LARVAL DIAPAUSE OF MATERNAL ORIGIN: INDUCTION OF DIAPAUSE IN *NASONIA VITRIPENNIS* (WALK.) (HYMENOPTERA: PTEROMALIDAE)

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INTRODUCTION

Factors which induce diapause in insects are, in most cases, only effective when applied to the individuals which are destined to cease development (Lees, 1955), although in many examples—for instance the vine leafroller, *Polychrosis botrana* (Komarova, 1949)—the sensitive stage and the resulting diapause are separated by several intervening instars. However, there are a few insects known in which factors affecting the maternal generation induce diapause in the progeny. Examples of this type of induction are found in *Spalangia drosophilae* and *Cryptus inornatus* (Simmonds, 1946, 1948) in which the age of the female parent and the temperature and dietary conditions she experiences before oviposition affect the incidence of diapause in the larvae she produces. There is also evidence of a maternal influence on production of diapause larvae in *Lucilia sericata* (Cragg & Cole, 1952), *Phlebotomus papatasii* (Roubaud, 1928) and *Aedes triseriatus* (Love & Whelchel, 1955). The most recent example was provided by Schneiderman & Horwitz (1958) working with the parasitic wasp, *Nasonia* (= *Mormoniella*) *vitripennis*, which has a facultative diapause at the end of the fourth and final larval instar. Working with strains of *Nasonia* from North America (Massachusetts), Schneiderman & Horwitz tested many environmental and intrinsic factors affecting both larval and maternal generations and concluded that chilling the females whilst the eggs were developing in the ovaries, or depriving them, for a few days, of hosts in which to oviposit, increased the likelihood of the females producing diapause offspring. According to these authors neither daylength nor the age of the female parent was effective in inducing diapause and, although Wylie (1958) claimed that low temperature caused diapause in mature larvae, Schneiderman & Horwitz showed that none of the factors they tested were effective in the larvae themselves. In a strain of *Nasonia* isolated in London, however, it has been shown (Saunders, 1962) that diapause larvae are more frequent among the offspring of old females, and in the present experiments it was found that daylength affecting the maternal generation was undoubtedly the most important factor inducing diapause in this species. It became clear, therefore, that a re-assessment of the factors inducing diapause in *Nasonia* was necessary.

Although as yet there is no direct evidence, the state of diapause in the larvae of *Nasonia* seems to be of the normal larval-pupal type involving a cessation of neurosecretory activity in the brain (Schneiderman & Horwitz, 1958) and the resulting inactivation of the brain-prothoracic gland system as in *Hyalophora cecropia* (Williams,
1952) and *Cephas cinctus* (Church, 1955). It is clear, however, that females of *Nasonia* producing diapause offspring must lay eggs which are qualitatively different from those which give rise to developing progeny, and the transduction of the environmental stimuli in the maternal generation into the neuro-humoral mechanism of larval diapause must involve a chemical ‘factor’ passed through the egg. The experiments described in the present paper are part of an attempt to elucidate this mechanism of diapause induction in *N. vitripennis*.

**Materials and Methods**

(1) *Culture methods*

*Nasonia vitripennis* is an ectoparasite of the pupae of cyclorrhaphous flies, the immature stages living in the space between the pupa and the puparium. In the experiments described below the parasites were reared on pupae of *Sarcophaga barbata*.

Two strains of *Nasonia* were used. Most of the experiments were performed on a strain (C) isolated in October 1961 by Dr G. Salt at Cambridge, England. The second strain used was the ‘Woods Hole wild type’ (WH +) obtained from Prof. P. W. Whiting and isolated at Woods Hole, Massachusetts, in September 1951. This second strain was also that used by Schneiderman & Horwitz (1958) in their study of diapause.

Cultures of *Nasonia* were maintained in 3 x 1 in. tubes at 25° C. and about 60% R.H. by exposing about twenty 3-day-old *Sarcophaga* pupae to 15–20 female parasites. The host pupae were changed daily and incubated at the same temperature as the parent generation. Host *Sarcophaga* were reared, as adults, on fresh meat, sugar and water and, as larvae, on a medium made from agar, yeast and dried milk. After pupation the *Sarcophaga* pupae were allowed to develop at 25° C. for 3 days and then kept at 2° C. until required.

