The Effect of Diet on Egg Maturation and Resorption in *Mormoniella vitripennis* (Hymenoptera, Pteromalidae)

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SUMMARY

1. *M. vitripennis* females have been treated in one of the following ways: (*a*) fed on honey, (*b*) starved, (*c*) fed on host blood.

2. When fed on honey the ovaries contain 22 eggs after 2 days, then a slow cycle of maturation and resorption begins so that for 16 days their condition does not change. After 16 days resorption is more rapid and by 28 days there are only 1 or 2 mature eggs.

3. When starved, the parasites die in 5 days. Rapid resorption occurs and at death there are only 3 eggs in the ovaries.

4. When fed on host blood, eggs mature rapidly. After 5 days the ovaries contain 40 mature eggs even though 260 have been deposited. Parasites which are then starved, resorb eggs very rapidly and die in 48 hours, but those fed on honey live for at least 8 days and rapid resorption does not occur.

INTRODUCTION

MORMONIELLA VITRIPENNIS (Walker) is parasitic on the pupal stage of Muscoid flies. The female drills through the host puparium and lays a number of eggs on the enclosed pupa. The eggs hatch into larvae which feed on the host tissues and pupate inside the host puparium from which the resulting adult parasites eventually bite their way out. After ovipositing, the female parasite forms a feeding tube and sips up some of the host's blood. This blood meal is not essential for the maintenance of life, as the parasites will live for several weeks on a diet of honey, but it has a marked effect on the condition of the ovaries.

Roubaud (1917) records that when *M. vitripennis* females are fed on glucose only, they have 4–5 well-developed eggs in the ovaries after 12 days, but when fed on host blood, they have ovaries 'bulging with eggs' after 4–5 days. It was further shown by Flanders (1935) that when deprived of hosts, many parasitic Hymenoptera resorb the mature eggs present in the ovaries. The phenomenon has been recorded in several species, but the only quantitative work appears to be that of Grosch (1950), who counted the number of eggs in the ovaries of *Habrobracon juglandis* (Ashmead) at various stages of starvation.

The present quantitative work on the maturation and resorption of eggs in *M. vitripennis* was undertaken as a preliminary to a behaviour study. It is obvious that the behaviour pattern will be affected by the condition of the ovaries and it is therefore important to know what changes occur with increased age and with different diets. It is also advantageous if a method of

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rearing can be devised such that the condition of the ovaries at any time can be predicted without killing the parasite and dissecting it.

Standardization of material

By observing parasitized puparia in transmitted light it can be shown that *M. vitripennis* imagines spend 20–30 hours inside the host puparium before biting their way out, so that an error is introduced if the age of the imago is measured from the time it leaves the host. To avoid this error host puparia were opened when the parasites were still in the pupal stage, 48 hours before eclosion. The ages given are accurate to ± 2 hours.

As the overall length of the imago varies not only with the contents of the gut and ovaries, but also through muscular action, the parasites were measured in the late pupal stage, and only those having a length of 2·00 mm. $\pm$ 0·05 mm. were chosen. The resultant imagines were about 2·5 mm. long, and each was confined with a male immediately. Scrutiny of the spermatheca after the experiment showed that in every case mating had taken place. The parasites were reared, and all experiments were carried out at a temperature of 25° C. and relative humidity 75 per cent.

Puparia of the house fly *Musca domestica* L. were used as hosts. The fly larvae were reared on milk at a temperature of 25° C. and were kept at that temperature until required for experiments. They were used 24 hours after the onset of pupation, and measured 6·00 $\pm$ 0·2 mm. in length.

Method of recording

For ease of comparison a method of recording was devised whereby the condition of the ovaries could be expressed as a series of numbers indicating the number of eggs at five different stages of maturation and resorption. The ovarioles are polytrophic and the greater part of them is packed with numerous minute oocytes measuring less than 0·1 mm. in length, accompanied by a slightly larger group of nurse cells. These oocytes have not been recorded, partly because counting them would be extremely laborious, but mainly because there was never any obvious change in their number throughout the experiments.

The freshly killed insects were dissected and the ovaries removed in a drop of saline. The early stages of maturation and the late stages of resorption show up plainly, but as the transitional stages are poorly defined the ovaries were stained for 1 minute in acetocarmine. Mature eggs take up no stain, but immature and resorbing eggs stain deeply.

