALT (1941) observes "The various effects brought about by the host upon the parasites bred, have been generally ignored, because the host is generally looked upon as a passive victim, influenced by, not itself influencing

the parasite". An analysis of the experimental data, as well as the condition of the ovaries as determined by dissections appear to throw some light on this aspect of the problem, and suggests that the condition and availability of food material in the host influences the growth and formation of the ovaries of parasites bred on them. In the case of insects like moths, that lay their full quota of eggs in a few days, the dissection of the ovaries gives an approximate value for fecundity. But in the case of *M. gelechiae* which lives for a long time and lays eggs during the entire period of its life, the number of eggs in the ovaries at the time of dissection does not indicate the number she is capable of laying. However, it gives some idea of the condition of the ovary as judged by the development of ovarioles. The number of ovarioles and their relative development in *M. gelechiae* is an important factor in assessing the fecundity. It is shown that the under sized weak female have only one pair of fully developed ovarioles, the other pair being reduced. In extreme cases the ovaries are completely reduced and distorted. This finding is of extraordinary significance as it explains why the fecundity of several parasites bred from the same host varies so much and may be abnormal.

The fecundity of *M. gelechiae* bred on *P. gossypiella* was very high when compared to the fecundity of the parasites bred on other hosts like *S. nivella, C. cephalonica* and *D. punctiferalis*. This may be partly attributed to the protein content of the host, which is the primary factor in the production of eggs in many insects. In the case of pink boll worm which feeds on the cotton seeds, a high percentage of protein and fat may be present in the caterpillar on which the parasitic grubs develop. Therefore it can be assumed that the parasitic grubs accumulate the necessary quantity of protein material for the future development of the adult. As in Hymenopterous insects, *M. gelechiae* also feed on the blood of the host that exudes during the act of stabbing. As Imms (1937) observes "that nitrogenous food of this kind is necessary to ensure complete maturation of eggs." Very recently Hagen (1950) by feeding *Chrysopa california* with synthetic food of high protein value, was able to improve the fecundity of the predator to a great extent. This recent observation lends additional support to the view advanced in these studies. Just as the fecundity is affected, the longevity may also be influenced in the same manner by the quality of the food of the host.

It has been experimentally shown that the parasite can transfer certain pigments from the host to its offspring. It is also seen that the pigmentation is not germinally fixed. This suggests the direct transference of some pigmented material from host to the yolk of the egg during the development of the egg in the ovary. Dr. Wigglesworth (in a communication to the Division) suggests that the pigments concerned may be carotinoid in nature. This opens up possibilities for further research in this fascinating problem.

II. The effect of different hosts on the development and behaviour of *Microbracon gelechiae Ashmead*

Introduction

A number of workers have suggested that the host has an effect on the selective behaviour of its parasite. This is the so called host selection principle. According to this theory, if a species is capable of breeding on 2 or 3 more species, individuals tend to oviposit upon the kind on which they themselves developed. WHEELER (1923) was probably the first to apply this idea to entomophagous insects, when he used it as an explanation of the predilection of solitary wasps for a particular species of prey, but there is no experimental evidence to support his suggestion. HASE (1925) and SALT (1941) have given sufficient data to proove that at least in the case of *Trichogramma* which has more than 60 alternate hosts, the principle of host selection does not hold good. However, the positive application of this principle to a parasite has been readily demonstrated by Thorpe & JONES (1937) experimenting on *Nemeritis canescens*, the larval parasite of the Mediterranean flour moth, *Ephestia kuehniella*. In the present paper the effect of hosts on the development of *M. gelechiae* has been described. The data show that the theory holds good in the case of *M. gelechiae* which has been bred continuously for 8 years on *Corcyra cephalonica* as an alternate host.

Results

The various hosts previously mentioned were daily supplied to the parasite for oviposition in separate cages, and the development of the grubs was closely watched. A short summary of the development on each host is given below:

1. *Corcyra cephalonica*: The eggs hatched within the usual incubation period of 24 hours and the larval period lasted for 3 to 4 days. The adults energed on the 10th and succeeding days.

2. *Scirpophaga nivella*: The eggs hatched within the usual incubation period. The larval stage lasted for 3 to 4 days. The grubs attained maturity after 11 days.

3. *Pectinophora gossypiella*: The larval stage was at no time more than 3 days. The adults emerged on the 9th and succeeding days.

4. *Dichocrosis punctiferalis*: Parasitization was very much delayed. The larval period was unusually long and lasted nearly 5 to 6 days. The adults started emerging only on the 14th and succeeding days. On one occasion the adults began to emerge on the 18th and succeeding days.

5. *Galleria melonella*: Development was rather slow. Adults emerged on the 13th and succeeding days.

6. *Plusia orichalcea*: Development normal   Adults began to emerge on the 12th day and onwards.

