THE INDUCTION AND TERMINATION OF FACULTATIVE DIAPAUSE IN THE CHALCID WASPS *MORMONIELLA VITRIPENNIS* (WALKER) AND *TRITNEPTIS KLUGII* (RATZEBURG)*

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Many insects exhibit a facultative diapause which is under the control of specific environmental factors. When the insect enters diapause growth and development cease. The end of diapause is signalled by the resumption of development. In most instances the environmental agencies that cause diapause affect the generation that will enter diapause, but occasionally a stimulus applied to the maternal generation affects the post-embryonic stages of the progeny. Commonly in facultative diapause, normal growth proceeds for several moults after the application of the stimulus, and development ceases only at a later stage. Whatever the mechanism which finally prevents moulting and produces diapause, it does not interfere with intermediate moults. The present report defines the extrinsic factors that cause facultative diapause in the last larval instar of two parasitic wasps and analyses in detail the mechanism of diapause termination.

MATERIALS AND METHOD

(1) *Experimental animals*

Mormoniella vitripennis (Walker) (=*Nasonia brevicornis* Ashmead) and *Tritneptis klugii* (Ratzeburg) (=*Caelopisthia nematicida*) are closely related chalcid wasps in the family Pteromalidae. Both have a facultative larval diapause (Girault & Saunders, 1909; Graham-Smith, 1919; Altson, 1920; Cousin, 1932; Evans, 1933; Roberts, 1935; Van der Merwe, 1945; Moursi, 1946*b* (*Mormoniella*); Hewitt, 1912 (*Tritneptis*)). *Mormoniella* has been the subject of considerable genetic (e.g. Whiting, 1949, 1955*a–d*) and some physiological study (e.g. Jacobi, 1939; Moursi, 1946*a*, *b*; Schneiderman & Williams, 1952; Schneiderman, Horwitz & Kurland, 1956; Schneiderman, Kuten & Horwitz, 1956; Ray, Hunter & Stephens, 1954; Ferschl & Wolsky, 1956). Its post-embryonic development has been described in great detail by Tiegs (1922) and its general biology by various workers (Girault & Saunders, 1910; Roubaud, 1917; Parker, 1924; Parker & Thompson, 1928; Cousin, 1933; Fukuda, 1939). *Tritneptis* is currently used to control saw-fly damage to

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spruce trees in Northern Ontario, but little is known of its biology (Hewitt, 1912). Both wasps are external parasites upon other insects, are easily reared in the laboratory, and at room temperature have a life cycle of about 2 weeks. They are relatively flightless and can be handled with a fine camel-hair brush. *Mormoniella* can be reared at temperatures from 15 to 30° C. whereas *Tritneptis* thrives only below 30° C.

The life history of *Mormoniella* is typical of both wasps and is briefly described below. The female is parasitic on pupae of muscoid Diptera like the flesh-fly *Sarcophaga bullata* (Parker). After piercing the puparium she lays her eggs on the developing fly pupa. One to three days later the egg hatches and the first-instar larva begins feeding as an ectoparasite on the fly pupa. The larva feeds and moults four times (Van der Merwe, 1945) during the next 3 days, finally ceases feeding and enters a resting stage which persists for about 24 hr. The larval tissues then begin to break down and simultaneously the imaginal disks proliferate. Only a few cell divisions are necessary to break down the thin partition between the mid-gut and the invaginated rectum, and defaecation next occurs. The pre-defaecating larva is greyish in colour but becomes white when faecal material is voided. Defaecation marks the end of the resting stage. The short post-defaecation period (20 hr.) is occupied by the great cellular activity of prepupal development and terminates with the pupal moult. Within 12 hr. the newly moulted white pupa becomes pinkish in colour. Epidermal differentiation and pigmentation then proceed for about 5 days, whereupon the fully formed adult wasp resorbs its moulting fluid and emerges. The time sequence for the development of *Mormoniella* at 25° C. from egg to adult is presented in Table 1, and typical developmental stages are illustrated in Fig. 1. At room temperature the adult will live without food for about 6 days. Females supplied with fly puparia survive about twice as long, feeding on the pupal fluids. Eggs are laid from the second day after adult emergence until death.

Table 1. *Time-table for the development of* Mormoniella *at* 25° C.

Day	Stage
0	Egg
2–3	First-instar larva
5–6	Last-instar larva
6–7	Larval defaecation
8	White pupa
9	Pink pupa
10	Red eyes
11	Red-brown eyes
12	Black head and thorax
13	All black
14	Adult emergence

The life history and development of *Tritneptis*, which is summarized in Table 2, follows a similar pattern except that the hosts are saw-fly prepupae.

When diapause occurs in either species it is manifest at the end of the last larval instar, after the feeding period has ended and just prior to defaecation. The diapausing larva differs from the normal larva in appearance. The fat-body becomes

semi-transparent (Van der Merwe, 1945) and, as first pointed out by Whiting (personal communication), the cuticle acquires a sticky waxy coating. But the truly diagnostic feature of the diapausing larva is that it does not immediately moult into a pupa. These diapausing larvae will survive for many months at room temperature. In the case of *Mormoniella*, diapausing larvae kept at room temperature for

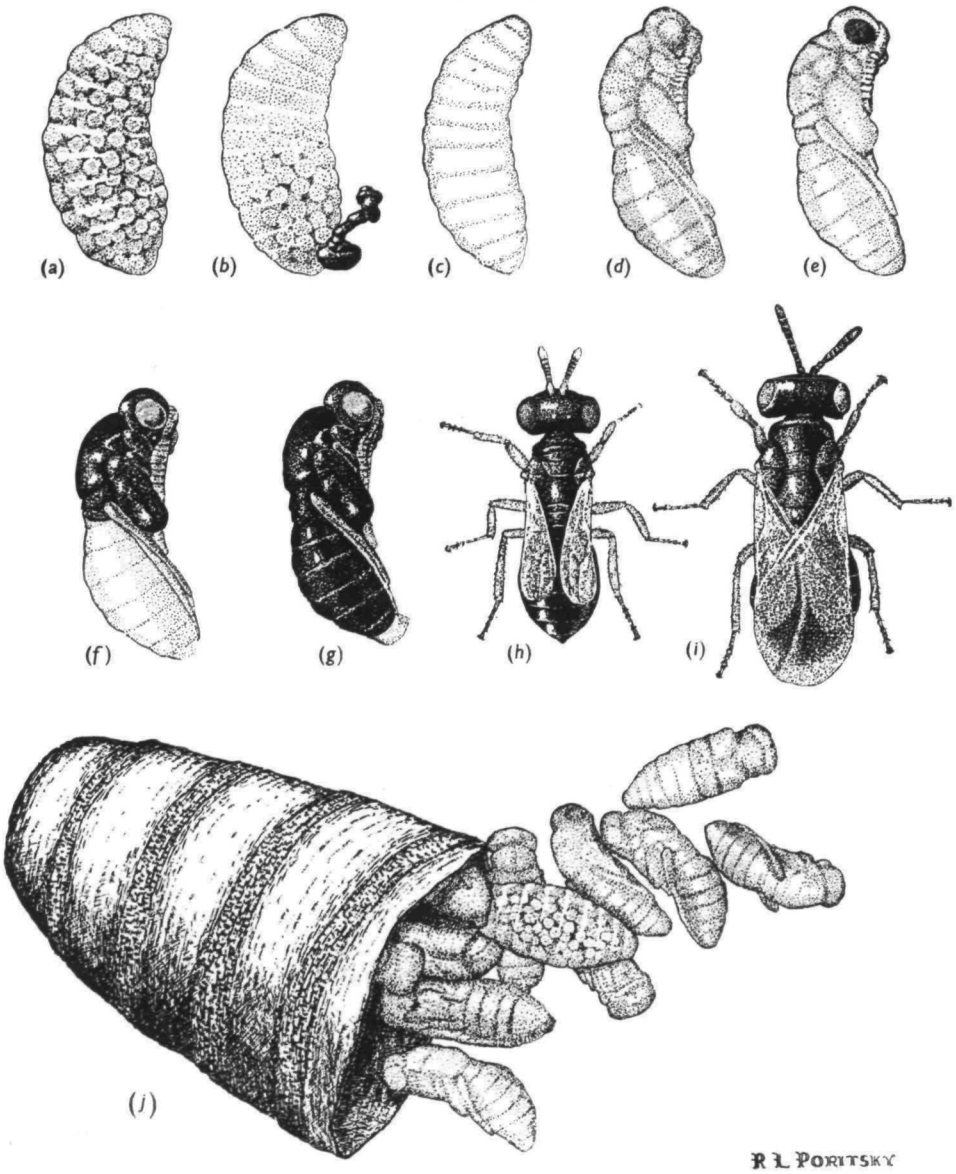

Fig. 1. Stages in the development of *Mormoniella*. (*a*) Diapausing larva. (*b*) Defaecating larva. (*c*) Early prepupa. (*d*) Pink pupa. (*e*) Red eyes. (*f*) Black head and thorax. (*g*) All black. (*h*) Adult male. (*i*) Adult female. (*j*) *Sarcophaga* puparium broken open to reveal enclosed diapausing larvae and pupae. The size of the larva is 2·2 mm.

more than 2 years have been capable of developing into normal adult wasps when appropriately stimulated. Either larval defaecation or the pupal moult may serve as convenient end-points for diapause, although other changes such as wrinkling of the larval cuticle are evident even earlier.

Table 2. *Time-table for the development of* Tritneptis *at* 25° C.

Day	Stage
0	Egg
1–2	First-instar larva
8–9	Larval defaecation
10	Orange pupa
11	Red eyes
12	Brown eyes
13	Black head and thorax
14	Striped abdomen
15	All black
16	Adult emergence

Except where specified, an inbred strain of *Mormoniella*, 'Woods Hole wild-type',* and a wild strain of *Tritneptis*† were used. In a few experiments a strain of *Mormoniella* collected locally, 'Ithaca wild-type', and several mutant strains were employed.