(2) *Analysis of progeny*

In order to analyse the progeny of single females for diapause or non-diapause offspring, newly emerged mating pairs were enclosed in 2 x ½ in. glass tubes with two 3-day-old *Sarcophaga* pupae. In each experiment, all the females were derived from a single pairing; the males which are normally present in small numbers in the offspring of a fertilized female because of the haplo-diploid mechanism of sex-determination, were obtained from virgin females. The host pupae supplied to the pairs were changed every 24 hr. and incubated in 2 x ½ in. tubes which were kept in wooden racks to facilitate handling and the recognition of individual ‘broods’. Apart from experiments involving temperature changes or the use of a daily photoperiod, both parents and offspring were incubated in continuous darkness at 25° C. In all experiments the larvae were reared at 25° C. The photoperiods used were either natural daylengths obtained in a north-facing laboratory, or artificial photoperiods obtained in a low-temperature cabinet fitted with a ‘strip light’ controlled by a Lomex time-switch. Host-deprived females were kept on a glucose-only diet during the period of deprivation and then supplied with host pupae to obtain their progeny. Temperatures below ambient were controlled either in the low-temperature cabinet or in a water bath by the use of a Shandon ‘Circotherm’ and a Townson and Mercer ‘Minus 20’ Refrigeration unit. Temperatures above ambient were maintained in an incubator.
Relative humidity was maintained in Kühner jars over sulphuric acid and water, or saturated salt solutions (Solomon, 1951).

In the early experiments the developing adult progeny were counted at emergence and the hosts were then opened for diapause larvae. However, since it was found that mixed broods were infrequent, especially in the C strain, a scoring method (+ for diapausing broods, – for developing broods) was adopted in the later experiments, and the proportion of females producing diapause larvae per day was calculated rather than the proportion of larvae entering diapause. This has a greater biological significance since the environmental factors affect the adults and not the larvae.

**RESULTS**

*The effects of age and environmental factors on the incidence of diapause*

(a) Age

Since temperature, photoperiod and nutritional conditions are known to affect the incidence of diapause in *Nasonia*, the effects of age were studied in continuous darkness at 25°C. (Fig. 1a). Each female was supplied with two pupae of *Sarcophaga barbata* daily.

In both the WH+ and the C strains a small proportion of females produce diapause larvae at the beginning of reproductive life and then switch to the production of developing larvae, usually within 48 hr. In the WH+ strain outbreaks of diapause occur throughout life at 25°C. These outbreaks (termed ‘spontaneous diapause’ by Schneiderman & Horwitz, 1958) are characterized by their temporary nature and the females usually return to the production of developing progeny after 1 or 2 days. In the WH+ strain the overall trend at 25°C, therefore, shows no increase in the proportion of females producing diapause larvae with age, although the outbreaks of ‘spontaneous diapause’ are often more frequent in the second half of life. Schneiderman & Horwitz (1958) also found that there was no connexion between age and diapause induction in the WH+ strain at 25°C.

In every experiment involving the C strain, however, a very definite trend was observed in which young females (after the first few days) produce developing progeny but change to the production of diapause larvae later in life. This pattern of diapause production confirms that found in a strain of *Nasonia* isolated in London (Saunders, 1962) and resembles that in *Spalangia drosophilae* and *Cryptus inornatus* (Simmonds, 1946, 1948). In each female of the C strain the change-over from the production of developing progeny to diapause larvae is very sudden and mixed broods only occur at the point of change-over. Once a female begins to produce diapause larvae she continues to do so until she dies. This change-over during the life of the female resembles the maternal ‘switch mechanism’ controlling the production of parthenogenetic and sexual forms of the aphid, *Megoura viciae* (Lees, 1959).