Table I. The development of parasites

Sr. No.	C. cephalonica			P. gossypiella		
	Date of oviposition	Date of emergence	No. of days taken for development	Date of oviposition	Date of emergence	No. of days taken for development
1.	28. 6. 50	8. 7. 50	10	2. 12. 49	12. 12. 49	10
2.	28. 6. 50	8. 7. 50	10	3. 12. 49	13. 12. 49	10
3.	29. 6. 50	10. 7. 50	11	12. 12. 49	22. 12. 49	10
4.	29. 6. 50	10. 7. 50	11	6. 1. 50	16. 1. 50	10
5.	29. 6. 50	10. 7. 50	11	7. 1. 50	17. 1. 50	10
6.	30. 6. 50	10. 7. 50	10	9. 1. 50	18. 1. 50	9
7.	30. 7. 50	11. 7. 50	11	10. 1. 50	20. 1. 50	10
8.	30. 7. 50	11. 7. 50	11	19. 1. 50	29. 1. 50	10
9.	1. 7. 50	13. 7. 50	12	23. 1. 50	2. 2. 50	10
10.	2. 7. 50	12. 7. 50	10	22. 1. 50	1. 2. 50	10
11.	2. 7. 50	13. 7. 50	11	31. 1. 50	10. 2. 50	10
12.	2. 7. 50	13. 7. 50	11	1. 2. 50	11. 2. 50	10
Average No. of days taken for development		10.75				9.91

7. *Chilo zonellus*: Development normal. Parasites attained maturity on the 11th day and onwards.

8. *Gnorimoschema operculella*: This is the natural host and the life-history took 11 days to be completed.

Table I shows the date of oviposition and the date of emergence of the parasites bred on some 4 hosts.

The indirect effect of host food on the development of its parasites

In the course of these studies it was observed that the full grown caterpillars of *Plusia orichalcea* feeding on gram (*Cicer arietinum*) were readily accepted by *M. gelechiae* and the subsequent development of parasitic grubs was quite normal. When *Plusia* caterpillars feeding on tobacco (*Nicotiana tabacum*) were provided, the parasites accepted them and laid egg, but the grubs on hatching out, after feeding for some time died. To study the indirect effect of the food plant of the host on the parasites, the following experiments were carried out.

Experiment No. 2

Six full grown larvae of *Plusia* spp., each collected from tobacco and gram were exposed separately to 3 gravid females for oviposition. After 24 hours the larvae were found to be parasitized. Each larva bearing eggs was kept separately in small cavity blocks. It was found that in the case of the tobacco caterpillar, after the usual incubation period the eggs

reared on different hosts

S. nivella			D. punctiferalis		
Date of oviposition	Date of emergence	No. of days taken for development	Date of oviposition	Date of emergence	No. of days taken for development
2. 12. 49	14. 12. 49	12	18. 11. 49	4. 12. 49	16
1. 12. 49	13. 12. 49	12	19. 11. 49	6. 12. 49	17
3. 12. 49	15. 12. 49	12	28. 11. 49	11. 12. 49	13
4. 12. 49	15. 12. 49	11	2. 12. 49	16. 12. 49	14
6. 12. 49	17. 12. 49	11	8. 12. 49	20. 12. 49	12
7. 12. 49	18. 12. 49	11	9. 12. 49	27. 12. 49	18
8. 12. 49	19. 12. 49	11	10. 12. 49	24. 12. 49	14
11. 12. 49	24. 12. 49	13	10. 12. 49	27. 12. 49	17
13. 12. 49	24. 12. 49	11	30. 12. 49	13. 1. 50	14
14. 12. 49	26. 12. 49	12	31. 12. 49	13. 1. 50	13
15. 12. 49	27. 12. 49	12	18. 1. 50	2. 2. 50	15
16. 12. 49	28. 12. 49	12	20. 1. 50	2. 2. 50	13
		11.66			14.66

hatched, but all the parasite grubs died within three days after feeding on the host whereas those that were feeding on the gram caterpillars normally developed and reached maturity. This experiment was repeated three times. The results are shown in the following tables.

Table II. The development of *M. gelechiae* on *Plusia* feeding on gram

Sr. No.	No. of hosts exposed	No. of eggs laid	No. of parasites maturing
1	3	8	5
2	3	10	4
3	3	7	6
Total	9	25	15

Table III. The development of *M. gelechiae* on *Plusia* feeding on tobacco

Sr. No.	No. of hosts exposed	No. of eggs laid	No. of parasites maturing
1	3	8	Nil
2	3	6	Nil
3	3	7	Nil
Total	9	21	

It may be seen from the above Table II that out of 25 eggs deposited on the hosts 15 developed into adults.

It is seen from the Table III that out of 21 eggs oviposited none attained maturity. There was cent percent mortality among them.

Experiment No. 3

In another experiment *Plusia* larvae of very young stages (2nd and 3rd instar) were collected from tobacco leaves and were given for oviposition. It was interesting to find that the grubs normally developed and emerged out as adults. The results of the experiment is tabulated below.

