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PHOTOPERIODIC CONTROL OF DEVELOPMENT IN THE NEW ZEALAND LEAFROLLER MOTH
PLANOTORTRIX OCTO DUGDALE (LEPIDOPTERA, TORTRICIDAE)

Michael Charles Morris

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in zoology, University of Auckland.

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Lastly thanks to God for providing me with the inspiration and desire to enjoy the study of His creation.
ABSTRACT

The aim of this study is to test for photoperiodic control of larval and pupal development in the New Zealand moth Planotortrix octo Dugdale.

The photoperiodic response curves for larval and pupal development and especially for instar number at 17°C and 21°C indicate that a photoperiodic mechanism is involved. Superimposed on this response is the suggestion that daylength affects development rate directly, with larvae and pupae developing faster under longer photophases. This effect is especially strong for pupal development (Chapter 3).

The effects of thermophotoperiods (Chapter 4), night interruption and resonance experiments (Chapter 6) provide further evidence for photoperiodic involvement. The response to resonance experiments suggests the involvement of an hourglass rather than a circadian mechanism.

Larvae reared under short days accumulate significantly more lipids in the 5th and 6th instars than larvae reared under long days (Chapter 4). This finding, combined with the suppressed development rate and higher instar number under short days, suggests that a weak form of diapause may be present in this insect. This is significant in being the first recorded incidence of a photoperiodically induced diapause in a phyllophagous New Zealand insect for which a year round food supply is available (Chapter 1).

By transferring insects from long to short days I found that long days have more influence than short days on larval development (Chapter 7).

An attempt was made to measure juvenile hormone titres under long and short days using a Galleria bioassay. The test used was not sensitive enough however to measure any significant amounts of juvenile hormone (Chapter 8).

Simulations of the experimental results were performed using a damped circadian oscillator model (Chapter 9). This model was considered the most appropriate to use, based on the experimental results and on a review of the literature (Chapter 2). Simulations showed good similarities with experimental results in most cases, but could not account for resonance responses.
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<th>Abbreviation</th>
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<tbody>
<tr>
<td>DD</td>
<td>Continual darkness.</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography - mass spectroscopy.</td>
</tr>
<tr>
<td>GPD</td>
<td>General purpose diet.</td>
</tr>
<tr>
<td>GU</td>
<td>Galleria unit.</td>
</tr>
<tr>
<td>h</td>
<td>Hours.</td>
</tr>
<tr>
<td>INDSUM</td>
<td>Diapause induction titre.</td>
</tr>
<tr>
<td>JH</td>
<td>Juvenile hormone.</td>
</tr>
<tr>
<td>Lx</td>
<td>Duration of instar x.</td>
</tr>
<tr>
<td>LD$x_1:x_2$</td>
<td>A light-dark cycle where $x_1$ and $x_2$ are the time of the light and dark period respectively in hours.</td>
</tr>
<tr>
<td>LDLD$x_1:x_2:x_3:x_4$</td>
<td>As above only the light periods are $x_1$ and $x_3$ and the dark periods are $x_2$ and $x_4$.</td>
</tr>
<tr>
<td>LL</td>
<td>Continual light.</td>
</tr>
<tr>
<td>PPRC</td>
<td>Photoperiodic response curve.</td>
</tr>
<tr>
<td>PTTH</td>
<td>Prothoracicotropin hormone.</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay.</td>
</tr>
<tr>
<td>T</td>
<td>Period of the light/dark cycle.</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Free running period of a biological rhythm.</td>
</tr>
<tr>
<td>$\varphi_i$</td>
<td>Photoinducible phase.</td>
</tr>
<tr>
<td>Wx</td>
<td>Head capsule width of instar x.</td>
</tr>
<tr>
<td>ZT</td>
<td>Zeitgeber time.</td>
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1.1. Photoperiodism in Living Systems

Early records on the effects of daylength on a variety of plants have been reported by Garner and Allard (1920, cited Saunders, 1982a). Since this pioneering study, daylength has been found to control many seasonally related physiological functions in a range of organisms. Processes under photoperiodic control include reproductive cycles in mammals and birds and flowering and germination in plants (see Vince-Prue, 1975; Follett and Follett, 1976; Saunders, 1977 for reviews).