Two experiments were performed to investigate the nature of this maternal effect. In the first experiment, 15 virgin females of the C strain were allowed to oviposit under exactly the same conditions (continuous darkness at 25°C and two host pupae per day) as 15 fertilized controls. In this experiment the pattern of diapause production was identical in the two groups (Fig. 1b) even though the virgin females produced all-male progeny.
Fig. 1. The production of diapause larvae by females of *Nasonia vitripennis* in continuous darkness at 25°C. (a) O, 10 C strain females; ●, 10 WH+ strain females. (b) O, 15 fertilized C strain females; ●, 15 virgin C strain females.
Fig. 2. The production of diapause larvae by females of *Nasonia vitripennis* in continuous darkness at 25°C. (a) O—O, WH+ females mated to C males (cross (1)); O--O, WH+ females mated to WH+ males (WH+ control); •—•, F₁ females from cross (1) mated with F₁ males from cross (1); •--•, F₁ females from WH+ control mated with F₁ males from WH+ control. (b) O—O, C females mated to WH+ males (cross (2)); O--O, C females mated to C males (C control); •--•, F₁ females from cross (2) mated with F₁ males from cross (2); •--•, F₁ females from C control mated with F₁ males from C control. In all cases there were 20 mating pairs.
In the second experiment two crosses were made: (1) 20 virgin females of C strain x 20 males of WH + strain; (2) 20 virgin females of WH + strain x 20 males of C strain. Each mating pair (parental generation) was isolated and supplied daily with host pupae; and each day’s brood ($F_1$ generation) was scored as + (diapausing) or as — (developing). In this way the proportion of females of the parental generation which produced diapausing offspring was ascertained for each day of reproductive life.

The developing $F_1$ larvae were allowed to reach maturity. Twenty pairs were taken from the offspring of cross (1) and allowed to breed; similarly, 20 pairs from the offspring of cross (2). Each mating pair was isolated as before and each day’s brood was scored as + or —. In this way the proportion of females of the $F_1$ generation which produced diapausing offspring was ascertained for each day of reproductive life.

As controls the offspring of WH + females x WH + males and of C females x C males were also scored for two generations.

The results of this experiment (Fig. 2a, b) showed that the patterns of diapause production were the same for C strain females mated whether with C strain males (control) or with WH + strain males (cross (1)); and similarly for WH + females, mutatis mutandis. But the patterns of diapause production shown by the $F_1$ females from the crosses were intermediate between those shown by C females and those shown by WH + females. These two experiments show that, between one generation and the next, the mechanism of diapause induction is a purely maternal one and the males play no part in determining the diapause-characteristics of their immediate progeny. The mechanism is, however, under ultimate genetic control because the males contribute towards the genotype of their grandchildren and the strain characteristics. Between the mother and her progeny, the mechanism is probably a cytoplasmic one.

Further evidence for the underlying genetic control of diapause-production in Nasonia is provided by the differences between the two strains. Throughout these experiments females of the C strain have produced a much greater proportion of diapause larvae among their progeny than have females of the WH + strain under the same conditions. However, it is not known whether this represents a natural difference between the populations, or whether it is the result of the 13 years of continuous laboratory culture of the WH + strain which might have selected a relatively diapause-free line.

(b) The effects of temperature and photoperiod

Chilling newly emerged females of the WH + strain at 10° C in darkness for 5 days after emergence causes an increase in the proportion of females which produce diapause larvae when returned to 25° C. This effect is temporary (Fig. 3b) and, although a few females may continue producing diapause larvae throughout life, the majority of them switch to the production of developing progeny after a few days. This result agrees with that of Schneiderman & Horwitz (1958). Chilling the C strain for a similar period at 10° C causes a similar increase of diapause (Fig. 3a) which tends to wear off a few days after return to 25° C. Since females of both strains continue to lay eggs giving rise to diapause larvae for several days after returning to 25° C., these results demonstrate that the period at 10° C affects the maternal physiology and not that of the eggs deposited on the host. Females of the C strain also show an increased tendency to produce larvae again later in life, although this increase with age occurs later than that in the control group kept throughout imaginal life at 25° C. (Fig. 3a). This
Fig. 3. The effect of a period of chilling (in continuous darkness) on the production of diapause larvae by females of *Nasonia vitripennis*. (a) C strain: ○—○, 15 females at 25° C.; •—•, 15 females chilled at 10° C. for 5 days and then returned to 25° C.; •—•, 10 females chilled at 2° C. for 5 days and then returned to 25° C. (b) WH + strain. ○—○, 10 females at 25° C.; •—•, 16 females chilled at 10° C. for 5 days then returned to 25° C.
result suggests that the period of chilling has two effects. First, it causes a delay in egg production and accentuates the small initial production of diapause larvae seen at 25° C. and, secondly, it delays the effect of age. Chilling at 2° C. for the first 5 days had no effect, showing that this temperature is too low to induce diapause (Fig. 3a).