Immature eggs were divided into two groups, namely, 'three-quarter-mature eggs' in which the ratio between the length of the oocyte and its group of nurse cells was greater than 3, and 'half-mature eggs' in which this ratio lay between 1 and 3. If this ratio was less than 1, no record was made. Resorbing eggs were also divided into two groups—those which were in the early stages of resorption, as shown by their shape and size, and those in later stages which had become smaller and in which the oval outline had been lost.

The number of eggs falling into each category was written down in order, the half-mature eggs first, and the mature eggs underlined for extra clarity. As an example the ovary shown in fig. 1, which has 6 half-mature eggs, 7 three-quarter-mature eggs, 24 mature eggs, 2 in the early stages of resorption and 2 in the late stages, would be recorded as 6–7–*24*–2–2. This is clearly not an accurate method of recording, but it is quick and gives a good general picture of the condition.

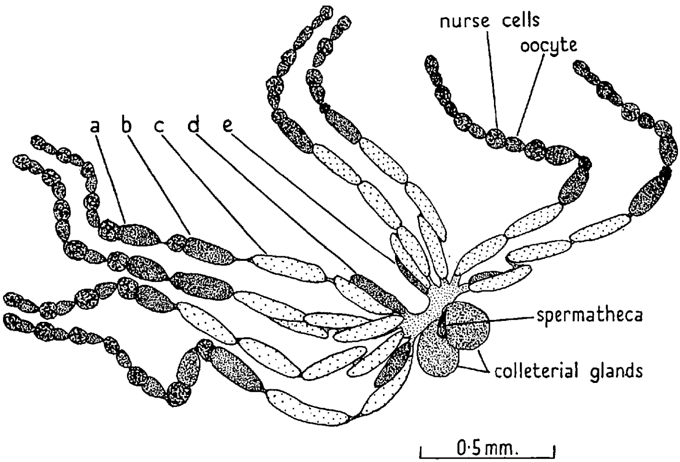

FIG. 1. The ovaries of *M. vitripennis* showing eggs at various stages of maturation and resorption. The distal part of each ovariole has been omitted (semi-diagrammatic).
a = Half-mature egg. *b* = Three-quarter-mature egg. *c* = Mature egg.
d = Egg in early stage of resorption. *e* = Egg at late stage of resorption.

The subdivisions were chosen arbitrarily before the work started and it was subsequently apparent that the difference between a 'half-mature egg' and a 'three-quarter-mature egg' was greater than that between a 'three-quarter-mature egg' and a mature one. When eggs are maturing rapidly the difference in time between a 'three-quarter-mature egg' and a mature egg is probably less than 1 hour, and a better estimate of the number of eggs available for oviposition is obtained if these two categories are lumped together. This has been done when calculating the egg maturation rates.

The ovaries of parasites fed on honey only

The condition of the ovaries of standard females deprived of hosts and fed only on honey, is shown in table 1. The first eggs mature when the imago is between 1 and 2 days old. Resorption begins at about 3 days and continues indefinitely. Eggs continue to mature at a low rate and there appears to be a slow cycle of maturation and resorption, the two processes just keeping pace with each other for the first 16 days so that the general condition of the ovaries remains more or less constant. The change in maturation rate during the first 3 days can be obtained from table 1 by observing the increase in the number of mature and three-quarter-mature eggs on consecutive days. The rates for the first 3 days are 9, 13, and 3 respectively. It is not possible to obtain an accurate estimate for longer than 3 days because there is no way of knowing how many eggs have been resorbed, but the rate is clearly not more than 1–2 per day (see fig. 2).

TABLE 1

The condition of the ovaries of standard-sized M. vitripennis *females deprived of hosts and fed only on honey*

Age in days	*Sample size*	*Mean condition of ovaries* *a b c d e*
0	4	0–0– *0*–0–0
1	13	8–9– *0*–0–0
2	31	7–4–*18*–0–0
3	6	7–1–*24*–1–0
4	6	5–0–*20*–1–2
5	6	4–1–*19*–0–1
6	6	2–2–*19*–2–2
7	7	2–0–*16*–1–2
8	7	3–2–*15*–1–1
9	6	3–1–*14*–0–2
10	12	5–1–*16*–2–1
12	7	4–2–*15*–2–1
16	6	2–1–*15*–3–2
20	3	3–1– *5*–2–3
22	1	1–1– *2*–0–2
28	2	1–0– *1*–0–3

a = No. of half-mature eggs. *b* = No. of three-quarter-mature eggs.
c = Mature eggs. *d* = Eggs in early stage of resorption.
e = Eggs in late stage of resorption.