(2) *Hosts*

The flesh-fly *Sarcophaga bullata* (Parker) served as host for *Mormoniella*. Flies were raised on raw hamburger or brewers' yeast and powdered milk, permitted to develop at room temperature for 4–5 days after pupation and then stored at 6° C. Animals treated in this manner can emerge after several months of storage and the puparia can be used for host material for a year.

Tritneptis, a parasite of saw-flies, was raised upon the larch saw-fly, *Neodiprion lecontei*.† *Lecontei* saw-flies also enter diapause, and the diapausing prepupa encased in a tough cocoon normally served as host material. These cocoons may be stored at temperatures as high as 6° C., but pupation occurs after several months and storage at a temperature of 2° C. is preferable. An attempt was made to raise *Tritneptis* on *Sarcophaga*. The wasps managed to parasitize the fly puparia, and while occasionally an individual completed its development it was stunted and failed to produce another generation on *Sarcophaga* (cf. Cousin, 1933, for *Mormoniella* on *Galleria*).

(3) *Rearing conditions*

Wasps were reared in cotton-plugged vials. All vials were kept in opaque cardboard containers, unless specifically stated otherwise, and stored in incubators regulated to within 1° C. and maintained at 70% humidity. In a few experiments where more precise thermo-regulation was required a water-bath was employed and the vials were placed in large cotton-plugged opaque flasks.

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(4) *Procedure for anaerobic experiments*

In several experiments both adults and diapausing animals were kept without oxygen for specific periods. Quarter-litre flasks furnished with a side-arm and two stop-cocks were used. Animals were enclosed in the flask to which was added 25 ml. of a powerful oxygen absorbent (Fieser, 1924). The flasks were then promptly flushed with nitrogen (99·9%) and sealed. In all but a few experiments a small vial containing a saturated solution of lead acetate and a fluted filter paper were added to absorb the small amounts of hydrogen sulphide liberated by the Fieser solution.

THE INDUCTION OF DIAPAUSE IN *MORMONIELLA*

Both in nature (Altson, 1920) and in mass laboratory cultures at 25° C. a persistent but irregular proportion of *Mormoniella* larvae enter diapause. In individual matings at 25° C. a similar irregular incidence of diapause is commonly found. A single female may produce both diapausing and non-diapausing offspring in the same puparium, or sometimes only one or the other. During the past 6 years experiments were conducted with the offspring of more than 5000 individual females to uncover factors that increase or decrease the incidence of this facultative diapause. Studies were made of the effects on the offspring of nutrition, temperature, humidity, photoperiod, etc. These extrinsic factors, as well as the intrinsic ones of heredity, age, size, etc., were also appraised in the maternal generation, in order to determine the presence of a maternal influence. This report summarizes the principal findings.

(1) *Factors affecting the larval generation*

(A) *Superparasitization*

It had been suggested by Cousin (1932) that successive parasitization of the same host puparium caused developing larvae of *Mormoniella* to enter diapause because of the reduced food supply available to them. This seemed unlikely because of the frequent occurrence of puparia containing only two or three plump diapausing larvae and no other individuals, i.e. diapause amidst ample food. Nevertheless, the possibility was tested by parasitizing individual puparia on 3 successive days with wild-type *Mormoniella* and two eye-colour mutants, oyster-eye and orange-eye (*oy* and *or*—336·2 of Whiting, 1955*b*). The experiment was conducted at 30° C. No diapausing larvae were produced, although many puparia had been superparasitized and yielded all three kinds of adults.

(B) *Age and condition of host*

In some hymenopterous parasites diapause is thought to depend upon the stage of development of the host at the time of parasitization (Flanders, 1944). This is not the case in *Mormoniella*, for the incidence of diapause was not affected by the age of the developing fly within the puparium, nor by the length of time the puparium had been stored in the cold. Duplicate experiments were conducted at 25 and

30° C., and the same proportion of diapause occurred in fresh puparia of various ages and in puparia which had been stored at 6° C. for almost a year.

In an effort to decrease the nutritional value of the host, puparia were damaged by puncturing the head region and the injuries sealed with paraffin to reduce desiccation. Although the punctured pupae soon died they could commonly be parasitized for at least 1 day after damage. Few wasps developed within each punctured puparium because of rapid desiccation and decay, but those surviving on this obviously poor host material showed no increased tendency to enter diapause.

(C) *Humidity and photoperiod*

Parasitized puparia were placed at relative humidities varying from about zero ($CaSO_4$) to nearly 100% (water) at 25 and 30° C. The incidence of diapause was the same at all humidities tested. The exposure of parasitized puparia to varying photoperiods also failed to affect the frequency of diapause.

(D) *Temperature*

Puparia at various times after parasitization at 25 and 30° C. were kept at 15, 20, 25 or 30° C. The rates of development naturally varied with the temperatures, but the incidence of diapause was the same at all temperatures. Thus the temperature at which larval development took place did not affect the incidence of the diapause.

In further experiments several extrinsic factors were varied simultaneously, e.g. temperature, humidity, etc., without effect. Since the extrinsic conditions to which larvae were exposed failed to affect the incidence of diapause, attention focused on the maternal generation.

(2) *Factors affecting the maternal generation*

(A) *Intrinsic factors*

Careful analysis of the frequency of diapause in the offspring of females during their entire life at several temperatures revealed no correlation between the age of the female and the incidence of diapause. Fecundity was similarly studied and there was no difference in the frequency of diapause offspring between highly fecund and almost infertile females.

The significance of size was next examined to determine whether larger females produced a different proportion of diapausing offspring than smaller females. Animals were divided into two groups: those measuring 2·3–2·6 mm. in length and those measuring 1·8–2·0 mm. The proportion of diapause in the offspring of these two groups was not significantly different. Finally, an effort was made to select out a diapause-producing strain, but this too proved fruitless. Diapause was evidently phenotypic (but see Discussion for experiment of Moursi (1946*b*)). Our attention then turned to the effects of extrinsic agencies on the maternal generation.

(B) *Host deprivation*

In addition to ovipositing on *Sarcophaga*, female *Mormoniella* commonly feed on the pupal juices through punctures made with their ovipositors. Depriving

females of hosts thus results in both starvation and possibly also oösorption (Flanders, 1944) and reduces by half their normal life span of about 2 weeks at 25–30° C. In order to ascertain the effects of host deprivation on the incidence of diapausing progeny, the following experiment was carried out at 30° C. Into each of twenty small vials were placed a newly emerged male and female *Mormoniella*. Two *Sarcophaga* pupae were added to each of five vials after 1, 2, 3 or 5 days, and daily thereafter until the twelfth day.

Table 3. *The effect of various periods of host deprivation on the incidence of diapause in the progeny of* Mormoniella *females maintained at* 30° C.

Days of deprivation	Total offspring	% diapause
1	1786	0
2	2125	34·6
3	1992	67·1
5	1866	97·3

The data summarized in Table 3 reveal a correlation between host deprivation and the incidence of diapause. The first group showed no diapause. In the group that was deprived for 2 days one animal produced only diapause offspring, three produced only developing animals; one showed an intermediate condition producing both developing and diapausing offspring on the first day and thereafter only diapausing ones. Females deprived of hosts for 3 days produced a still greater proportion of diapause offspring, and females deprived for 5 days produced nearly 100 % diapausing progeny for the duration of the experiment.

In this experiment 5 days without food caused a profound change in the physiology of the female as measured by the frequency of diapause in her offspring. In subsequent experiments the results were not so clear-cut, but there was, nevertheless, a strong tendency for starved females to produce more diapausing offspring.

(C) *Chilling the female*

Newly emerged males and females from a continuously breeding population were placed in pairs in small vials; five pairs were stored at 25° C. throughout and five pairs were exposed to 10° C. for 5 days and then stored at 25° C. Puparia were present throughout the experiment but no oviposition or feeding took place at 10° C. Puparia were collected for about a week at 25° C. In this experiment, in the group exposed to 10° C. prior to oviposition, all of the progeny produced at 25° C. entered diapause, while in a group kept continuously at 25° C., 30% of the animals developed without arrest. A second experiment was performed in identical fashion with newly emerged animals which had been in diapause for more than 100 weeks and then permitted to develop at 25° C. The wasps reared at 25° C. throughout yielded no diapause offspring, while the wasps chilled for 5 days and then returned to 25° C. produced 21% diapause offspring. These two experiments suggest that chilling a female enhances the chance that her progeny will enter

diapause. To be sure, there are large differences in the proportion of diapause between the experiments, but in these experiments and in numerous others like them, chilling of the females invariably led to a marked and significant increase in the incidence of diapause in their offspring.

More detailed experiments were conducted to analyse this chilling effect and to determine what developmental stage of the female was sensitive to low temperature. To this end, animals at the following developmental stages (see Table 1) were placed at 10° C. for 5 days: red-brown eye pupae, black head and thorax pupae, black pupae, newly emerged adults, 1-day-old adults, 2-day-old adults. Each group consisted of six vials with one mating pair per vial. After the 5-day period of chilling five vials from each group were placed at 30° C. and the sixth at 25° C. Two *Sarcophaga* puparia were added to each vial daily. The results recorded in Table 4 reveal that chilling had no effect upon red-brown eye pupae or upon pupae at the stage of black head and thorax: all of their offspring developed into adults without the intervention of a diapause. The group of all black pupae also produced only developing progeny, with the exception of one female, which was observed to have emerged as an adult before the group was removed from the low temperature. This animal first gave rise to both diapausing and developing progeny and then produced diapausing progeny exclusively. This foreshadowed the results in the next group, newly emerged adults, where all of the progeny produced immediately after chilling entered diapause. In three of the four fertile animals, the chilling effect began to wear off after several days: that is, they produced developing animals after a period of producing only diapausing ones. Similar results were obtained with the 1-day-old and 2-day-old adults. Table 5 records the fate of the animals placed at 25° C. instead of 30° C. after chilling. The results are substantially the same as those of Table 4, although confused somewhat by a high spontaneous incidence of diapause at 25° C., which will be considered presently.