Experiments were also conducted in which females were kept throughout their reproductive life at temperatures lower than 25° C. In both strains lowering the temperature to 20° C. or 15° C. caused the females to produce an increased proportion of diapause larvae, but the manner in which this was accomplished was dependent upon illumination. Although an increased proportion of C strain females kept in continual darkness at 15° C. and 20° C. produced diapause larvae throughout life the change-over from developing to diapause larvae was not completed until a few days later than it was at 25° C. (Fig. 4a and 5a). However, at these lower temperatures egg production is lower and the length of reproductive life is extended, so that a much greater proportion of the progeny enter diapause at 15° C. or 20° C. than at 25° C. Females of the WH + strain kept at 15° C. or 20° C. in darkness also produced diapause larvae at the end of life, the switch from developing to diapause larvae occurring between the 20th and 25th days (Figs. 4b and 5b). This result suggests that WH + females do not show an increase in the production of diapause larvae with age at 25° C. because they do not live long enough; lowering the temperature extends reproductive life and the maternal switch in this strain becomes apparent.

The most important factor affecting induction of larval diapause in *Nasonia* becomes apparent when the daylength experienced by the maternal generation is studied. An artificial short daylength of 6 hr. light per 24, or a natural short (December to January) daylength caused the maternal switch mechanism in the C strain to operate sooner. Of the 45 females incubated at short daylength at 15° C. eight produced diapause larvae throughout life and the remainder had completed the switch to diapause larvae by the 9th day (Fig. 5a). In the WH + strain the change-over from the production of developing to diapause larvae was brought forward in short daylength from about the 20th to 25th day to between the 9th and the 17th (Fig. 5b). Therefore, at short day-length, the greater proportion of the progeny of both strains entered diapause. Rearing females of both strains in a water bath at 15° C. and exposed to natural long (May to June) daylength in a north-facing laboratory resulted in the complete suppression of diapause (Figs. 5a and b).

Two experiments were also performed in which females of the C strain were transferred from short to long daylength, or from long to short daylength, during imaginal life. Fifteen females were given short-day cycles for 9 days at 15° C. and then transferred to long daylength at the same temperature. After the nine short-day cycles the switch to the production of diapause larvae was complete (Fig. 6). After transfer to long daylength they produced diapause larvae for a further 8 days and then began to switch back to the production of developing progeny. In the converse experiment 15 females received 10 long-day cycles and were then transferred to short daylength. They continued producing developing progeny for 5 days after transfer and then rapidly switched to the production of diapause larvae. These results show that the mechanism which controls the production of diapause and developing larvae in *Nasonia* is reversible during imaginal life, and that 9 or 10 short-day cycles are sufficient to complete the switch to diapause larvae.
From this evidence it is clear that *Nasonia* is a typical 'long day' insect in its responses to photoperiod (de Wilde, 1962). Short days and cool conditions increase the production of diapause larvae, and long days and high temperatures avert diapause and allow uninterrupted growth. Conditions of continual darkness are 'intermediate' in

**Fig. 4.** The effect of temperature on the production of diapause larvae by females of *Nasonia vitripennis* kept in continuous darkness. (a) ○—○, 15 C strain females at 25°C.; ●—●, 30°C strain females at 20°C. (b) ○—○, 14 WH+ strain females at 25°C.; ●—●, 29 WH+ strain females at 20°C.

character and, especially in the WH+ strain at 25°C., show a fluctuation from one type of larva to another. However, because of the transovarian mechanism of induction and the evident importance of daily egg production in *Nasonia*, the manner in which the environmental factors express themselves differs from that in more normal
long-day' insects in which the sensitive stage and the resulting diapause occur in the same individual.