The importance of this to the parasite is that at any time during that period a fairly large number of freshly matured eggs is always available. The number of immature eggs is small, however, so that once the supply of mature eggs has been exhausted, there will be an interval before more eggs are ready. After 16 days the condition of the ovaries rapidly deteriorates and by the end of 28 days the condition has become 1–0–*1*–0–3.

A noteworthy point is the extreme constancy of the ovaries of females aged 48 hours. In a batch of 31 females the mean number of mature and three-quarter-mature eggs together was 22 with a standard deviation of only 2·05. This fact has been utilized in the later experiments where it was desirable to know the number of eggs in the ovaries without dissecting the female.

The ovaries of starved parasites

If females are starved from eclosion the shortage of immature eggs is apparent at 48 hours, when the condition of the ovaries is 1–0–*16*–0–0. Resorption begins at 48–72 hours and by 5 days, a few hours before death, the condition has become 2–0–*3*–8–2. The situation in *M. vitripennis* appears to differ from that found by Grosch (1950) in *Habrobracon juglandis*, for he records that the mature eggs were resorbed by the sixth day, the immature ones by the ninth day, and death followed on the tenth day.

The ovaries of small parasites

The importance of standardizing the size of the females was shown by considering the ovaries of a batch of females which measured only 1·6 mm. as pupae. Table 2 shows that, although the general trend is similar, the ovaries never contain as many eggs as those of the larger females. This agrees with the findings of Jacobi (1939), who showed that the reproductive power of small *M. vitripennis* females is less than that of large ones.

TABLE 2

The condition of the ovaries of 'small' M. vitripennis *females deprived of hosts and fed only on honey*

Age in days	*Sample size*	*Mean condition of ovaries* *a b c d e*
0	2	0–0–*0*–0–0
1	4	2–2–*1*–0–0
2	2	2–3–*2*–0–0
3	4	1–1–*8*–0–0
5	6	1–0–*3*–0–0
7	2	2–0–*3*–1–1
8	2	1–1–*4*–0–2
12	4	1–1–*3*–0–1
16	4	0–1–*3*–1–1
20	3	0–0–*2*–0–1
24	4	0–0–*1*–0–1
30	1	0–0–*0*–0–0

a = No. of half-mature eggs. *b* = No. of three-quarter-mature eggs.
c = No. of mature eggs. *d* = No. of eggs in early stage of resorption.
e = No. of eggs in late stage of resorption.

The ovaries of parasites fed on host blood

For these experiments newly eclosed females were first fed on honey for 48 hours, and then confined in a 3-inch by 1-inch tube with an ample supply of puparia for periods of between 1 and 5 days. Females of this type were chosen because (1) the condition of the ovaries could be predicted (as shown above), and (2) parasites less than 36 hours old display little interest in puparia. After the experiments the females were immediately dissected, but the puparia were kept at 25° C. until the unparasitized hosts had emerged. The remaining hosts were then opened to discover whether they had been stung by the parasite or had died from natural causes. If the host had been stung, the number of parasite progeny was counted and recorded. There is no polyembryony in *M. vitripennis*, and as a test batch of fifty hosts showed that egg mortality is negligible, the number of progeny can be considered equal to the number of eggs laid.

TABLE 3

The conditions of the ovaries of standard-sized M. vitripennis *females which were fed on honey only for the first 48 hours after eclosion and then given host blood for the number of days shown.*

Age in days	*Duration of blood meal in days*	*Sample size*	*Mean condition of ovaries* a b c d e	*Mean number of eggs laid during period*
3	1	14	12– 8– *8*–0–0	21
4	2	14	13–12–*10*–0–0	50
5	3	3	16–22– *8*–0–0	144
6	4	3	10–20–*13*–0–0	199
7	5	3	15–19–*19*–0–0	250

a = No. of half-mature eggs. *b* = No. of three-quarter-mature eggs.
c = No. of mature eggs. *d* = No. of eggs in early stage of resorption.
e = No. of eggs in late stage of resorption.

The average condition of the ovaries at the end of the experiment is shown in table 3. The most noticeable difference between these and the ovaries of females fed on honey only is in the large number of immature eggs present. The number of mature eggs is, of course, not large as the parasites have been ovipositing continuously. The mean number of eggs laid during the experiments is given in column 5. Although the samples were small, an estimate of the maturation rates can be obtained by adding the number of eggs laid during the experiment to the number in the ovaries after the experiment, and subtracting 22—the number in the ovaries at the start of the experiment. Mature eggs and three-quarter-mature eggs are lumped together here for the reason given previously. From these figures, which give the number of eggs maturing over the whole period, the daily rate can be obtained. This is a better method than giving the parasites a fresh batch of hosts every day and counting the number of eggs laid, partly because of the danger of disturbing the parasite and partly because it would not be possible to take into account the number of eggs still in the ovaries at the end of each day.