All these organisms often show common responses to photoperiod in that there is a sharp distinction between two types of physiological function at either side of a critical photoperiod. This critical photoperiod has been observed in plants, mammals and birds (Vince-Prue, 1975; Saunders, 1977; Elliot, 1981) as well as being widespread in insects (Beck, 1980; Saunders, 1982a), suggesting that photoperiodism has common elements of control.

In insects, photoperiodism plays an important part in seasonal control. The photoperiod is often used as an indicator of season, allowing insects to react in advance to unfavourable conditions.

1.1.1. Migration in Insects

Migration is commonly employed by insects to escape from unfavourable extremes of temperature (Dingle, 1985), and is frequently associated with seasonal changes in morphology and behaviour.

During unfavourable conditions the wings of some migratory species are larger in proportion to the body weight, while in favourable conditions wings can be small or absent. This is believed to be an adaptation which allows easy migration from winter conditions (Harrison, 1980; Dingle, 1985). Photoperiodically induced wing polymorphism is documented in aphids (Lees,
Seasonal behavioural changes brought about by photoperiod include migratory restlessness (Harrison, 1981; Dingle, 1985) and calling behaviour. Mating behaviour has been studied extensively in the army worm *Pseudaletia unipuncta* by Delisle and McNeil (1986, 1987). These authors found a delay in the female calling response under short days and low temperatures. McNeil (1987) believes this is an adaptation allowing it to migrate to its winter breeding grounds before starting to mate.

1.1.2. Diapause in Insects

Diapause has been defined as a dormancy which is brought about by token stimuli such as photoperiod. This can be contrasted with quiescence in which dormancy is directly brought about or terminated by unfavourable conditions (Tauber *et al.*, 1986).

Diapause is the most commonly studied photoperiodic phenomenon in insects. Saunders (1982a) lists over 300 species in 15 orders in which photoperiodic induction of diapause has been demonstrated or reasonably inferred.

Characteristics of diapause include a reduced rate of development (Beck, 1980; Rock and Shaffer, 1983; Danks, 1987), accumulated food reserves such as fat and glycerol (Danilevski, 1965; Downer and Matthews, 1976; Adedokum and Denlinger, 1985) and a reduced metabolic rate (Danilevski, 1965). Diapause can be found during any stage of the life cycle, but within species it normally occurs at one specific stage (Roberts, 1978; Saunders, 1982a). It is anticipatory and usually starts while conditions still permit growth and development (Roberts, 1978). Often, but not always it requires a specific stimulus such as photoperiod or prolonged chilling to terminate it (Tauber and Tauber, 1976).

The stage of the life cycle that is sensitive to photoperiod usually occurs before the diapause stage. It is often the early larval stages which are sensitive (Saunders, 1982a) but in some species such as *Nasonia vitripennis* (Saunders, 1966) and in Calliphorid flies (Vinogradova, 1985) the females of the previous generation show the sensitivity to photoperiod.
Fig. 1.1.

1.1.3. Photoperiodic Response Curves

A photoperiodic response curve (PPRC) plots the percentage of one particular strategy (e.g. diapause) adopted by a population of insects, against the photoperiod. Beck (1980) classified these curves into four types, which are reproduced in Fig. 1.1.

Most insects show the Type 1 or long-day response, characterised by a drop off in diapause during long days. Often these also show reduced diapause in very short photoperiods. In some cases there is no diapause at short daylengths and the result is a Type 3 curve. Since the short days are outside normal ecological cycles, insects with Type 2 curves effectively function as long-day insects under natural conditions.

Some Type 1 insects also show increased diapause in very long days. These include *Bombyx mori* (Masaki, 1984), *Pectoniphora gossypiella* (Pittendrigh and Minis, 1971), *Leptinotarsa decemlineata* (Danilevski, 1965) (Lepidoptera) and *Aphis fabae* (Vaz Nunes and Hardie, 1987) (Hemiptera: Aphididae)

Some insects diapause over the summer when conditions are too hot or dry for development. In the laboratory these short-day insects show a diapause response at long days, a Type 2 curve. This response has been documented in about 40 insect species (Masaki, 1980).