Table IV. The development of *M. gelechiae* on *Plusia* (young larvae) feeding on tobacco

Sr. No.	No. of hosts exposed	No. of eggs laid	No. of parasites maturing
1	3	8	3
2	3	5	2
3	3	9	4
Total	9	22	9

It may be seen from the above table that though there was a high percentage of mortality among larval stages, their development was normal and a few developed into adults.

The effect on the behaviour of the parasite

The effect of different hosts on the selective behaviour of the parasites was experimentally studied and the results are recorded below.

The female parasite as soon as it comes in the vicinity of the host is attracted towards it, and this initial attraction of the parasite to the hosts as in other braconids appears to be mainly due to the odour of the host larva. After reaching the region in which the host is located, the female parasite tries to find the exact spot where the host is located by vibrating its antennae. There is little doubt that the olfactory sensillae appear to be located in the antennae for it was found that when these organs were amputated the parasites were unable to detect the presence of the host and generally showed little interest in oviposition.

Preliminary studies on the oviposition response of the female parasite to the stimuli emanating from the host showed that the odour of the larva was one of the dominant factors in host selection. The behaviour of gravid females towards the smell of the different hosts was therefore tested by means of a modified McINDO's olfactometer (Fig 5).

Mode of experimentation

The parasites tested were introduced into tube A and were allowed to settle down for a while. The hosts to be tested were placed in one or the other arm of the tube G G. A slow draught of air was drawn in through the tube H H, regulated by means of a
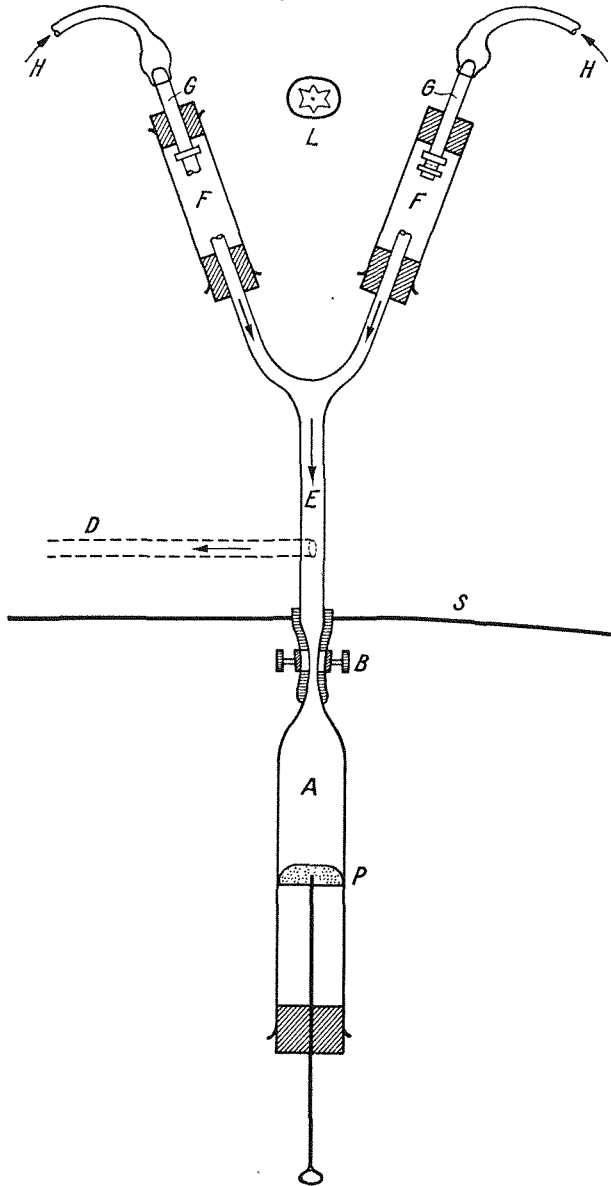
Fig. 5. McIndos's olfactometer modified after Thorpe & Jones (1937)

system of clips and wash bottles. The rate of flow of air passing through the tubes in these experiments were kept at 60 to 65 bubbles per minute. When the flow was regulated the clip B was slowly opened so that the parasites in A could make their way towards the light L from which they were protected from screen S. On reaching the glass tube E, they were exposed to the alternative odours and having made their choice

were eventually trapped in one or the other of the tubes FF. The position of the object used for testing the odour was alternated from one arm to another at each trial. At the end of each trial the Y tube was thoroughly washed with alcohol and dried.