The temporary nature of the chilling effect was clearly seen in numerous experiments in which females were permitted to oviposit at 30° C. for several days, exposed to 10° C. for 5 days and then returned to 30° C. for further oviposition. Such females produced no diapausing offspring, or only a few, before chilling. After chilling they produced many diapausing offspring for several days and then returned to producing developing offspring. Control experiments showed that low temperature and not simply change of temperature was important.

In addition to the clear effects of chilling adults in inducing diapause, comparison of the 25 and 30° C. groups (Tables 4 and 5) showed that oviposition and rearing at 30° C. suppressed the normal 'spontaneous' incidence of diapause at 25° C. This fact was confirmed in the following experiments.

Thirty mating pairs, which had developed at 25° C. until emergence, were stored individually at 15° C. where they were permitted to oviposit daily. Each day three groups of ten parasitized puparia were taken; one group was stored at 15° C., another group was stored at 25° C. and another at 30° C. The data recorded in Table 6 reveal the same results at all three temperatures: oviposition at 15° C. caused almost all the progeny to enter diapause. The immediate removal of the eggs

Table 4. *The effect of chilling thirty* Mormoniella *females at various stages of adult development for 5 days at 10° C. on the incidence of diapause in progeny oviposited and reared at 30° C.*

Days	Number and kind of progeny*									
	Red-brown eye stage					Black head and thorax stage				
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	34*a*	0	42*a*	35*a*	0
3	35*a*	0	40*a*	0	26*a*	37*a*	•	60*a*	59*a*	0
4	28*a*	63*a*	45*a*	0	37*a*	31*a*	49*a*	62*a*	72*a*	38*a*
5	36*a*	41*a*	36*a*	0	42*a*	57*a*	61*a*	40*a*	64*a*	21*a*
6	39*a*	55*a*	24*a*	0	49*a*	71*a*	36*a*	52*a*	57*a*	48*a*
7	44*a*	56*a*	37*a*	0	58*a*	54*a*	20*a*	58*a*	56*a*	0
8	34*a*	0	7*a*	0	0	39*a*	30*a*	47*a*	49*a*	0
9	45*a*	0	11*a*	0	0	46*a*	58*a*	55*a*	40*a*	0
	Total offspring, 888; % diapause, 0					Total offspring, 1578; % diapause, 0				
Days	All black stage					Newly emerged adults				
1	0	0	0	0	0	0	0	0	0	0
2	30*a*	0	41*d*; 11*a*	0	0	0	0	0	57*d*	0
3	71*a*	0	44*d*	57*a*	0	49*d*	64*d*	52*d*	58*d*	0
4	32*a*	0	63*d*	83*a*	0	43*d*	67*d*	51*d*	60*d*	0
5	23*a*	30*a*	40*d*	53*a*	41*a*	44*d*	53*d*	49*d*	46*d*	0
6	58*a*	55*a*	49*d*	52*a*	50*a*	0	66*d*	54*d*	40*d*	0
7	0	56*a*	41*d*	0	53*a*	50*d*	49*d*	56*d*	11*d*; 10*a*	0
8	53*a*	49*a*	53*d*	47*a*	42*a*	59*d*	50*d*; 12*a*	19*a*	30*a*	0
9	52*a*	42*a*	0	0	40*a*	49*d*	32*d*; 20*a*	6*a*	32*a*	0
	Total offspring, 1411; % diapause, 23·5					Total offspring, 1338; % diapause, 90·4				
Days	1-day-old adults					2-day-old adults				
1	0	27*d*	21*d*; 30*a*	32*d*	40*d*	0	47*d*	0	32*d*	43*d*
2	30*d*	40*d*	52*a*	47*d*	47*d*	45*d*	50*d*	0	17*d*	72*d*
3	49*d*	60*d*	42*a*	52*d*	43*d*	38*d*	57*d*	0	54*d*	67*d*
4	28*d*	23*d*	59*a*	31*d*	37*d*	31*d*	59*d*	0	64*d*	59*d*
5	33*d*	47*d*	55*a*	32*d*	52*d*	66*d*	16*a*; 43*d*	0	65*d*	57*d*
6	0	50*d*	62*a*	65*d*	38*d*	52*d*	60*a*	0	24*d*	55*d*
7	40*d*; 3*a*	48*d*	65*a*	51*d*	33*d*	23*d*	51*a*	0	25*d*	48*d*
8	32*d*; 8*a*	37*d*; 18*a*	61*a*	54*d*	29*d*; 5*a*	27*d*	42*d*; 10*a*	0	47*d*	53*d*
9	31*d*; 20*a*	42*d*; 10*a*	0	42*d*	25*d*; 7*a*	22*d*	32*d*; 11*a*	0	52*d*	50*d*; 20*a*
	Total offspring, 1885; % diapause, 72·5					Total offspring, 1686; % diapause, 90·0				

* *a*, non-diapausing progeny that developed without arrest; *d*, diapausing progeny.

for development at higher temperatures failed to influence the subsequent development of these eggs. Once destined for diapause, even 30° C. failed to override the effect of chilling. Chilling may cause only a temporary change in the maternal physiology, but the resulting effect on the egg is apparently an irreversible one. Parallel experiments showed that females that were raised at 25° C. and that oviposited at 30° C. produced eggs that gave rise almost exclusively to developing offspring, regardless of the temperature at which the eggs were incubated. Clearly, low temperature acts on the female and not on the developing offspring.

Table 5. *The effect of chilling six* Mormoniella *females at various stages of adult development for 5 days at 10° C. on the incidence of diapause in progeny oviposited and reared at 25° C.*

	Number and kind of progeny*					
Days	Red-brown eye stage	Black head and thorax stage	All black stage	Newly emerged adult	1-day-old adult	2-day-old adult
1	0	0	0	0	46*d*	41*d*
2	22*a*	47*a*	14*d*; 52*a*	25*d*	35*d*	26*d*
3	52*a*	59*a*	10*d*; 12*a*	0	58*d*	42*d*
4	41*a*	57*a*	40*d*; 21*a*	41*d*	15*d*	30*d*
5	35*a*	16*d*; 42*a*	16*d*; 43*a*	55*d*	50*d*	44*d*
6	65*a*	19*d*; 41*a*	0	29*d*	10*d*	0
7	0	25*d*; 40*a*	0	12*d*	0	48*d*
8	25*d*; 62*a*	27*d*; 54*a*	0	50*d*	0	0
9	15*d*; 56*a*	14*d*; 37*a*	0	0	30*d*; 37*a*	0
10	5*d*; 66*a*	12*d*; 38*a*	0	0	0	0
Total off-spring	444	528	208	212	281	231
% diapause	10·1	21·4	38·5	100	86·8	100

* *a*, non-diapausing progeny that developed without arrest; *d*, diapausing progeny.

Table 6. *The effect of exposing females to 15° C. during oviposition on the incidence of diapause in progeny reared at various temperatures*

	Temperature at which progeny were reared		
	15° C.	25° C.	30° C.
Total offspring	4140	2875	2276
% diapause	88·2	100	99·3

These several experiments with temperature have been repeated many times with varying techniques, but with substantially the same results: (*a*) exposing adult females to low temperatures greatly enhances the chance that their progeny will enter diapause; (*b*) the incidence of diapause is greater in the progeny of females ovipositing at lower temperatures; (*c*) the effect of low temperature on the

female wears off after a time. The common factor in these experiments appears to be the temperature at which oögenesis takes place; when oögenesis occurs at low temperature, diapause is more likely in the offspring.

(D) *Humidity, photoperiod, social factors*

Although low temperature was an exceedingly important factor in inducing diapause, it might not be the only factor, as was revealed by a careful study of offspring of several hundred females at low temperatures. Occasionally, even in cultures reared at 30° C., an outbreak of spontaneous diapause occurred. Likewise, in cultures reared at 15° C. large numbers of developing adults were sporadically produced. These exceptions led us to suspect that factors other than temperature and nutrition might be involved. Without describing numerous fruitless experiments, suffice it to say that many environmental factors were studied including humidity, photoperiod, as well as social factors, such as population density, numbers of puparia, crowding, etc. No additional combination of factors that increased or decreased the incidence of diapause was disclosed. On the basis of our present data it seems safe to conclude that the temperature history of the adult female is clearly the most critical factor in the induction of facultative diapause in *Mormoniella*, but that other factors probably operate.

THE INDUCTION OF DIAPAUSE IN *TRITNEPTIS*

(1) *Effects of temperature*

The discovery of a successful means of inducing diapause in *Mormoniella* led us to similar experiments with *Tritneptis*. Adults which had emerged at 25° C. were placed at 15, 20 and 25° C. with several *lecontei* cocoons upon which they oviposited. After the parasites had completed their development the cocoons were opened. All of the offspring in the 25° C. group developed promptly into adults, while 99% of the offspring in the 15° C. group entered diapause. The 20° C. group yielded both kinds of animals. The results of this experiment were clear enough except that they did not discriminate between the influence of low temperature on the parent and on the developing larva. Either or both might have responded to the stimulus of low temperature. The following experiment resolved this question. Promptly upon emergence, mating pairs reared at 25° C. were placed in vials at 15 and 25° C. and given cocoons periodically. Immediately after parasitization, cocoons from each group were placed at both 15 and 25° C., where the parasites completed their development. None of the parasites which were placed at 25° C. to complete their development entered diapause, while nearly all of the animals from cocoons which were placed at 15° C. entered diapause. The temperature at which the parents had been raised was of no significance. Low temperature did not act on the parents, but on their offspring: when the larvae developed at low temperature they usually entered diapause; when they developed at high temperatures they never did. Identical results were obtained with adults which had been reared at 15° C. The next experiment was designed to determine

how long an exposure to low temperature was necessary and what stages of egg or larval development were sensitive.