Other insects show a Type 4 curve where diapause is absent only during restricted photoperiods. Species in which this has been found are *Leucoma salicis*, *Euproctis similis*, *Ostrinia nubilalis* northern strain (Danilevski, 1965), *Carposina niponensis* (Toshima et al., 1961 and *Stathmopoda aposema* (Muggleston, 1988), all of these being Lepidoptera. A Type 4 response has also been documented for ovarian diapause at low temperatures in *Drosophila melanogaster* (Saunders et al., 1989).

1.1.4. Effects of Temperature

The diapause response can be modified by the environmental temperature. In general, diapause increases with lower temperatures in long-day insects and decreases in short-day species (Danilevski, 1965; Beck, 1980; Saunders, 1982a). However, diapause incidence in some long-day species can often drop under low
temperatures. This has been documented in *Grapholitha molesta* (Dickson, 1949), *Chloridia obsoleta* (Danilevski, 1965) and *M. vicinae* (Lees, 1986).

Laboratory experiments on some insect species show that the photoperiod can be replaced by daily temperature cycles in the dark. Thermoperiodic diapause induction is well documented in insects (Saunders, 1973b; Chippendale et al., 1976; DuMortier and Brunnarius, 1977b; Masaki and Kikukawa, 1981; Beck, 1982) and in one mite (Van Houten et al., 1988). All of these species show Type 1 or Type 3 curves for diapause induction. A thermoperiodic response affecting antifreeze production has also been demonstrated in the beetle *Dendroides canadensis* (Horworth and Duman, 1986). Conversely, thermoperiodic responses have been sought and found to be absent in *Sarcophaga argyrostoma* (Diptera) (Saunders, 1978, 1984) and *Platynota idaeusalis* (Lepidoptera) (Rock, 1983).

Combining a thermoperiod with a photoperiod can affect the diapause response depending on the phase relationship between the two cycles. In many species, the diapause response is intensified when the thermophase or high temperature pulse coincides with the photophase, and reduced when the scotophase is warmer. This is documented in several Lepidoptera (Beck, 1962; Goryshin, 1964; Chippendale et al., 1976; Thurston, 1976) as well as in two Diptera species (Ratte, 1979; Bradshaw, 1980) and a weevil (Cobb and Bass, 1968).

### 1.1.5. Photoperiod and Development Rate

The growth rate of immature life cycle stages can often be controlled by daylength. This is particularly common among Odonata where the duration of one nymphal stage is finely tuned by daylength changes in order to synchronise emergence (Corbet, 1962; Lutz, 1974a,b). Danks (1987) cites several species including 33 Lepidoptera where development rate is controlled by photoperiod. Many of these are diapausing species, but photoperiod can also affect the development rate of some non-diapausin insects (see Atwal, 1953; Hanna et al., 1977; Ochieng'-Odero, 1988). These authors only tested the response at a few photoperiods, so a true PPRC has not been demonstrated.
1.2. Diapause in New Zealand Insects

Note: A substantial part of this section has been published (Morris, 1989).

1.2.1. Introduction

Diapause has been extensively studied in northern hemisphere insects and several books on the subject have been published (Danilevski, 1965; Beck, 1980; Saunders, 1982a; Tauber et al., 1986; Danks, 1987). In contrast, the evidence for photoperiodically induced diapause in New Zealand insects is slim, suggesting that diapause is rare or absent in New Zealand.

Overwintering strategies in New Zealand insects were first reviewed by Roberts (1977, 1978). She cited only two cases where true diapause appeared to have been accurately inferred, both non-phytophagous species. She went on to suggest that its rarity in phytophagous insects was a result of the availability of a year round food supply from the predominantly evergreen native flora. The predominance of non-diapause insects and evergreen plant species was attributed by Dumbleton (1967) to the warmer climate in New Zealand during the Pleistocene when diapause was evolving in the northern hemisphere.

In support of this hypothesis, Ramsay (1978) and Watt (1978) report vastly reduced incidences of seasonality in New Zealand Orthoptera and Coleoptera respectively, when compared with northern hemisphere species. Gibbs (1980) also notes that diapause is absent in all 11 species of New Zealand butterflies. Winterbourne (1978) and Towns (1981) looked at the life cycles of New Zealand freshwater insects and noted the lack of seasonal synchrony compared with similar habitats in the northern hemisphere. Diapause is a major factor in achieving synchronisation in insects (Lutz, 1974a,b; Tauber et al., 1986; Danks, 1987) so the presence of seasonality or synchronisation of life cycle stage in field studies is a useful indicator of diapause incidence. The lack of synchronisation in New Zealand insect therefore suggests a paucity of true diapause.