Table V. Response of *M. gelechiae* (reared on *S. nivella* for 4 generations) to larvae of *S. nivella* and *C. cephalonica* in olfactometer

Sr. No. of the expt.	No. of females entered the arm of olfactometer containing			Remarks
	S. nivella	*C. cephalonica*	Total	
1	5	10	15	
2	6	15	21	Out of 181 choices *Corcyra* was
3	5	17	22	preferred by 116 females
4	10	15	25	(64.2%) as against *S. nivella*
5	12	16	28	by 65 (35.8%)
6	13	18	31	
7	6	13	19	
8	8	12	20	
Total	65 = 35.8%	116 = 64.2%	181	

Table VI. Response of *M. gelechiae* (reared on *C. cephalonica* to larvae of *C. cephalonica* & *G. operculella* in olfactometer

Sr. No. of the Expl.	No. of females entered the arm of the olfactometer containing			Remarks
	C. cephalonica	*G. operculella*	Total	
1	13	9	22	
2	11	5	16	
3	15	6	21	Out of 211 choices *Corcyra*
4	18	7	25	preferred by 154 females
5	13	7	20	(72.9%) as against *G. oper-*
6	16	2	18	*culella* by 57 (27.1%)
7	18	7	25	
8	16	2	18	
9	14	4	18	
10	20	8	28	
Total	154 = 72.9%	57 = 27.1%	211	

Standard deviation = ± 1.136
t value   =   8.540

In order to determine the behaviour of parasites reared on *S. nivella* for 4 generations to a choice of two hosts namely, *S. nivella* and *C. cepha-lonica*, the following experiments were conducted.

In one of the arm of the olfactometer 2 final instar larvae of *S. nivella* were placed and in the other 2 final instar larvae of *C. cephalonica*. 35 gravid females reared continuously for 4 generations on *S. nivella* were used in these experiments. Table V shows the results of these trials.

Next the reactions of gravid females which have been continuously reared on *C. cephalonica* since 1944, to two hosts viz., *C. cephalonica* and *G. operculella*, its natural host, was investigated. 30 gravid females were used in each of these experiments and the results are given in the Table VI.

The standard deviation calculated from the formula $S d = \dfrac{\varepsilon (x - \bar{x})^2}{N (N - 1)} = \pm 1.201$ and the "t" value for degrees of freedom is 6.763. Since the t value for 7 d. F at 5% is 2.365, at 1%, 3.499, and at 0.1%, 5.405, the results are highly significant (Table V).

Since the "t" value for 5 degrees of freedom at 5% is 2.262, at 1%, 3.250, and at 0.1%, 4.781, the results are highly significant (Table VI).

Discussion

The developmental period of the parasite on different hosts has been studied extensively by various workers in the field. PANTEL (1910) studying the development of a Tachinid on two hosts, *Acronycta* and *Vanessa*, and SMITH (1912), the development of *Perilampus* on two other hosts, have shown that the parasites mould their development on the development of their hosts. So also in the present studies it is shown that the total number of days taken for the development of *M. gelechiae* varies from host to host; the parasite takes nearly 15 to 18 days to complete its develpment on *Dichocrosis* as against the usual 10 to 11 days on *Corcyra*. This retarded development may be due to marked physiological differences in the host. These studies have also demonstraded that the food plant on which the host is fed may also indirectly affect parasite development. When the host was feeding on gram the parasite was able to develop normally to the adult stage; but when the same host was fed on tobacco the parasites were unable to develop. However, a small percentage of parasites completed their growth on the second and third instar larvae of *Plusia* feeding on tobacco. The indirect effect of the food plant on the development of the parasite was first noticed by MORGAN (1910) and GILMORE (1938). They attributed the mortality to the toxic substances contained in the food plant of the host. The present observations confirm their findings. But the fact that a small number of parasites were able to develop on younger larvae has to be considered. Possibly the younger caterpillar which have just started feeding may contain only minute quantities of nicotine, and hence the parasites were able to develop. The mortality of the parasite on older larvae may be due to the accumulated nicotine contents. However, a chemical analysis of the body contents of different hosts, will have to be made before arriving at a definite conclusion.

The olfactometer experiments to test the selective behaviour of the hosts suggest that continuous breeding on an unnatural host for several hundred generations will affect the tendency of the parasite to select its original host. In the first set of experiments, *M. gelechiae* bred on *S. nivella* for 4 generations did not show any preference to *S. nivella*. But *M. gelechiae*

bred on *C. cephalonica* for 7 years continuously under controlled conditions, tend to develop into a distinct race which shows preference to the new adopted host on which it was bred, even when the natural host *G. operculella* is available. But it would be hasty to formulate any definite conclusion on the basis of experiments under critical controlled condition. The behaviour of parasites under the complex conditions of the natural environment may differ greatly from what is observed under artificial laboratory conditions. However, these results undoubtedly give some clue to the behaviour of the parasite bred on different hosts.

III. The effect of hosts on the sex-ratio of
Microbracon gelechiae Ashmead

Introduction

An important aspect of mass breeding of beneficial parasites under laboratory conditions using suitable alternate laboratory hosts is the one that concerns the sex-ratio of the parasite. There is always the danger of depletion of the stock if the output of females becomes low. Many workers in the field have given various suggestions and theories on this aspect of the problem and none seems to have arrived at a definite conclusion. Two of the most prominent workers in this field, CLAUSEN (1941) and FLANDERS (1939) have dealt with the subject in detail.