Isolated mating pairs which had been reared at 25° C. were given a fresh cocoon daily and kept at 25° C. At intervals of 1, 3 or 5 days after oviposition these cocoons were placed at 15° C. They remained at 15° C. for 5, 15 or 35 days, whereupon they were returned to 25° C. to complete their development. Examination of the cocoons yielded the results summarized in Table 7. Exposure to 15° C. for 5 days was just sufficient to induce diapause in some of the larvae which had remained at 25° C. for 5 days prior to chilling. Most of the larvae that were returned to 25° C. after 15 and 35 days of chilling exhibited diapause. What development there was in these animals invariably occurred in cocoons containing *lecontei* that had pupated prior to parasitization, an observation to which we shall return presently. In this experiment all the *Tritneptis* within a single cocoon behaved in the same manner as far as diapause was concerned.

Table 7. *The effect on the incidence of diapause of exposing* Tritneptis *larvae of various ages to* 15° C. *for* 5, 15 *and* 35 *days*

Days at 25° C. prior to chilling	Days at 15° C. prior to return to 25° C.	Type of larva resulting from treatment	
		Developing	Diapausing
0	0*	+	0
1	5	+	0
3	5	+	0
5	5	+†	+
1	15	0	+
3	15	+†	+
5	15	0	+
1	35	0	+
3	35	+†	+
5	35	+†	+

* Unchilled controls.
† Development occurred only in cocoons that contained *lecontei* which had pupated.

In a parallel experiment, *Tritneptis* that had been reared at 15° C. were permitted to oviposit at this temperature. The parasitized cocoons were then transferred to 25° C. at various times after oviposition. Parasitized cocoons that had been kept at 15° C. for 12 days or less before being placed at 25° C. gave rise to developing offspring exclusively. Cocoons stored at 15° C. for longer than 12 days yielded diapausing animals only. Thus, about 5 days of development at 25° C. and 12 days at 15° C. brought *Tritneptis* larvae to a temperature-sensitive developmental stage, where a period of chilling caused them to enter diapause. The upper limit of this temperature-sensitive period was determined in the following experiment.

Cocoons containing eggs of an approximately known age were obtained by collecting parasitized *lecontei* twice daily from vials containing large numbers of *Tritneptis*. After parasitization the cocoons were kept at 25° C. for from 1 to 8 days and were then stored at 15° C. for 30 days. (*Tritneptis* larvae commonly

defaecate on the ninth day at 25° C. (Table 2).) The results summarized in Table 8 reveal that cocoons kept for 8 days at 25° C. before chilling yielded developing offspring only; cocoons stored for only 4 days before chilling yielded diapausing offspring only; at intermediate stages both kinds of offspring were found. Thus chilling animals which have progressed beyond the seventh day of development at 25° C. fails to induce diapause.

Table 8. *The effect on the incidence of diapause of exposing* Tritneptis *larvae of various ages to* 15° C. *for* 30 *days*

Days at 25° C. prior to chilling	% of larvae going into diapause
1	100
2	100
3	100
4	100
5	62
6	50
7	20
8	0

Efforts to delimit further the temperature-sensitive period failed principally because of the impossibility of obtaining *Tritneptis* larvae of exactly known age. For although all animals within a single *lecontei* cocoon were commonly at the same stage of development, cocoons exposed to identical conditions frequently contained *Tritneptis* which differed in developmental stage by as much as 2 days. It was therefore virtually impossible to estimate closer than to within 2 days the developmental stage of the larvae within an unopened cocoon. Experiments that required larvae of precise ages were consequently ruled out.

This finding of varying rates of development among wasps in different cocoons probably accounts for the fact that *Tritneptis* from cocoons parasitized at the same time often reacted differently to exposure to low temperature in diapause-induction experiments. These occasional aberrant results are understandable when one recognizes that the larvae were probably in different developmental states.

Recently it proved possible to raise *Tritneptis* on *lecontei* prepupae that had been removed from their cocoons immediately after parasitization and placed in moist chambers. The wasp larvae developed normally, though slightly more rapidly than in the cocoon, and development was not at the same rate in all the parasites of a single prepupa as it was in the enclosed cocoon. This technique should permit selection of larvae of known age and thereby precisely define the temperature-sensitive period.

(2) *Other factors*

Although the experiments just considered showed that chilling of the larva was apparently a sufficient stimulus for diapause induction in *Tritneptis*, other factors were tested to see whether special conditions need accompany chilling and whether another agency could substitute for chilling. Careful study of numerous adult females revealed that size, photoperiod, temperature history, etc., had no influence

on the incidence of diapause in their offspring. Similar experiments conducted on larvae failed to reveal factors that could affect or substitute for the action of chilling. The influence of host condition was also assessed by using both *lecontei* prepupae and pupae in various conditions. The only fact that emerged has already been noted, namely, that in some cases chilled *Tritneptis* larvae grown on *lecontei* pupae failed to enter diapause.

These findings lead to the conclusion that chilling *Tritneptis* larvae at a particular stage in larval development induces diapause.

TERMINATION OF DIAPAUSE IN *MORMONIELLA*

At temperatures above 15° C. *Mormoniella* larvae remain in permanent diapause until death ensues after a year or more. However, if the larvae are exposed to temperatures below 15° C. for several months and then returned to higher temperatures, diapause ends and the larval-pupal and pupal-adult transformations take place. In our experience, chilling is the only treatment that will terminate diapause. Exposure to low temperature effects changes within the insect which render it competent to develop. For our present purposes it is not necessary to specify the site of action of low temperature, although it is very likely the brain itself (Schneiderman, 1957 and Discussion). Low temperature permits physiological changes to take place within the insect which prepare the larva for the resumption of morphogenesis when it is returned to higher temperatures. The experiments next described were designed to analyse these processes that bring about the end of diapause, processes that we shall refer to as 'diapause-ending processes'.*

The larvae used were removed from their host puparium when they were firmly in diapause, i.e. at least 1 week after adult development would have been completed had growth not been arrested. *Mormoniella* were resistant to this treatment and, so far as could be judged, behaved in all respects as if still enclosed within puparia. Defaecation which just precedes the larval-pupal moult was chosen as a convenient point to mark the end of diapause.

(1) *The effects of constant low temperature*

Several thousand larvae that had been in diapause for 1 month were divided into groups of forty and placed at 2, 5, 10, 15 and 25° C. in shell vials. At 2-week intervals forty were removed from each temperature, placed at 25° C., and observed daily. The results are recorded in Figs. 2 and 3. None of the animals placed at 15 or 25° C. escaped from diapause. The termination of diapause was completed most rapidly at 2° C., where the greatest proportion of animals broke diapause after 8 weeks of chilling and where the number of days at 25° C. required for the breaking of diapause was by far the smallest. Also, as has been observed in many other species (Salt, 1947; Andrewartha, 1952), the data show that as the period of exposure to

* The term 'diapause development' coined by Andrewartha (1952) for these same processes was of great significance in that it drew general attention to the processes that lead to the end of diapause. But since the term might be construed to denote the processes that lead to diapause, the less ambiguous term 'diapause-ending processes' was chosen.

low temperature increased, diapause terminated more rapidly upon return to 25° C. and a larger fraction of the chilled larvae eventually developed. In simplest terms the diapause-ending processes are favoured by low temperature.

If the low-temperature treatment is continued beyond the 16 weeks necessary to complete the diapause-ending processes at all effective chilling temperatures, the animals continue in diapause because the early stages of pupation will not occur at temperatures below 15° C. The dormant larvae remain alive for many months at the low temperatures, but viability gradually falls if post-diapause development is prevented for more than a year. After about 2½ years mortality is almost complete.

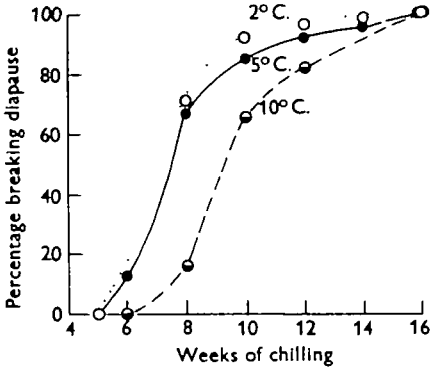

Fig. 2

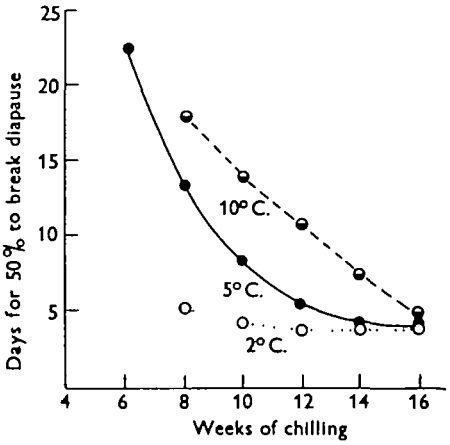

Fig. 3

Fig. 2. Effects of various periods of chilling at several temperatures on the percentage of *Mormoniella* larvae breaking diapause.

Fig. 3. The time in days for 50% of the larvae described in Fig. 2 to break diapause.

(2) *The effects of alternating temperatures*

Diapausing larvae, 2–4 weeks after they had entered diapause, were exposed to alternating periods at 5 and 25° C. according to the following schedule:

(*a*) 5° C. continuously.
(*b*) 5° C. for 7 days, 25° C. for 1 day, 5° C. for 7 days, etc.
(*c*) 5° C. for 7 days, 25° C. for 2 days, 5° C. for 7 days, etc.
(*d*) 5° C. for 7 days, 25° C. for 4 days, 5° C. for 7 days, etc.
(*e*) 5° C. for 7 days, 25° C. for 7 days, 5° C. for 7 days, etc.
(*f*) 5° C. for 2 days, 25° C. for 2 days, 5° C. for 2 days, etc.

After a cumulative exposure to 5° C. for *N* weeks, sixty animals were removed from each set of conditions, placed at 25° C. and their development observed. The results summarized in Fig. 4 reveal that the effects of subthreshold chilling in terminating diapause can be undone by warming. Two days at 25° C. undid a great deal of the effects of 7 days at 5° C., while 7 days at 25° C. almost completely undid the effects of 7 days at 5° C.