Studies on New Zealand populations of the Argentine stem weevil *Listronotus bonariensis* by Goldson and Emberson (1980) demonstrated the existence of a true diapause, but no variation in critical daylength with latitude. The authors suggest that this diapause is a "relict" response which has not evolved further
since the species arrived in New Zealand, as it confers no adaptive advantage for this species under New Zealand conditions.

1.2.2. Evidence for Diapause in New Zealand Insects

The above published reports (Section 1.2.1) support Dumbleton's (1967) hypothesis that diapause is rare in New Zealand insects. However, recent experimental work on two species of Lepidoptera suggest that another review of the literature and an assessment of the applicability of this theory may be warranted.

**Lepidoptera.** Experimental work on two phyllophagous native moths has revealed the influence of photoperiod on larval duration and instar number. Ochieng-Odero (1988) tested the duration of the final instar in 'Cnephasia' jactatana Walker (Tortricidae) under five photoperiods. The duration of the final instar in this species show a Type 3 (Beck, 1980) photoperiodic response, and the author suggests the circadian system is involved.

Field observations on the endemic species *Ovocrambus vitellus* (Pyralidae) and *O. flexuosus* revealed a vastly lengthened final instar in winter populations (Gaskin, 1975).

Although presence of a full diapause response in the above two species is far from conclusive, there is some evidence of a diapause mechanism being involved. Danks (1987) cites several instances where instar number and larval duration are influenced by photoperiod, with the comment that this is normally associated with diapausing species. Rock and Shaffer (1983) suggest that lengthened instar durations can be regarded as part of diapause. The experimental demonstration of a photoperiodic response curve in *C. jactatana* also suggests a diapause mechanism and not merely a direct effect of photoperiod.

A photoperiodically induced diapause has recently been reported in laboratory studies of *Stathmopoda aposena*, a native moth feeding on seasonally produced Kowhai seed pods (Muggleston, 1988). This species has a lengthened prepupal stage and diapauses over all but a narrow range of photoperiods. Its photoperiodic response curve is that of a long-short day (Type 4 of Beck, 1980) species, a rare phenomenon among Lepidoptera (Muggleston, 1988).
The introduced tortricid *Laspeyresia pomonella* exhibits a diapause, a trait that it evolved in the northern hemisphere. In contrast with the Argentine stem weevil mentioned earlier, different populations in New Zealand show a variation in critical day length with latitude (Roberts, 1978). Diapause has continued to evolve in this species, suggesting that this strategy can be advantageous in New Zealand conditions. This may be related to the seasonally limited fruit diet of *L. pomonella* compared with the leaf eating *L. bonariensis*.

**Coleoptera.** The indigenous weevil *Praolepra uniformis* is one example of diapause mentioned by Roberts (1977). *P. uniformis* exists in close synchrony with its host plant, diapausing inside mature fruit and emerging 16-18 months later.

**Freshwater Insects.** As mentioned earlier, field studies of New Zealand freshwater insects reveal a lack of synchrony of life cycles compared with northern hemisphere species. Exceptions have been found in *Zelandobius confusus* and *Z. furcillatus* (Plecoptera) and in *Zelandopsyche ingens* (Trichoptera), all of which show synchrony of life cycle stages. *Z. ingens* is also reported to overwinter in the final instar (Winterbourne, 1978).

Among native Odonata, diapause has been reported in the field in the final and penultimate instars of *Xanthocnemus zealandia* (Deacon, 1978; Rowe, 1987), and in the egg stage of *Austrolestes colensonis* (Deacon, 1978) and *Procordula smithii* (Rowe, 1987).

The native Corixidae *Sigara arguta*, *Anisops assimilis* and *A. wakefieldi* show ovarian regression similar to their British counterparts, though spring ovarian development takes place earlier in New Zealand (Young, 1978).