The mean daily estimates are shown graphically in fig. 2. It is seen that when the parasites are fed on host blood the maturation rate rises to a peak of

102 on the third day and then drops to little more than half that figure on the two following days. In the same way the mean daily number of hosts stung by the parasites can be estimated, and it is found that these figures also rise rapidly from 2·4 on the first day, to a peak of 12·2 on the third day, dropping again to 9·0 by the fifth day.

These results are in broad agreement with those of previous authors. Smirnov and Kuzina (1933) record that one *M. vitripennis* female stung 98 *Calliphora* puparia in 12 days, and produced 745 offspring—a mean oviposition

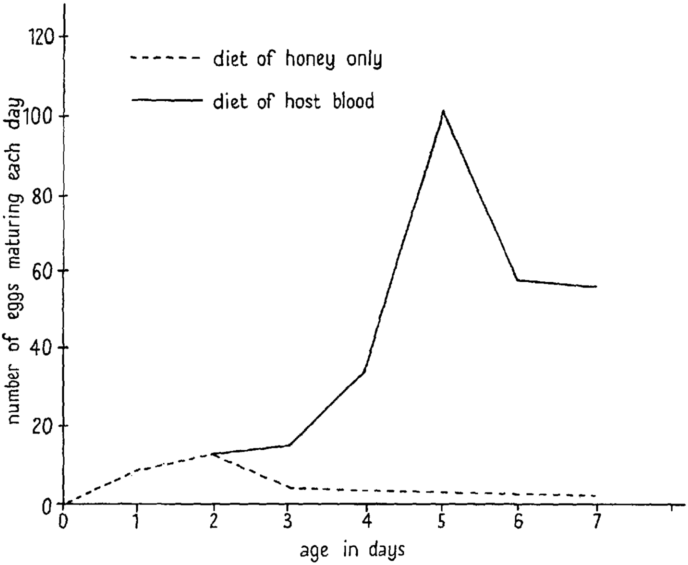

FIG. 2. The effect of diet on the egg maturation rate of *M. vitripennis*; a comparison between the daily egg maturation rates of females which have been (*a*) fed on honey only, and (*b*) allowed to feed on host blood after a diet of honey for 2 days.

rate of over 60 per day. Van der Merwe (1943), using *Lucilia* puparia as hosts, gives the mean oviposition rate as 50 per day. Cousin (1933), who gives the average as only 15–17 per day, limited the parasites to two *Lucilia* puparia daily. None of these workers appears to have standardized the size of the females.

The effect of host scarcity

A situation likely to arise in nature is that in which a parasite finds a number of hosts, oviposits, and feeds, and is then unable to find more for several days.

It is interesting to consider what happens to the ovaries under these circumstances. Newly eclosed females were fed on honey for 48 hours, given ample hosts for the next 48 hours, and then starved. No female survived more than 48 hours' starvation and dissections showed that resorption was proceeding very rapidly whilst few eggs were maturing (see table 4).

TABLE 4

The condition of the ovaries of standard-sized M. vitripennis *females which had been given honey for the first 48 hours after eclosion, host blood for the next 48 hours, and finally starved.*

Age in days	*Period of starvation in hours*	*Sample size*	*Mean condition of ovaries* *a b c d e*
4·0	0	14	13–12–10– 0–0
4·7	16	2	3– 3–25– 7–1
5·0	24	5	8– 0–34– 3–1
5·7	40	7	3– 0– 5–10–4

a = No. of half-mature eggs. *b* = No. of three-quarter-mature eggs.
c = No. of mature eggs. *d* = No. of eggs in early stage of resorption.
e = No. of eggs in late stage of resorption.

TABLE 5

The condition of the ovaries of standard-sized M. vitripennis *females which had been given honey for the first 48 hours after eclosion, host blood for the next 48 hours, and then returned to a diet of honey*

Age in days	*Duration of second honey-meal in days*	*Sample size*	*Mean condition of ovaries* *a b c d e*
4·0	0	14	13–12–10–0–0
4·7	0·7	3	4– 5–33–3–0
5·0	1·0	3	3– 1–46–0–1
6·0	2·0	4	5– 1–39–1–2
8·0	4·0	2	3– 1–40–3–1
9·0	5·0	2	2– 1–37–3–2
12·0	8·0	2	2– 1–23–1–2

a = No. of half-mature eggs. *b* = No. of three-quarter-mature eggs.
c = No. of mature eggs. *d* = No. of eggs in early stage of resorption.
e = No. of eggs in late stage of resorption.