These results and the results obtained with constant chilling are consistent with the hypothesis that two reactions are at work (Schneiderman, 1957). One reaction is favoured by low temperature: this is the primary reaction of the diapause-ending processes which leads to the synthesis and accumulation of some substance (probably within the insect's brain). When this substance reaches threshold concentration it enables the insect, when returned to high temperature, to moult. The substance is apparently broken down at high temperatures. 'Chilling' at temperatures above 15° C. never terminates diapause, and we may conclude that at temperatures above 15° C. the rate of the breakdown reaction exceeds the rate of the synthetic reaction, so that the substance never accumulates to threshold concentrations. Below 15° C. the synthetic reaction outstrips the breakdown reaction and the substance accumulates to threshold levels. In other words, the positive temperature coefficient (Q_{10}) of the synthetic reaction is less than that of the breakdown reaction, and at about 15° C. the rates of the two reactions are equal. The following experiments were designed to test the hypothesis and to define further the synthetic and the breakdown reactions.

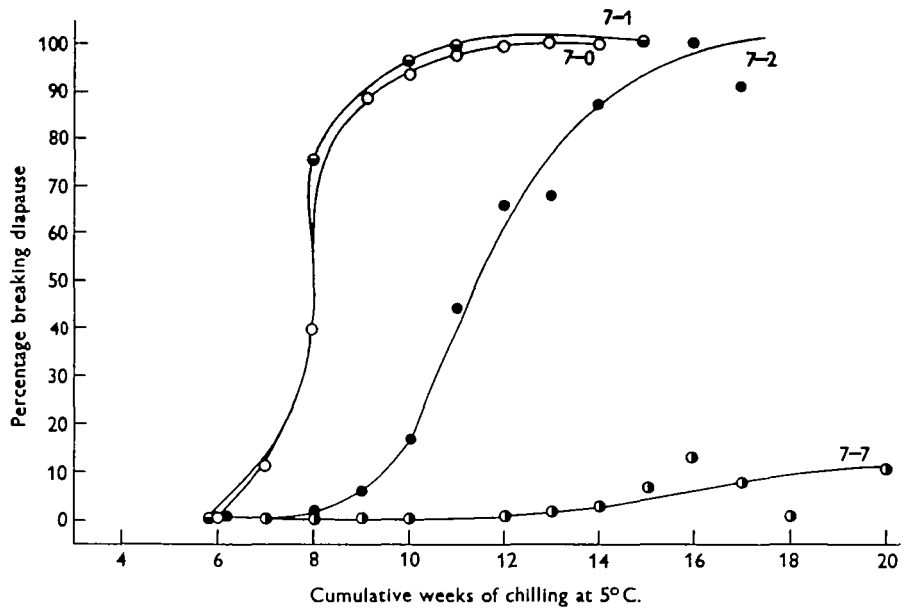

Fig. 4. Effects of alternating periods of chilling and warming in terminating diapause in *Mormoniella*. Data for 7–4 are substantially the same as those for 7–2; data for 2–2 are substantially the same as those for 7–7.

(3) *The synthetic reaction*

If low temperature acts by effectively promoting some synthetic reaction in the insect, this synthetic reaction—presumably requiring energy—ought to be inhibited when the insect's metabolism is decreased. A simple method of inhibiting metabolism during low-temperature exposure is to chill larvae at very low temperatures. To this end groups of diapausing larvae were stored at −2 to −6° C. for 6, 12 and

16 weeks. They failed to break diapause when returned to 25° C., although they did survive the chilling experience. To see whether any of the diapause-ending processes had taken place at the very low temperature, some of the larvae which had been chilled below zero for 16 weeks were given 2 and 4 additional weeks of chilling at 5° C. before being returned to 25° C. These animals also failed to break diapause when returned to 25° C. Apparently little of the diapause-ending processes took place at subzero temperatures presumably because metabolism proceeded at too slow a pace. Exposure to subzero temperatures caused no permanent damage, for when the larvae were rechilled at 5° C. for 8 or 10 weeks they developed normally.

A more effective method of lowering the insect's metabolism during chilling is to chill animals anaerobically. While this procedure is not possible with many diapausing insects which die promptly of oxygen lack, *Mormoniella* is singularly resistant to anaerobiosis. Even at 25° C. the LD 50 is about 7 days and 10% survive as long as 17 days. At low temperatures the survival was far longer as the following experiments showed. Groups of forty diapausing larvae were maintained in anaerobic flasks (see Methods) at 5° C. for periods up to 28 weeks. Parallel control groups were kept in air at 5° C. After treatment the larvae were returned to air and placed at 25° C. along with controls. As Table 9 shows, anaerobiosis at 5° C. for

Table 9. *The effect of chilling in the absence of oxygen on diapausing larvae of* Mormoniella

Weeks of chilling at 5° C.	% surviving 3 weeks after return to air at 25° C.	% developing
3	100	0
8	85	0
10	85	0
16	62	0
20	26	0
28	8	0

up to 10 weeks had little effect on the viability of the animals, and within a day of being returned to air at 25° C. they regained their motility. Exposure for 28 weeks resulted in considerable mortality, but nonetheless nearly 10% of the animals survived. Although they remained alive and apparently healthy for many months, not one of the anaerobically chilled larvae pupated, while the air-chilled control groups broke diapause and developed normally. Thus anaerobiosis prevented chilling from ending diapause. When the anaerobic animals were rechilled in air for an adequate period many initiated development and some of them developed normally, indicating that the anaerobic régime caused no permanent damage to their endocrine system. From the results of these experiments it appears that the diapause-ending processes in *Mormoniella* demand oxidative metabolism, and have a temperature optimum at about 2° C.

Although the diapause-ending processes occur most rapidly at temperatures below 15° C., evidence that they may also proceed at higher temperatures is

provided by the following experiments. Diapausing larvae were removed from puparia when the non-diapausing larvae of he same brood had reached the black pupal stage, i.e. the larvae had been in diapause for about 4 days. These diapausing larvae of known age were maintained at 25° C. At intervals, groups were placed at 5° C. for 6 and 8 weeks of chilling and then returned to 25° C.

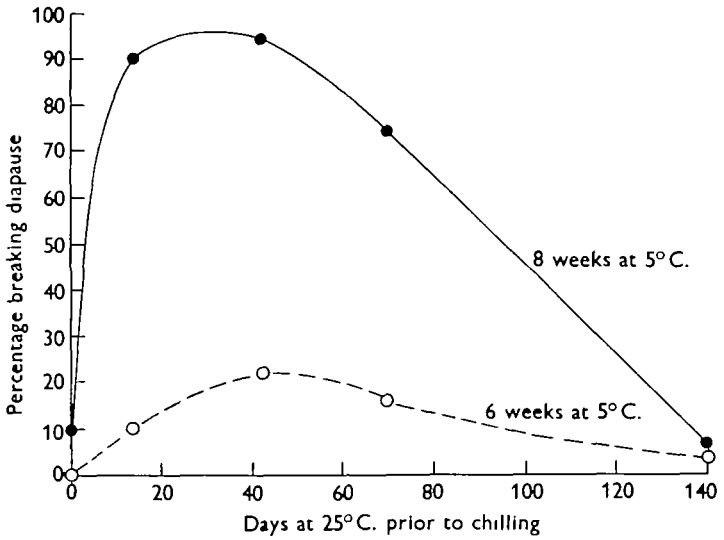

Fig. 5. Effects of incubation at 25° C. prior to chilling on the percentage of *Mormoniella* larvae breaking diapause after 6 and 8 weeks of chilling.

The results are presented graphically in Fig. 5. Incubation at 25° C. for several weeks reduced the duration of chilling necessary to break diapause, i.e. 25° C. apparently promoted the diapause-ending processes, albeit very slowly. After 10 weeks, however, this promoting effect began to wear off. These findings are consistent with the view that some diapause-ending processes can take place at high temperatures (see also Discussion).

(4) *The breakdown reaction*

To examine the nature of the breakdown reaction the following experiments were conducted. Their basis was the observation that the effects of chilling could be undone by warming and the fortunate fact that *Mormoniella* is resistant to anaerobiosis. Animals were chilled for a subthreshold period (a period too short to enable the larvae to escape from diapause). Following this, they were placed at elevated temperatures for various intervals under both aerobic and anaerobic conditions. In the first experiment of this kind, groups of twenty-five diapausing larvae, 2–4 weeks after they entered diapause, were chilled at 5° C. for 24 days, and then subjected to specific experimental conditions. After this they were returned to 5° C. for an additional 32 days of chilling (a total period of chilling of 8 weeks). Thereupon they were placed at 25° C. and their development observed. The larvae

were exposed to the following experimental conditions between the two periods of chilling: incubation at 25° C. for 4, 8 or 25 days in air, or 4 or 8 days anaerobically; incubation at 30° C. for 3 days either in air or anaerobically. Control groups were placed at 25° C. after 0 day, 24 days, 7, 8, 9 or 10 weeks of continuous chilling at 5° C. The results are tabulated in Table 10A and Fig. 6. It can be clearly seen in

Table 10. *The effect on diapausing larvae of* Mormoniella *of interrupting* 8 *weeks of chilling at* 5° C. *after* 24 *days by aerobic and anaerobic incubation at* 25 *or* 30° C. *for specific periods*

A

Total weeks of chilling	Conditions of incubation	% of survivors developing
7	—	12
8	—	40
9	—	83
10	—	87
8	4 days aerobic at 25° C.	4
8	4 days anaerobic at 25° C.	78
8	8 days aerobic at 25° C.	16
8	8 days anaerobic at 25° C.*	58
8	3 days aerobic at 30° C.	8
8	3 days anaerobic at 30° C.	92
8	5 days aerobic at 30° C.	9
8	25 days aerobic at 25° C.	12

B

Total weeks of chilling	Conditions of incubation	% of survivors developing	Days required for half to initiate development
$3\frac{1}{2}$	—	0	—
6	—	9	9
7	—	10	7
8	—	78	7
9	—	95	6
10	—	97	4
8	4 days aerobic at 25° C.	35	8
8	4 days anaerobic at 25° C.	91	4
8	8 days aerobic at 25° C.	38	7
8	8 days anaerobic at 25° C.†	62	5
8	40 days aerobic at 25° C.	15	—
8	3 days aerobic at 30° C.	43	8
8	3 days anaerobic at 30° C.	88	4
8	5 days aerobic at 30° C.	45	8

* Only 50% survived. † Only 65% survived.