**Hymenoptera.** Harris (1974) found a prepupal diapause in the native spider-eating wasps *Sphictostethus nitidus* and *Priocnemis carbonarius*. These were mentioned by Roberts (1977) as one of the two records of diapause in New Zealand. It is not clear from the description whether diapause is facultative or obligatory.
The prepupal stage of the native bee *Leioproctus boltoni* has also been found to overwinter. Pupation does not occur until October, even when the temperatures were raised (Donovan 1968), suggesting that diapause could be involved.

A similar lengthened prepupal stage has been observed in Auckland populations of the Mason wasp *Pison spinolae*. Pre pupae in the winter generation cease development as early as February (Cowley, 1959). The onset of dormancy while the weather is still warm suggests a true diapause and not a winter quiescence, especially as diapause is terminated in August with subsequent development during cooler months than February. The author notes that *P. spinolae* is well established in New Zealand, but also found in the eastern region of Australia, leaving its endemicity in doubt.

**Orthoptera.** A facultative egg diapause has been found in the native grasshopper *Phaulacridium imaginale* (Ramsay, 1978) and in the native phyllophagous crickets *Pteronemobius nigrovus* and *P. bigelowii* (McIntyre, 1978). Parkes (1972) also compared two populations of *Pteronemobius* spp. Field studies on the inland population revealed seasonal synchrony and an egg diapause. In contrast, the population inhabiting the warmer coastal environment showed no synchrony or diapause. From these observations it appears that diapause in *Pteronemobius* species is influenced by temperature. The role of photoperiod has not been studied.

### 1.2.3. Conclusion

Diapause in insects can come about as a protection against adverse climatic conditions or shortage of food (Roberts, 1978). According to Dumbleton’s (1967) hypothesis, adaptations to the former should be rare, while the presence of a year round food supply would make the latter adaptation unnecessary in New Zealand leaf-eating species (Roberts, 1978).

Most published reports support this hypothesis with the few cases of diapause being restricted to insects with a seasonally limited food supply. However, the presence of diapause in native phyllophagous species and especially photoperiodic control of development in phyllophagous Lepidoptera reopens the question of the frequency of diapause in indigenous species and the value of diapause in New Zealand conditions. One possible reason for diapause in some New Zealand species could be the advantage of synchronising life cycle stages.
This would ensure that adults emerge over a short period and would facilitate reproduction (Tauber et al., 1986).

Many observations of overwintering in New Zealand come from field studies where it is difficult to distinguish between diapause and quiescence. A closer look at these species under laboratory conditions might give further insight into the nature of the diapause response in New Zealand and the relative frequency of diapause in New Zealand insects compared with overseas species.

1.3. Hormonal Control of Diapause

During normal insect development, larval moulting is initiated by the secretion of prothoracicotropic hormone (PTTH) from neurosecretory cells in the corpora cardiaca. This stimulates the prothoracic glands into producing ecdysone, the moulting hormone. During larval diapause, the secretion of PTTH is inhibited, resulting in non-activated prothoracic glands. The larva does not moult, and instead undergoes a period of suppressed development (see Saunders, 1982a; Denlinger, 1985 for reviews).

1.3.1. Juvenile Hormone and Larval Development

Larval-pupal metamorphosis and the role of JH has been extensively studied in the tobacco hornmoth Manduca sexta by Nijhout and Williams (1974a,b). In this species JH titres are high during the first stages of the final instar. These high titres delay the production of PTTH, which initiates the pupal moult. Normally PTTH is released as JH titre declines, and pupation is initiated. If the titre is kept artificially high by injections of JH, PTTH secretion is delayed and the final instar duration prolonged (Nijhout and Williams, 1974b).

Inhibition of PTTH secretion by JH has also been documented in final instar Mamestra brassicae (Hiruma et al., 1978) and in Galleria mellonella (Sehnal et al., 1981). The effect of JH in prolonging the final instar and inducing extra larval moults or larval-pupal intermediates is well documented in other Lepidoptera by Sehnal (1976).

The release of PTTH prior to the next moult in penultimate instar M. sexta occurs just after the corpora allata stop producing JH. This suggests that the
same mechanism of PTTH inhibition may occur in other instars beside the final one.

1.3.2. Juvenile Hormone and Larval Diapause

The role of JH in larval diapause has been studied intensively in Diatraea grandiosella (Chippendale and Yin, 1973; Yin and Chippendale, 1973) and Chilo suppressalis (Yagi and Fukaya, 1974). In diapause individuals, the JH titre remains high during the final instar, and the larval period is prolonged. An additional one or two instars can also develop in which there is no growth (Chippendale and Yin, 1973).