The rapid death of the females under these circumstances as compared with their survival for 5 days when starved from eclosion is presumably linked with the size of the fat-body. Newly eclosed females have a large reserve of fat which disappears after 4–5 days.

In nature the parasites might be expected to feed on nectar or honey-dew if hosts were scarce, and the effect of this can be demonstrated by providing them with honey. Standard-sized females after being fed on honey for 48 hours were given ample hosts for 48 hours and then returned to a diet of honey only. They lived for at least 8 days—the experiments were not continued longer—and during most of that time the number of mature eggs did not fall greatly. A slow cycle of maturation and resorption occurred and the general condition of the ovaries was little changed at least for the first 5 days (see table 5).

It appears that under starvation conditions the eggs are resorbed and their contents used in general body metabolism. If honey is available for this general metabolism the parasites can live for a considerable period and maintain a large number of eggs in the ovaries. The importance of this to a parasite whose host is found in isolated groups is obvious.

Discussion

The method of rearing has a very great effect on the condition of the ovaries of *M. vitripennis* and this in turn will affect the behaviour pattern. A detailed correlation between the ovary condition and the behaviour pattern has not been carried out, but it has been observed that females which have been deprived of hosts for 5 days are unable to parasitize more than 1 or 2 hosts in the next 36 hours whereas those which have had ample hosts parasitize 12–15 in the same time. Presumably after the mature eggs in the ovaries have been deposited there is a delay whilst others are maturing.

If a female *M. vitripennis* is dissected soon after feeding on host blood the mid-gut is distended with a pale straw-coloured fluid. As digestion proceeds the colour gradually darkens until it becomes black at the end of 48 hours. If fed on pure sucrose the crop becomes distended whilst the mid-gut remains flaccid and does not darken. An interpretation of this could be that carbohydrates are digested in the crop, but host blood, and probably all proteins, are digested in the mid-gut. Females fed on honey showed a slight discoloration of the mid-gut after 3 weeks. This does not occur when females are fed on pure sucrose, and may indicate that the small amount of protein in the honey was being used for egg production. This could be checked by making a comparison with the condition of the ovaries of females which had been fed on pure sucrose only. Under natural conditions, however, females feeding on nectar or honey-dew would also take in small quantities of protein which might have the same effect as the protein in honey.

An egg which has been resorbed leaves no trace in the ovariole, so that the number of eggs being resorbed at any one time bears no relation to the number of eggs already resorbed. Flanders (1942*a*) states that in *Encyrtus fuliginosus* the complete exochorion is extruded into the body cavity and that 10–100 of these may be found free in the body fluids. During the present work no trace of exochorions of resorbed eggs could be found in the body fluid. As the exochorion in this species is smooth and transparent it may be difficult to find; on the other hand, careful scrutiny of stained preparations of ovaries shows that the egg loses its shape and the chorion apparently disappears at an early stage of resorption.

Flanders (1942*b*) found that some non-viable eggs are laid when a parasite has been deprived of hosts. He suggests that these are eggs in the early stages of resorption in which the chorion is still tough enough to withstand the passage through the ovipositor. Partly deflated eggs of *M. vitripennis* have occasionally been found on a host, but it has been impossible to decide whether these were in the early stage of resorption, or whether they had been damaged during oviposition.

No attempt has been made to determine the exact process by which egg resorption occurs. The contents are not taken back by the nurse cells, as these have disappeared; it is not initiated by special cells in the wall of the ovariole because often a mature and a resorbing egg are found lying side by side; it seems likely that a change in the structure of the egg starts the process. No attempt has been made to discover how this is affected by the general condition of the insect, but work along these lines is continuing.

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REFERENCES

Cousin, G., 1933. Bull. Biol., **67**, 371.
Flanders, S. E., 1935. Ann. ent. Soc. Amer., **28**, 438.
—— 1942*a*. Ibid., **35**, 251.
—— 1942*b*. J. econ. Ent., **35**, 283.
Grosch, D. S., 1950. Biol. Bull. Wood's Hole., **99**, 65.
Jacobi, E. F., 1939. Arch. nèerl. Zool., **3**, 197.
Merwe, J. S. van der, 1943. J. ent. Soc. S. Afr., **6**, 48.
Roubaud, E., 1917. Bull. Biol., **50**, 425.
Smirnov, E., and Kuzina, O., 1933. Zool. J., **12**, 96.