Fig. 6, and in the table, that a brief period of aerobic warming undoes some of the effect of 24 days of chilling. Even more striking, and quite unexpectedly, the animals incubated anaerobically showed much more development than the corresponding air controls, which were continuously chilled. These results were confirmed and amplified in the following experiments.

Groups of 100 diapausing larvae had 8 weeks of chilling at 5° C., interrupted after 24 days by warming under specific experimental conditions. The several conditions and the development of the animals at 25° C. after this régime are recorded in Table 10B. It can be judged from the table that a brief interval of aerobic warming caused a measurable setback in development, while 40 days of warming had a considerable effect. Furthermore, as in the previous experiment, anaerobic warming markedly accelerated the termination of diapause. These experiments suggest that the breakdown reaction is aerobic and that the diapause-ending processes are accelerated by brief anaerobic warming.

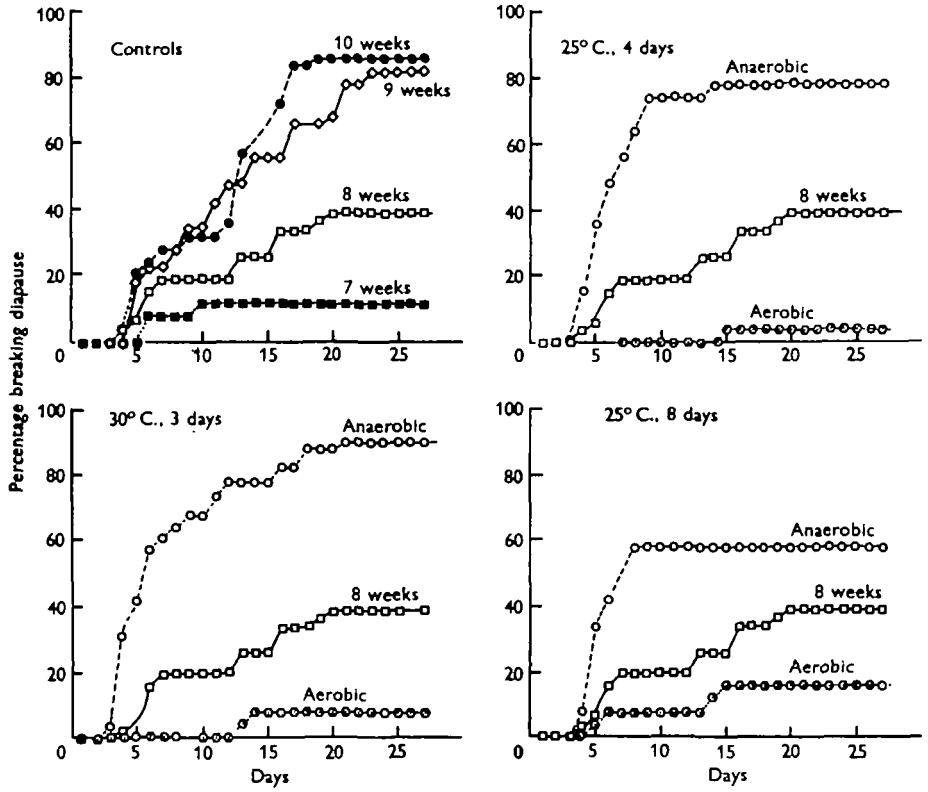

Fig. 6. Effects of interrupting 8 weeks of chilling after 24 days by warming for specific intervals under aerobic and anaerobic conditions.

Further insight into the breakdown reaction was provided by the following experiment, which was designed to ascertain whether the build-up reaction of the diapause-ending processes ever became irreversible. *Mormoniella* larvae were chilled at 5° C. for 30 weeks. This meant that the diapause-ending processes were completed and nearly 100% would break diapause when placed at 25° C. Groups of twenty-five of these larvae were given thermal shocks by exposing them to 35, 38, 40, or 45° C. for from 1 hr. to several days. The results recorded in Table 11

reveal that once the diapause-ending processes have been completed in *Mormoniella*, they cannot be undone, even by exposure to high temperature.

Table 11. *The effects of thermal shocks on the termination of diapause in chilled larvae of* Mormoniella

Temperature of shock (° C.)	Duration of shock	% remaining in diapause	% developing	% dead
25*	—	0–10	90–100	0
35	8 days	7	71	22
35	8 days	9	62	29
38	1 day	0	91	9
38	2 days	0	53	47
40	4 hr.	0	60	40
40	1 day	0	65	35
40	1 day	8	46	46
45	2 hr.	5	45	50

* Controls put directly at 25° C.

Experiments were next undertaken to ascertain when the diapause-ending processes became irreversible. Since it was usually reversible after 24 days at 5° C., groups of diapausing larvae were chilled at 5° C. for a period longer than 24 days. They were then warmed either aerobically or anaerobically and returned to 5° C. until they had received a total of 8 weeks of chilling. Thereupon they were placed at 25° C. and their development observed. The data revealed that after 38 days at 5° C., the effects of chilling could no longer be reversed by brief periods of aerobic warming. Results of a typical experiment are presented in Fig. 7.

From these several experiments it appears that the diapause-ending processes consist of at least two phases: a phase that can be reversed by brief periods of aerobic warming, which is succeeded by a phase that is substantially indifferent to aerobic warming.

(5) *Further analysis of the effects of anaerobic warming*

From the experiments considered thus far, it is not clear whether anaerobic warming resembles chilling in promoting the diapause-ending processes or is something quite distinct. Experiments were conducted to determine whether animals exposed to subthreshold chilling could be caused to break diapause by simply warming them anaerobically. Larvae were divided into groups of about fifty and put at 5° C. 3 weeks after entering diapause. The results of a typical experiment are presented in Table 12. None of the larvae chilled at 5° C. for 38 days developed when placed directly at 25° C. in air. However, if after chilling these larvae were maintained anaerobically for 4 days at 25° C., then 8% broke diapause when they were returned to air. The anaerobic treatment enabled these larvae to complete their diapause-ending processes, and in this sense it resembled chilling. The table also reveals the accelerating effects of anaerobiosis on the 45- and 57-day groups and thus confirms and extends the findings reported in the previous

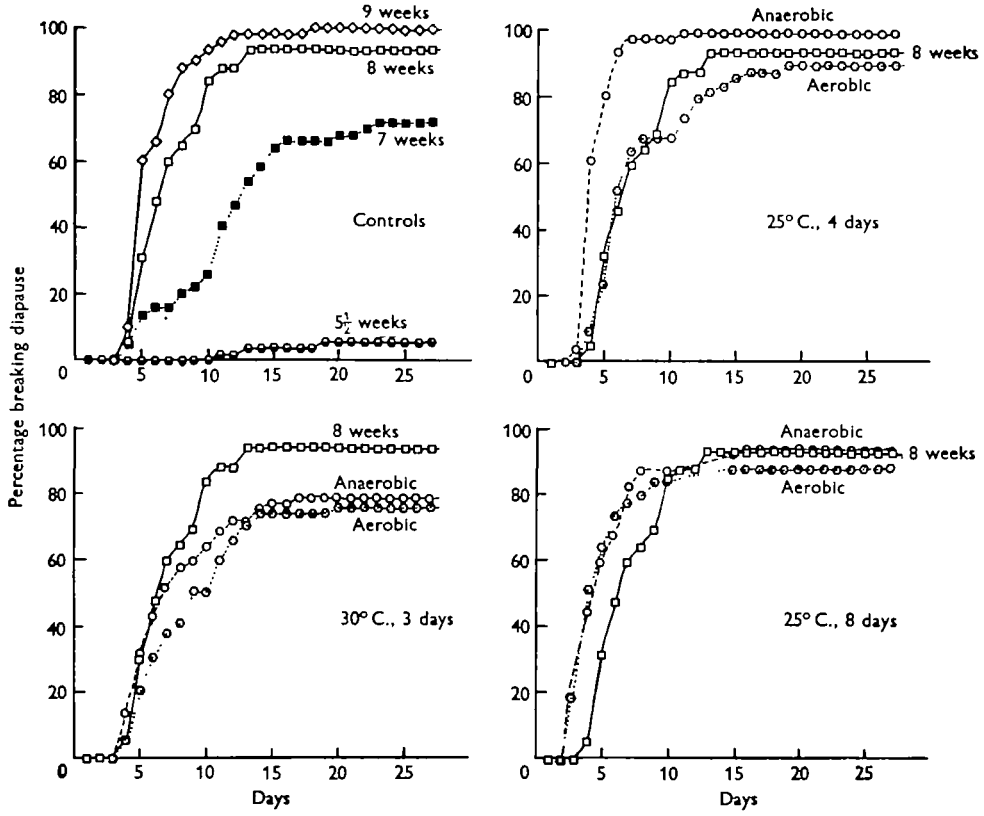

Fig. 7. Effects of interrupting 8 weeks of chilling after 38 days by warming for specific intervals under aerobic and anaerobic conditions.

Table 12. *The effect of aerobic and anaerobic incubation at* 25° C. *on diapausing larvae of* Mormoniella *chilled at* 5° C. *for specific periods*

Total days of chilling	Days at 5° C. prior to incubation	Conditions of incubation	Days at 5° C. after incubation	% of survivors developing
25	25	—	0	0
25	25	4 days anaerobic	0	0
31	31	—	0	0
31	31	4 days anaerobic	0	0
32	25	4 days air	7	0
32	25	4 days anaerobic	7	0
38	38	—	0	0
38	38	4 days anaerobic	0	8
45	45	—	0	4
45	38	4 days air	7	4
45	38	4 days anaerobic	7	28
49	49	—	0	12
56	56	—	0	60
57	25	4 days air	32	12
57	25	4 days anaerobic	32	92

Table 13. *The effect on diapausing larvae of* Mormoniella *of interrupting* 8 *weeks of chilling at* 5° C. *after* 20 *days by consecutive periods of aerobic and anaerobic incubation at* 25° C.