Historical

In certain species of parasitic Hymenoptera, females preponderate. Indeed, in a population of thousands of females, there may only be single male. In others there is ordinarily a slight preponderance of females. In "Arrhenotokous hymenoptera" where, as a rule, the males are derived from unfertilized eggs, the sex of the progeny depends on whether the egg has been fertilized or not. CHEWREUV (1913) was the first, who found that the great majority of *Pimpla* species from large cocoons were females whereas those from smaller ones were males. He attributed the marked disparity in the sex-ratio of *Pimpla* to selective oviposition by the parent female. That the sex-ratio is markedly affected by a shortage of food resulting from superparasitism, has been shown by SALT (1936) in the case of *Trichogramma evanescens Westw.*, BRUNSON (1937) while studying the sex-ratio of *Tiphia popilliavora Roh.* found that the eggs which were laid on the 3rd instar larva of the Japanese beetle, *Popillia japonica* mainly produced females, whereas the eggs laid on the 2nd instar larva mainly produced males. When he interchanged the eggs from one instar to the other, he did not find any appreciable change. He came to the conclusion that the sex is determined when the egg is laid and that it could not be altered by changing the quantity or quality of the food after oviposition. FLANDERS (1945) suggested that the maximum percentage of

females could be obtained under mass production conditions by regulating such factors as heat, light and parasite density, which prevented impregnation of female parasites during and shortly after mating. VANDEL (1932) states that when superparasitism is high the male sex predominates because of selective elimination of the female rather than through the direct influence of a sex determining mechanism. GROSCH (1948) while dicsussing dwarfism and differential mortality in *Habrobracon brevicornis* concluded that expressions of differential mortality of sex types under dwarfing conditions indicate that haploidy and not maleness is the quality or characteristic which enables animals to better survive partial or complete larval starvation by metamorphosis. FLANDERS (1939) concludes that when the spermatheca in hymenopterous insects is stored only with female producing sperm it becomes a sex changing mechanism.

Observation and Results

The effect of hosts on the viability or the percentage of emergence of adult was studied. Female parasites, unmated as well as mated, were kept in separate rearing jars and a known number of host larvae were provided daily. The number of eggs laid was counted every 24 hours and as far as possible natural environmental conditions were provided for the development of the parasitic grubs. The following experiments were conducted.

Experiment 1

This experiment was conducted to determine the viability of the eggs of mated females. The results are tabulated below.

Table I. Viability of *Microbracon gelechiae*, eggs of mated females

Total No. Experiments conducted	No. of eggs laid	No. of grubs pupated	No. of adults emerged	No. of males	No. of females
20	253	156	144	46	98

Percentage of viability 56.19
Percentage of females 68.5
Percentage of males 31.5

It is seen from the above Table that the mortality during larval stages is very high and only 56.19% developed into adults.

Experiment 2

In this experiment, unmated females were allowed to oviposit and the development of grubs was kept under close observations. The results are tabulated below (Table II).

4*

Table II. Viability of *Microbracon gelechiae*, eggs of unmated females

Total No. of Experiments conducted	No. of eggs laid	No. of grubs pupated	No. of adults emerged	No. of males	No. of females
20	154	137	136	136	—

Percentage of viability   88.3
Percentage of males   100.0
Percentage of females   Nil

Table III. The sex-ratio of *Microbracon gelechiae* reared on
P. gossypiella

Sr. No. of experiment	No. of grubs per host	No. of males	No. of females
1	9	1	7
2	6	4	2
3	7	2	5
4	8	5	3
5	7	2	5
6	6	6	0
7	6	3	3
8	9	4	5
9	7	3	4
10	7	2	5
Total	72	32	39

Sex ratio Male   45%
Female 55%

Table IV. The sex ratio of *M. gelechiae* reared on
C. cephalonica

Sr. No. of experiment	No. of grubs per host	No. of males	No. of females
1	7	2	5
2	6	2	4
3	7	1	6
4	7	1	6
5	7	2	5
6	7	0	7
7	9	3	6
8	8	2	6
9	6	5	1
10	5	0	5
Total	69	18	51

Sex ratio Male   26.1%
Female 73.9%

Table II shows that the mortality during larval stage was very low and 88.3% of the eggs developed into adults. All the adults were males.

Experiment 3

Next the effect of two laboratory alternate hosts namely, *Scirpophaga nivella* and *Pectinophora gossypiella* on the sex-ratio of *Microbracon gelechiae* was investigated. In every case, care was taken to see that almost the same number of grubs developed on each host and the hosts were selected from the culture before giving them for oviposition. The following experiments were conducted and the results are tabulated on Table III—V.

It is seen from Table III that males and females were producced in almost equal numbers.

The results clearly indicate that females predominate in the parasite population developing on *Corcyra* (Table IV).