(c) The effect of host deprivation

Schneiderman & Horwitz (1958) showed that depriving females of the WH + strain of hosts for a few days after emergence caused an increase in the production of dia-

![Graph a](image1.png)

![Graph b](image2.png)

Fig. 5. The effect of temperature and photoperiod on the production of diapause larvae by females of *Nasonia vitripennis*. (a) O—O, 30°C strain females at 25°C in darkness; O—O, 42°C strain females at 15°C in darkness; O—O, 45°C strain females at 15°C and 6 hr. light per 24; O—O, 15°C strain females at 15°C and natural short (December-January) daylength; x—x, 15°C strain females at 15°C and natural long (May-June) daylength. (b) O—O, 30 WH + strain females at 25°C in darkness; O—O, 45 WH + strain females at 15°C in darkness; O—O, 44 WH + strain females at 15°C and 6 hr. light per 24. x—x, 12 WH + strain females at 15°C and natural long (May-June) daylength.
Larval diapause of maternal origin

They also showed that increasing the period without hosts raised the proportion of diapause larvae in the progeny.

In the present experiments this result has also been obtained with the C strain. Depriving females for 4 days at 25°C (Fig. 7a) and then providing them with host pupae caused an increase in the proportion of females producing diapause offspring. A period of host deprivation in the middle of the reproductive life had a similar although less well-marked effect, and alternate days without hosts (Fig. 7b), an experiment designed to simulate a situation of host shortage, resulted in a higher proportion of females producing diapause larvae throughout life.

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**Fig. 6.** The effect of transferring C strain females from short to long, and long to short, day-lengths (15°C). •—•, 15 females at 6 hr. light per 24; •—•—•, 15 females at 6 hr. light per 24 for 9 cycles, then transferred to natural long day length at A; O—O, 15 females at natural long daylength; O—O—O, 7 females at natural long daylength for 10 cycles, then transferred to 6 hr. light per day at B.

**Fig. 7.** The effect of host deprivation on the production of diapause larvae by females of the C strain (in darkness at 25°C). (a) O—O, 10 females with 2 host pupae per day; •—•, 16 females deprived of hosts for the first 4 days, then supplied with 2 host pupae per day. (b) O—O, 10 females with 2 host pupae per day; •—•, 20 females supplied with, and deprived of, hosts on alternate days.
Larval diapause in *Nasonia vitripennis* is induced by four factors which affect the maternal generation but have no effect upon the developing immature stages themselves. Three of these factors—daylength, temperature and host deprivation—are directly involved. If these factors are analysed in relation to daily egg production the fourth factor, maternal age, becomes apparent, and diapause larvae are produced in a distinct age-pattern whereby young females produce developing offspring and then switch over at some time to the production of eggs which give rise to diapause larvae. Since it is independent of temperature, this age-pattern does not seem to be associated with senescence, but is rather the manner in which daylength, temperature and host deprivation express themselves via egg production.

Since the sensitive stage and the resulting diapause occur in successive generations, the effects of these factors must be passed from the parent female to the larva through the egg. The fact that virgin females produce diapause larvae in exactly the same manner as fertilized females, and that crosses between the two strains produce a pattern of diapause-production intermediate between the two in the *F*₁ generation but not in the *F*₂ generation, shows that this effect is solely a maternal one, although there is ultimate bi-parental control of the diapause-characteristics of the strain. In all probability this mechanism, whereby factors operating in the female parent dictate the pattern of diapause-production in the offspring, is a cytoplasmic one, and the eggs laid by diapause-producing females differ qualitatively, or perhaps quantitatively in a fraction of their chemical composition, from eggs laid by normal females. Since larval diapause in *Nasonia* appears to be of the normal larval-pupal type involving an inactivation of the neuro-secretory processes in the brain (Schneiderman & Horwitz, 1958), this chemical difference must effect, no doubt by an indirect route, the endocrine mechanisms of the larva. This state of affairs, therefore, differs significantly from that in the silk-worm, *Bombyx mori*, in which the female produces a 'diapause hormone' from the suboesophageal ganglion causing diapause in the egg before the development of the embryonic endocrine system (Fukuda, 1951; Hasegawa, 1951).