Total days of chilling	Conditions of incubation	% of survivors developing
49	—	0
56	—	8
70	—	94
56	4 days anaerobic	70
56	4 days air	24
56	4 days air followed by 3 days anaerobic	50
56	4 days anaerobic followed by 3 days air	16
56	4 days air followed by 3 days anaerobic followed by 4 days air	12

section. This result is consistent with the view that anaerobic warming has the same final effect on the diapause-ending processes as chilling.

If a brief period of anaerobic warming is qualitatively the same as a brief period of chilling in promoting the diapause-ending processes, then, like the effects of chilling, the effects of anaerobic warming should be reversed by a period of aerobic warming. To test this possibility, groups of fifty larvae that had been in diapause for one month were chilled at 5° C. for 20 days. Following this they were exposed to consecutive periods of aerobic and anaerobic incubation at 25° C., and they were then chilled for 35 more days. Finally, after a total of 8 weeks of chilling, they were returned to 25° C. for development. The conditions of incubation and the results are summarized in Table 13. The undoing of the subthreshold chilling by the 4 days of aerobic warming was not evident in this experiment. However, the accelerating action of 4 days of anaerobiosis was striking. Furthermore, it is clear from the table that, even after the animals had been incubated in air, anaerobiosis accelerated development. It is also evident that aerobic incubation undid the accelerating effect of anaerobiosis. In this respect anaerobic warming and aerobic chilling are similar—both are commonly reversed by aerobic warming.

TERMINATION OF DIAPAUSE IN *TRITNEPTIS*

Unlike *Mormoniella*, diapausing larvae of *Tritneptis* fail to survive for prolonged periods when removed from the cocoons of their host. Indeed, only 12 weeks of chilling at temperatures of 2–10° C. killed nearly half the isolated larvae, but there was little mortality among larvae chilled while still enclosed within their host cocoons. Sufficient experiments were conducted on both isolated and enclosed larvae of *Tritneptis* to demonstrate that exposure to low temperature terminates larval diapause much as it does in *Mormoniella*. Results of a typical experiment on isolated larvae are recorded in Table 14. It is also noteworthy that occasional

individuals developed after several months at 15° C., indicating that the diapause-ending processes have a higher threshold temperature in *Tritneptis* than in *Mormoniella*.

Table 14. *The effect of chilling at* 5° C. *for various periods on the termination of larval diapause in* Tritneptis

Weeks of exposure to 5° C.	% developing of those which survived for 2 weeks after being returned to 25° C.
5	4
6	5
7	24
8	50
9	45
10	100

DISCUSSION

(1) *The induction of diapause*

In the case of *Mormoniella* the experimental results fail to confirm the assertions of Cousin (1932) that diapause results from deficient larval nutrition, and of Van der Merwe (1945) that diapause is induced in larvae exposed to low humidity or falling temperatures. But they leave little doubt that exposing female wasps to low temperature during oögenesis causes their offspring to enter diapause at the end of the last larval instar. In some way the temperature stimulus appears to be communicated from parent to offspring through the egg. The low-temperature stimulus may be acting directly on the ovaries and thereby affecting the chemical composition of the egg prior to the initiation of cleavage. Or the stimulus may be acting on some other organ within the insect and affecting indirectly the composition of the egg.

A decision between these alternatives will have to await appropriate ovarian transplantation experiments. In any event, low temperature causes the female to lay an egg that is qualitatively different from an ordinary egg in that the larva emerging from it eventually enters diapause. It is also clear that the low-temperature effect wears off after several days and chilled females return to producing non-diapausing offspring. Since some females produce mixed broods for several days after chilling, the effect is not all-or-none as far as the female is concerned. Conceivably it is all-or-none for the eggs within one of the eight ovarioles, but it is more likely that there is a distribution of sensitivity among the eggs of a given female. Moreover, since some females produce a proportion of diapause offspring without any chilling, there is also a distribution of susceptibility to diapause induction among females. Some females are much more sensitive to temperature than others. In this latter connexion, it is worth noting that Moursi (1946*b*) failed to obtain any diapause in the offspring of female *Mormoniella* that had been chilled as pupae or adults. However, his experiments were conducted on wasps collected in southern California, and it is not unlikely that genetic differences between his population and the Woods Hole wild-type used in our experiments are at least in

part responsible for the apparent different susceptibilities of the females to diapause induction.

So far as we are aware, only a few examples are known of maternal stimuli being transmitted to the larval progeny (Simmonds, 1948; Cragg & Cole, 1952; Lees, 1955). The closest parallel to *Mormoniella* is found in the pteromalid *Spalangia drosophilae*. This pupal ectoparasite of the frit-fly (*Oscinella frit*) undergoes a facultative larval diapause in the third instar when the animals are reared continuously at temperatures below 24° C. (Simmonds, 1948). Furthermore, the incidence of diapause increases in the offspring of senile females. Simmonds concludes that '. . . the temperature to which a female has been subject during development and temperature changes prior to the commencement of oviposition affect the potentialities with regard to diapause of eggs laid subsequently. The temperature of development of eggs within the ovarioles. . . has some affect on the quality of the eggs and their potentialities for future development' (p. 401). These conclusions seem to apply to *Mormoniella*.

Although the present experiments provide few clues about how low temperature acting on the parent has a delayed action in producing diapause in the offspring after several moults, they establish that the phenomenon is real. Moreover, they suggest that the generally held view that 'the central nervous system is the vehicle which carries and eventually transmits the "directions" of the environment' in inducing larval and pupal diapause may be inadequate to explain some kinds of facultative diapause (Lees, 1955, p. 114). In the case of *Mormoniella*, it is difficult to regard low temperature as a 'token' stimulus which acts via the central nervous system (Lees, 1955, p. 31). More probably, as mentioned above, low temperature directly affects the metabolism of the developing egg or some organ that contributes to the substance of the egg. It is possible that this organ might be an endocrine organ within the female wasp that secretes a 'diapause hormone'. The production of such a hormone by the suboesophageal ganglion of the commercial silkworm causes the eggs in the ovaries to become 'diapause eggs', i.e. embryos developing from these eggs enter diapause at the very young germ-band stage (Fukuda, 1951; Hasegawa, 1951). If such a mechanism operates in *Mormoniella*, then low temperature might be acting as a token stimulus via the nervous system of the mother.

The mechanism of diapause induction in *Tritneptis* is far easier to explain than that of *Mormoniella*. In *Tritneptis*, to be effective, low temperature must act on the larva itself to produce diapause in that generation.

It is noteworthy that two wasps as closely related as *Mormoniella* and *Tritneptis* should use very different mechanisms to induce diapause. This fact supports the suggestion of Lees (1955) that diapause has frequently been evolved independently in closely related groups. In this connexion, attempts were made to induce diapause in a third chalcid in the family Eulophidae, *Dahlbominus fuscipennis** (= *Microplectron fuscipennis*), by the stimuli which proved effective for *Mormoniella* and *Tritneptis*. None of the stimuli was effective, although *Dahlbominus* has been

* Kindly supplied by Dr A. Wilkes and Dr J. C. Martin, Entomology Laboratory, Belleville, Ontario, Canada.

reported to have a facultative larval diapause (Morris & Cameron, 1935). Possibly, factors other than temperature are critical in this insect.

The arguments up to this point have not required any decision about the immediate cause of larval diapause in *Mormoniella* and *Tritneptis*. However, there is considerable evidence which has been summarized elsewhere (Lees, 1955, pp. 108–10, 1956; Church, 1955; Williams, 1956; Schneiderman, 1957) that the larval and pupal diapause of the Lepidoptera and Hymenoptera usually results from the inactivation of specific neurosecretory cells in the insect's brain. These cells normally produce and release a tropic factor, the brain hormone, which stimulates the prothoracic glands to secrete the growth and moulting hormone. When the neurosecretory cells cease producing their hormone, growth and moulting cease and diapause supervenes. Under this view, the larval diapause of *Mormoniella* and *Tritneptis* would stem from the inactivation of the larval neurosecretory cells. While this is probably true, we ought not to forget that diapause has probably evolved independently many times and, in the absence of appropriate surgical experiments, we should not completely rule out the possibility that inactivation of some link in the endocrine chain other than the brain may be the central cause of diapause in some insects (cf. for example, Ozeki (1954) and Rahm (1952)).

(2) *The termination of diapause*

Since only a few experiments were conducted on the termination of diapause in *Tritneptis*, this discussion will focus on *Mormoniella*. The original hypothesis to explain diapause-ending processes that furnished a starting point for the present experiments proposed two competing reactions with different positive temperature coefficients—a synthetic reaction and a breakdown reaction (cf. p. 535). Indeed, this has been the most favoured hypothesis to explain the action of low temperature in terminating diapause and has been suggested by numerous workers on various grounds (Andrewartha, 1943, 1952; Salt, 1947; Williams, 1947; Lees, 1955, 1956). The experimental results demonstrate the following about these two postulated reactions.

The synthetic reaction

(1) The synthetic reaction is in part obligatorily aerobic, for chilling fails to exert an effect unless it is performed aerobically in at least the initial stages. Thus larvae chilled anaerobically for many months, although they survived, failed to develop at 25° C. unless rechilled in air.

(2) The synthetic reaction can proceed in part in the absence of oxygen, for there was a marked increase in the proportion of animals that break diapause, and a decrease in the time required for the initiation of development when larvae were warmed anaerobically for a brief interval during the period of chilling. Furthermore, when larvae were chilled for a subthreshold period, a brief interval of anaerobic warming sufficed to enable some of the larvae to terminate diapause. This is consistent with the view that anaerobic warming and aerobic chilling favour the synthetic reaction.