Table V. The sex ratio of *M. gelechiae* on *Scirpophaga nivella*

Sr. No. of experiment	No. of grubs per host	No. of males	No. of females
1	9	4	5
2	9	3	6
3	7	1	6
4	6	2	4
5	9	6	3
6	10	5	5
7	5	5	0
8	9	7	2
9	5	5	0
10	6	4	2
Total	75	42	33

Sex ratio Male 56%
Female 44.1%

It is very interesting to see that though the host is much larger than the other hosts, males predominate slightly (Table V).

Experiment 4

In this experiment, the parasite was bred on different hosts under mass rearing conditions. The results are tabulated in the following table.

Table VI. Sex-ratio of *M. gelechiae* under mass rearing conditions on different hosts

Host	Total No. reared	No. of males	No. of females	Percentage of females
1. *Pedinophora gossypiella*	284	160	124	43.6
2. *Corcyra cephalonica*	243	77	166	68.8
3. *Scripophaga nivella*	296	179	109	36.9

It is evident from the data that there is not much difference in the sex-ratio of *M. gelechiae* when bred under mass rearing conditions. The results of these experiments more or less tally with the results obtained from the critical experiments conducted earlier.

Effect of induced superparasitism on the sex-ratio of *M. gelechiae*

It was seen during the course of these studies that the number of parasitic grubs sharing a single host for their development had a direct bearing on the sex-ratio of the adults that emerged. To determine the validity of this statement the following experiment. was conducted.

Experiment 5

Corcyra larvae of the final instar and of the same size were selected from the culture and were given for oviposition. The number of grubs per host was increased from 3 to 30 and the development closely observed. The following Table VII gives the results.

Table VII. No. of parasite grubs per host, % female produced and % development

No. of grubs per host	% of female progeny	% of development
3	73.6	53.1
5	49.3	47.0
10	47.7	44.0
15	38.5	37.1
20	32.3	25.8
25	11.1	18.0
30	9.8	22.8

Table VIII. No. of parasite grubs per host and percentage of female produced

No. of grubs per host x	% Female y	$(x)^2$	$(y)^2$	$(x\,y)$
3	73.6	9	5416.96	220.8
5	49.3	25	2430.49	246.5
10	47.7	100	2275.29	477.0
15	38.5	225	1482.25	577.5
20	32.3	400	1043.28	646.0
25	11.1	625	123.21	277.5
30	9.8	900	96.04	224.0
108 $S(x)$	262.3 $S(y)$	2284 $S\,x^2$	12867.52 $S\,y^2$	2669.3 $S(x\,y)$

Coefficient of correlation

$$r = \frac{S(x\,y) - \dfrac{S\,x \cdot S\,y}{7}}{\sqrt{S\,x^2 - \dfrac{(Sx)^2}{7}}\;\sqrt{S\,y^2 - \dfrac{(Sy)^2}{7}}} = -0.98 \quad \text{Significant at 1\% level.}$$

Correlation studies between the number of grubs per host, percentage of female and percentage of development were conducted as seen in the Tables VIII & IX.

Table IX. No. of parasite grubs per host and % development

No. of grubs per host (x)	% development (y)	x^2	y^2	$x\,y$
3	53.1	9	2819.61	159.3
5	47.0	25	2209.00	235.0
10	44.0	100	1936.00	440.0
15	37.1	225	1376.41	556.5
20	25.8	400	665.64	516.5
25	18.0	625	324.00	450.0
30	22.8	900	519.84	684.0
108	247.8	2284	9 850.50	3041.3
$S(x)$	$S(y)$	$S\,x^2$	$S\,y^2$	$S(x\,y)$

Coefficient correlation, using the same formula as shown above we get

$$r = -0.96 \text{ significant at 1\% level.}$$

Having found that there is a significant relation between the number of grubs per host, the number of females produced and the percentage of development of grubs into adults, the regression coefficients are calculated by the following formula.

$$b_{yx} = \frac{S(x\,y) - \dfrac{S\,x \cdot S\,y}{n}}{S(x)^2 - \dfrac{(Sx)^2}{n}}$$

$b_{yx} = -2.117$ for the data shown in Table VIII and

$b_{yx} = -1.25$ for the data shown in Table IX.

Substituting these values in the equation given below the regression equation for Table VIII and Table IX is found out with the help of these equations regression lines for the data shown above is drawn on the observed values is plotted on the same graph to find the nearness to the regression lines drawn early (Fig. 6 & 7).

$y \quad = \bar{y} + b_{yx} (x - \bar{x})$ where

$y \quad$ = calculated value of percentage of female progeny or the percentage of grubs developed into adults.

$\bar{y} \quad$ = Mean of the percentage of female progeny or the percentage of grubs developed into adults as observed in the development.

$\bar{x} \quad$ = Mean of the number of grubs per host larva.

$b_{yx} \quad$ = Regression coefficient of y on x.