Although the difference between the eggs laid by diapause-producing and normal females can only be determined by chemical investigation, two simple hypotheses present themselves. First, a substance, or substances, amounting to a 'diapause-inducing factor' (a hormone?) could enter the cytoplasm of diapause eggs and lead to the inhibition of neuro-secretion in the larval brain; or, alternatively, a substance which is present in the eggs of 'normal' females and necessary for development at the larval-pupal moult could be absent from the eggs laid by diapause-producing females. The results of the present investigation tend to favour the first hypothesis, and they are interpreted in this light. However, the mechanism of induction of diapause in *Nasonia* involves several different organ systems and, as in other, less spectacular, instances of delayed photoperiod response, must have a built-in mechanism for 'counting the instars' (Wigglesworth, 1948). It must, therefore, be more complicated than the simple hypothesis presented here. The alternative suggestion (i.e. a 'development factor') is also possible, and the final decision will have to await suitable chemical investigation.

Daylength and temperature are obviously the most important factors inducing
Larval diapause of maternal origin

diapause in *Nasonia* and, apart from the transmission of the effect through the cytoplasm of the egg, the way in which the insect responds is remarkably similar to that in more typical examples of photoperiod induction. *Nasonia* is a 'long-day' insect, diapause being induced by combinations of short daylength and moderate temperature and averted by long daylength and high temperature. Conditions of continual darkness have an 'intermediate' effect (Lees, 1955), with some females changing to the production of diapause larvae early and some late in life. At 15° C. 5–9 short-day cycles seem to be sufficient to switch C strain females to the production of diapause larvae, and 9–17 cycles for the WH+ strain.

Lees (1960, 1964) has demonstrated that the centre of photoperiod reception in the aphid, *Megoura viciae*, is situated in the head, most probably in the pars intercerebralis of the brain. Although de Wilde (1958) and Beck & Alexander (1964) have reported the existence of extracephalic photoreceptors, it seems probable that the brain is involved in most cases of photoperiodic induction, and that the factors inducing diapause in *Nasonia* affect the brain and not the ovaries directly. This being so, the 'diapause-inducing factor' is probably produced by the brain or an associated structure during short-day cycles of illumination, and is passed into the haemolymph and thence into the developing oocytes in the ovary. Since larvae can either enter diapause or develop directly without any intermediate conditions of prolonged development, there appears to be a threshold mechanism operating in the egg. When the concentration of the 'diapause-inducing factor' rises above this threshold the eggs give rise to larvae which enter diapause.

Temperature and host deprivation, according to this hypothesis, would affect the incidence of diapause larvae by altering the rate of egg production. Lowering the temperature lowers the rate of egg production and lengthens reproductive life. By extending the period of reproduction without greatly delaying the completion of the maternal switch, lower temperature increases the proportion of the progeny which enter diapause. Host deprivation has been shown to delay the onset of oviposition because of the lack of suitable protein normally obtained from host blood (Edwards, 1954). Assuming that the photoperiod processes continue in the brain during this period, the diapause-inducing factor would build up in the haemolymph and on resumption of ovarian development enter the eggs in high concentration and induce diapause in the progeny.

**SUMMARY**

1. The environmental factors responsible for the induction of larval diapause in *Nasonia vitripennis* are photoperiod, temperature and host deprivation. They are effective during the life of the female parent and not during larval development.

2. Short daylength and low temperature induce females to produce diapause larvae early in reproductive life and long daylength and high temperature avert diapause. Conditions of darkness are 'intermediate' in this respect. The effects of daylength are reversible during the life of the female.

3. Females switch from the production of developing to diapause larvae during their reproductive life, so that a distinct age pattern is apparent. The two strains of *Nasonia* used in this investigation showed different patterns of diapause production. Although this difference is shown to be under ultimate genetic control, the mechanism of in-
duction is purely maternal and the males play no part in determining the diapause characteristics of their immediate progeny.

4. Unlike other cases of photoperiodic induction of larval diapause, the mechanism cannot operate solely through the central nervous system and a chemical ‘factor’ (a hormone?) passed from the parent female through the egg to the larva is postulated.

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