(3) The temperature optimum for the aerobic phase of the synthetic reaction is about 2° C., and little of the diapause-ending processes occurs at temperatures below −6° C. or above 15° C. (cf. Lees, 1953, p. 476). Thus, in *Mormoniella* as in the eggs of the grasshopper *Austroicetes* (Andrewartha, 1943) the temperature ranges for the diapause-ending processes and for morphogenesis do not overlap, and the two cannot take place concurrently. This is in contrast to pupae of the *cecropia* silkworm (Williams, 1956) and to eggs of *Melanoplus* and *Locusta* (Andrewartha, 1943).

(4) Although diapause will never be terminated by keeping larvae continually at temperatures above 15° C., diapause-ending processes do take place slowly at these temperatures. Thus a period of exposure to temperatures above 15° C. *prior to chilling* decreased the amount of chilling necessary to terminate diapause. This finding is in agreement with the observations of Emme (1949) on the termination of diapause in the eggs of *Bombyx* and of Church & Salt (1952) in the eggs of *Melanoplus*.

An alternative explanation of this finding is that exposure to low temperature is not effective until the last instar *Mormoniella* larva has reached some definite stage of physiological development and that several weeks are required after the last larval moult for these changes to take place (cf. Andrewartha, 1943). But inasmuch as there is other evidence that diapause-ending processes can occur at high temperatures (see below) the explanation offered in the preceding paragraph seems more attractive. In any event the results emphasize that keeping diapausing larvae at 25° C. prior to chilling is not an indifferent procedure and must be taken into account in all experiments on diapause termination. They also make it clear that a diapausing larva at 25° C. is not in *status quo* but is undergoing slow but persistent physiological changes.

(5) The synthetic reaction can be completed in part at 25° C. *after chilling*. For when larvae are chilled for 6 weeks at 5° C., more than 12 days are required for those that initiate development to do so, while after 14 weeks of chilling development is initiated within 4 days. In the 6-week group, the synthetic reaction presumably was completed during the 12 days at 25° C. (cf. Browning, 1952, p. 352).

The breakdown reaction

(1) The breakdown reaction is apparently aerobic, for the effects of chilling seem to be undone only by aerobic warming.

(2) Part of the synthetic reaction becomes irreversible after about a month of chilling, for brief aerobic warming fails to exert an effect if it intervenes after the larvae have been exposed for more than a month to 5° C. Thus, part of the synthetic reaction becomes irreversible long before the diapause-ending processes are completed. In this respect *Mormoniella* behaves quite differently from diapausing prepupae of the saw-fly *Cephus cinctus*, in which the synthetic reaction can be reversed by exposure to high temperature apparently after the diapause-ending processes have been completed (Salt, 1947; Church, 1955). The fact that prolonged aerobic warming undoes a considerable proportion of the effect of subthreshold

chilling is of interest when considered in relation to species which have a long-enduring diapause of several years in nature (e.g. as long as 6 years for some prepupae of the saw-fly *Gilpina polytoma* and up to 12 years for larvae of the wheat-blossom midge *Sitodiplosis mosellana* (cited by Lees, 1955)). Apparently in these species the amount of the diapause-ending processes completed in one winter is below threshold. It is likely that much of the net effect of the diapause-ending processes is lost before the succeeding winter, but not all of it, so that some residuum is carried over from one winter to the next until a threshold is reached (cf. Lees, 1955, p. 60).

(3) Aerobic warming undoes the accelerating effects of anaerobic warming as well as the effects of aerobic chilling. This is consistent with the view that anaerobic warming and aerobic chilling effectively promote the same synthetic reaction.

The experiments provide no evidence for the site of action of low temperature. However, as has been pointed out elsewhere (Schneiderman, 1957), there is considerable evidence that in most diapausing Lepidoptera and Hymenoptera low temperature acts directly on the brain, without the intervention of the rest of the central nervous system and probably of the rest of the body. Under this view, diapause-ending processes involve reactions occurring within the brain of the insect.

It must be pointed out, however, that the criterion we have used to mark the end of diapause—namely, defaecation—involves many reactions besides the diapause-ending processes that we propose occur within the insect's brain. In addition to these processes, there is the actual secretion into the blood of the brain hormone, the activation of the prothoracic glands by the brain hormone and the secretion of the prothoracic gland hormone, and, finally, the interaction of the prothoracic gland hormone with the tissues of the insect. In our opinion, the effects of low temperature and of aerobic and anaerobic warming on the termination of diapause result primarily from the action of these agencies on processes occurring within the insect's brain. This does not, of course, gainsay the possibility that some of these factors might be acting on other processes. Thus it is conceivable, for example, that anaerobic warming might in some way increase the sensitivity of the prothoracic glands to the brain hormone. However, in the absence of evidence to the contrary, we prefer to think of these several agencies as acting primarily on the brain itself.

Our results do not permit us to pinpoint any specific reactions within the insect's brain, but they do enable us to rule out certain hypotheses, for example, the early proposals of Andrewartha (1952) and Andrewartha & Birch (1954) (cf. Schneiderman, 1957). They also rule out the simple scheme of two reactions with different temperature coefficients, which has served as a working hypothesis for this and other studies (Williams, 1956; Schneiderman, 1957). Clearly, the synthetic reaction must be complex, having both aerobic and anaerobic phases, a phase that is favoured by low temperature but is reversible, followed by a phase that is also favoured by low temperature but is irreversible. Actually, it would seem more appropriate to think in terms of *synthetic reactions* (cf. Lees, 1955, p. 64). It is notable that, while there are evidently several synthetic reactions involved in the diapause-ending processes, there is no evidence for multiple breakdown reactions.

It is likely that the various synthetic reactions lead to the synthesis of the same active substance within the insect's brain—a substance that is necessary for the production or release of the brain hormone. These synthetic reactions are opposed by an oxidative breakdown reaction. Low temperature acts by slowing down this breakdown reaction within the brain, thereby enabling the brain to accumulate the substance. The nature of the substance that accumulates within the chilled larval brains is of uncommon interest. If we make the assumption that at low temperatures the same substance is being synthesized in these larval brains as in the pupal brains of Lepidoptera, then it is probable that the substance is acetyl choline. For Van der Kloot (1955) has shown that chilling promotes the synthesis of a cholinergic substance within the diapausing pupal brain of the *cecropia* silkworm, *Hyalophora cecropia*, and this invariably precedes the activation of the neurosecretory cells within the brain. Under this view, the breakdown reaction is either the breakdown of acetyl choline or the removal of one of the intermediates in its path of synthesis. Since the breakdown reaction seems to be aerobic it is probably enzymatic and not a simple non-enzymatic breakdown of acetyl choline as has been suggested (Williams, 1956). The apparent oxygen requirement of the breakdown reaction also rules out the possibility that the reaction is the simple hydrolysis of acetyl choline (Schneiderman, 1957). It seems likely that specific descriptions of the chemical reactants involved in both the synthetic and breakdown reactions will have to await further direct study of the metabolism of the insect brain.

SUMMARY

1. Experiments have been conducted to determine the extrinsic factors that cause facultative diapause in two parasitic chalcid wasps, *Mormoniella vitripennis* and *Tritneptis klugii*, and to analyse the mechanism of diapause termination.

2. In both species diapause occurs in the last larval instar after the feeding period has ended and just prior to defaecation. The diagnostic feature of the diapausing larva is that it does not immediately moult into a pupa.

3. In *Mormoniella* exposing females to low temperature during oögenesis causes their progeny to enter diapause at the end of the last larval instar. Low temperature thus causes the female to lay an egg that is qualitatively different from an ordinary egg in that the larva emerging from it eventually enters diapause. This action of low temperature on the female wears off after several days and the wasp returns to producing non-diapausing offspring.

4. In *Tritneptis* low temperature also produces diapause, but in this species low temperature, to be effective, must act on the larva itself between the second and final instar to produce diapause in that generation.

5. The diapause of *Mormoniella* was considered in relation to maternally induced diapause in other species and two possible mechanisms for the action of low temperature were suggested, namely, a direct action on the ovaries or an indirect action through the maternal production of a diapause hormone.

6. It was found that exposure to low temperatures enables larvae of both species to break diapause and complete their development when subsequently placed at 25° C.

7. The mechanism of action of low temperature in terminating diapause was examined in *Mormoniella* by exposing larvae to various temperature régimes in the presence and absence of oxygen.

8. Ten weeks at 5° C. enabled nearly 90% of the larvae to terminate diapause when returned to 25° C.; after 6 weeks at 5° C. less than 10% developed. Chilling at 2° C. was more effective than 5 or 10° C., while temperatures above 15 or below −6° C. were ineffective.

9. Although diapause was never terminated by keeping larvae continually at temperatures above 15° C., a period of exposure to temperatures above 15° C. *prior to chilling* decreased the amount of chilling necessary to terminate diapause.

10. Larvae chilled in the absence of oxygen for as long as 28 weeks failed to break diapause but developed when subsequently rechilled in air.

11. After receiving a *threshold* exposure to low temperature larvae could not be returned to diapause by temperature shocks as high as 45° C.; however, the effects of *subthreshold* chilling were reversed by exposure to 25° C. Thus, animals chilled for a total of 20 weeks, with 1 week of warming after each week of chilling, failed to develop. Similarly, it was found that interrupting 8 weeks of chilling on the 25th day by 4 days of warming partially undid the chilling.

12. When warming was conducted in the absence of oxygen it failed to undo the effects of subthreshold chilling and the termination of diapause was markedly accelerated. Indeed, when larvae were chilled for a subthreshold period, a brief interval of anaerobic warming sufficed to enable some of the larvae to terminate diapause.

13. In addition to the above, a variety of other experiments were conducted with alternating periods of chilling and warming in the presence and absence of oxygen. These led to a hypothesis which seems to account for the action of low temperature in terminating larval diapause.

14. The hypothesis focuses attention on the neurosecretory cells of the insect's brain and suggests that low temperature slows down an aerobic breakdown reaction within the larval brain and permits the synthesis of a substance necessary for neurosecretory activity. The initial stages of the synthetic reaction are aerobic but later stages are favoured by anaerobic warming. The nature of these reactions was discussed.

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