$x \quad$ = Number of grubs per host larva.
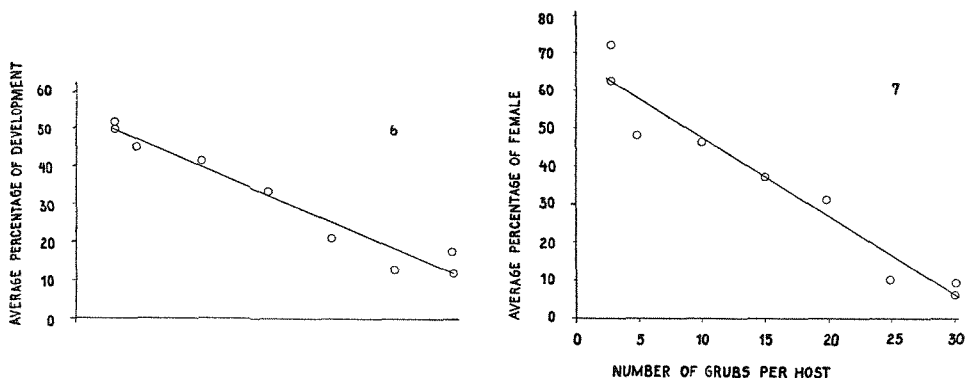
Fig. 6. Regression Line for the percentage of development on the number of Grubs per host. $y = 35.40 - 1.25 \ (x = 15.43)$. Fig. 7. Regression Line for the sex-ratio on the number of grubs per host. $y = 37.47 - 2.117 \ (x - 15.43)$

It is seen from Table VII that with the increase in the number of parasite grubs per host there is a definite decrease both in the percentage of development and the percentage of female progeny produced. Correlation studies confirm these findings and below is given the table showing the difference between observed and calculated values. Figs. 6 & 7 show that the observed values follow the same trend as the plotted regression line.

Table X.

No. of grubs per host	% female		% development	
	Observed	Calculated	Observed	Calculated
3	73.60	63.78	53.10	50.93
5	49.30	59.55	47.00	48.43
10	47.70	48.97	44.00	42.18
15	38.50	38.58	37.10	35.93
20	32.30	27.80	25.80	29.69
25	11.10	17.21	18.00	23.44
30	9.80	6.63	22.30	17.19

Morphology of the spermatheca of *Microbracon gelechiae*

In connection with the study of sex-ratio of *M. gelechiae*, the morphology of the spermatheca of the parasite, which controls the liberation of the sperm as in many of the hymenopterous parasites, was studied. A knowledge of the structure of the spermatheca and its associated glands will enable us to understand the mode of function of the spermatheca in controlling the fertilization of the eggs. In most of the hymenopterous parasites mating takes place during the preoviposition period, immediately following the emergence of adults. Generally the males emerge before the females and await the appearance of their mates. Mated females were dissected and several glycerine mounts of the spermatheca were prepared. The intricate structure of the sperm capsule and its ducts were studied under an oil immersionlens and drawings made.

The spermatheca, which is situated on the dorsal wall of the vagina, consists of three main parts namely, the sperm capsule, the associated sperm gland and the sperm duct which connects the sperm Capsule and the vagina. A number of minute canals of spermathecal glands open into a central reservoir which is an enlargement of the gland canal. The reservoir is very small when compared to the size of the sperm capsule. Though the capsule is chitinous in nature it is semi-transparent. When the spermatheca is dissected in physiological salt solution the sperms can be seen revolving round the capsule. The lumen of the sperm duct between the sperm capsule and the gland canal is spirally convoluted, corresponding in its structure to the undulations of an active sperm. A careful study of the spermatheca revealed that there is no visible valve, similar to the one occurring in chalcidoids or in aculeates (Flanders, 1939). In this type of spermatheca, the spermathecal gland empties into the lumen of the sperm duct which is so narrow and adjacent to the capsule that apparently only a single sperm can move out at a time. The sperm duct is uniformly of the same width throughout its length.

The discharge of sperm should coincide with the extrusion of the egg to ensure fertilization under normal circumstances. In *M. gelechiae* the discharge of a sperm appears to depend on the contractions of the spermathecal gland as the sperm capsule is devoid of a valve.

Discussion

It has been assumed that the sex-ratio is an approximately constant factor for any given parasite under field or laboratory conditions. A good deal of work is being done on this aspect of the problem within recent years by various workers. But the ever changing sex-ratio of insect parasites has puzzled investigators and they have not come to any definite conclusion as to the causes of its variation.

The present studies have indicated that the size of the host, which has been considered by many workers as an important factor in determining the sex of the parasite, may not have a great deal to do with it. BRUNSON (1937) has experimentally proved that the sex is predetermined when the egg is actually laid and it cannot be induced to change its sex by altering the quality or quantity of the food. The final sex-ratio of the progeny of any parasite is mainly depedent on two important factors: (1) fertilisation, regulated by the spermatheca and (2) the influence of differential mortality on the developing larvae.

As has been described earlier the discharge of the sperm is influenced by the contractions of the spermathecal gland. If this hypothesis is accepted, then so long as the sperm capsule contains the sperm and the rate of

oviposition does not exceed the rate of sperm discharge all the eggs that pass through the vagina must be fertilized. It is well known that in hymenoptera, the females are diploid and males are haploid. This means that all the progeny of a female should develop into females. But the data provided indicate that this is not so. This clearly shows that the source of stimulation for the glandular contractions must be external. It is possible that when the parasite stabs the host prior to oviposition, the fluid that oozes out of the body of the larva will rise up in the ovipositor canal due to capillary action and may influence the contractions of the spermatheca. The environmental factors like temperature, humidity, host etc., may also play their part in influencing the fertilisation of an hymenopteran egg.

When the capacity of the spermathecal gland is sufficient to allow fertilisation of all eggs, irrespective of the rate at which they are deposited, the sex of the progeny must be externally induced (FLANDERS, 1939). LEIBY (1926) suggests that in the oviposition of *Platygaster hiemalis* eggs may not receive a sperm if they pass through the vagina very rapidly. In such cases the sex of the progeny is partly internally induced. The fact that *M. gelechiae* does not deposit its eggs in quick succession suggests that the sex-ratio of the offspring is externally induced. ULLYETT (1936) has shown that the hymenopteron *Microplectron* is able to ascertain the shape and size of the host by antennal exploration and that it exhibits a definite preference for larger objects. BRUNSON (1937) and TAYLOR (1937) have shown that in *Tiphia* and *Pleurotropis*, the condition and the stage of the host determine the sex-ratio. In the present investigations also it is beyond doubt that the sex-ratio tends to vary from one host to another. It is evident that the production of males in many cases is an effect of inadequate external stimulation of the spermatheca which depends on the suitability of the host that the parasite encounters. These findings entirely agree with those of FLANDERS (1939).

When gregarious species like *M. gelechiae* attack small but suitable hosts, the male may predominate because of differential mortality resulting from superparasitism. VANDEL (1932) states that when many parasites inhabit a host, the male sex predominates because of selective elimination of the females rather than through the direct influence of a sex-determining mechanism. It has been experimentally proved on the basis of the data carefully collected that as the number of grubs per host larva is increased the percentage of males also increases. In the viability experiments it was seen that the offspring of a virgin female which all developed into males and are haploid, the grubs were able to survive the critical period of their larval life and that there was very low mortality. Whereas in the case of a mated female the viability was very poor and the mortality during larval stages was very high. GROSCH (1948) in a recent paper has shown that the diploid males of *Habrobracon brevicornis* have very low viability under starvation conditions. Hence it is the haploid male which

successfully meets the larval starvation by pupating and finally attains maturity. The mortality of female grubs on small hosts is higher because the female larvae cannot endure starvation. Hence the sex-ratios of parasites are partly dependent on differential mortality.

Summary

(1) The viability of unfertilized eggs is higher than that of the eggs of mated females.

(2) The effect of different hosts on the sex-ratio of *Microbracon gelechiae Ashm.* is shown.

(3) It is seen that as the degree of superparasitism increases the percentage of males also rises.

(4) The morphology of the spermatheca and the mechanism of the discharge of sperm are described.

(5) The various factors that determine the sex-ratio of a parasite and with particular reference to *M. gelechiae* are discussed. It is shown that the production of excess males in many cases is the direct effect of inadequate external stimulation of the spermatheca, which depends on host suitability. In gregarious species like *M. gelechiae* the male sex predominates because of differential mortality resulting from superparasitism. It is evident that the haploid male is less affected by larval starvation, than deploid males and females.

The sex-ratio of a parasite is determined by various external and internal influence. The action of the spermatheca in liberating the sperm and the differential mortality during the larval stages are mainly responsible for the ultimate sex-ratio of the parasite.

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Beiträge zur Kenntnis alter naturwissenschaftlicher Werke

Beitrag 6

Von L. E. RAUTENBERG, Berlin.

(Mit 5 Textfiguren)

DRÜMPELMANN, ERNST WILHELM und FRIEBE, WILHELM CHRISTIAN: Getreue Abbildungen und naturhistorische Beschreibung des Thierreichs aus den nördlichen Provinzen Rußlands, vorzüglich Liefland, Ehstland und Kurland betreffend.

Riga, Hartmann, 1806—1814. 2° (34 cm). 2 Bde. (je 4 Hefte) mit insges. 40 kolorierten Kupfertafeln. (Säugetiere 3 Tab., Vögel 25 Tab., Reptilien 3 Tab., Insekten 8 Tab. u. zwar Tab.: I, XI, XII, XXI, XXII, XXXI, XXXII, XXXIII.).

Es existieren Exemplare mit deutschem oder französischem Text, auch mit schwarzen Tafeln.