In the codling moth L. pomonella, larval diapause is induced but not maintained by a high level of JH. The JH titre is high in diapause-destined larvae, but declines once the diapause stage has been reached (Sieber and Benz, 1977).

These examples involve larval diapause in the final instar, but there is some indication that JH could also be involved in other instars. Under short-day conditions, the butterfly Lycaena deimo shows a supernumerary instar with no growth between the 2nd and 3rd moult (Endo et al., 1984) and in the moths Spilactia imparalis (Sugiki and Masaki, 1972) and Amanthes c-nigrum (Honek, 1979) the final instars of diapausing populations all show a reduced Dyar's coefficient indicative of a reduced growth rate. When the PTTH inhibition of penultimate instar M. sexta larvae is taken into account (Bollenbacher et al., 1987), there is good circumstantial evidence for the involvement of JH in controlling moulting and larval diapause in non-final instars.

1.4. The Study Animal

1.4.1. Classification

Planotortrix octo Dugdale 1990 (in press) (Lepidoptera, Tortricidae, Tortricinae) is endemic to New Zealand. It is found throughout New Zealand, including offshore islands (Dugdale, 1990) and is common around Canterbury, Otago and Bay of Plenty (Foster et al., 1986).

The morphological characteristics of the genus Planotortrix were defined by Dugdale (1966). Until this year, P. octo was classified as P. excessana Walker.
However, field trapping experiments reveal distinct differences in mate recognition systems among different strains of the "P. excessana" complex (Foster et al., 1986) suggesting several cryptic species could be involved. *P. octo* was described as *P. excessana* Type A by Foster et al. (1986) and Foster and Dugdale (1988) based on the pheromone chemistry and the male response in field trappings.

Dugdale (1990) has reclassified the "*P. excessana*" complex of Foster and Dugdale (1988) into four species, *P. excessana*, *P. octo*, *P. octoides* and *P. avicenniae*. *P. octo* is distinguished from *P. excessana* by its pheromone components.

### 1.4.2. Life Cycle

Thomas (1979) describes the "*P. excessana*" complex as having two to four overlapping generations per year depending on the latitude and host plant with an overwintering stage occurring mainly in the 2nd-4th instars. Green (1984) found three overlapping generations in an Auckland orchard. Clark (1936) describes a larval period in Canterbury of 45-60 days, with a longer overwintering period, and a pupal duration of two to three weeks.

Eggs are laid in batches of 2 to 170 (Thomas, 1979). Caterpillars develop through five or six instars in the field (Green, 1984) and in the laboratory (G. Clare, unpubl. data). The head capsule of the early instars gradually slip before being shed. This feature helps distinguish final and non-final 5th instar caterpillars.

*P. octo* can be sexed by examining the pupae. The female pupae have three abdominal sutures ventral to the wing pads while the males have four. This is a typical feature of tortricid pupae (Zenner-Polania and Helgesen, 1973).

Insects used during this study were originally collected from Canterbury, New Zealand. They had been reared by the DSIR Insect Rearing Division since 1982 (Hobson and Singh, 1987). They were reared from eggs to pupae on general purpose diet (GPD) (Singh, 1983) at 20°C and under a LD 18:6 photoperiod. Pupae were collected and placed in oviposition boxes at 18°C. The eclosed adults mate and oviposit in these boxes, and the eggs were stored at 10°C in DD until used for the study (Clare et al., 1987). An attempt was made to rear
P. octo for the study. When adults were mated at 20°C, the resulting eggs were sterile. It was later confirmed by P. Singh (pers. comm.) that this temperature is too high for successful breeding. Since eggs could be easily obtained from the DSIR, attempts to rear insects were abandoned.

1.4.3. Host Plants and Pest Status

The "P. excessana" complex is one of the New Zealand leafroller pests together with the related tortricids Ctenopseutis obliquana and the Australasian Epiphyas postvittana (Green, 1984). It is commercially important as a pest species on apples (Thomas, 1979) but it has also been reported feeding on blackcurrant (Baker and Batten, 1978), pines (Pinus radiata) (Clark, 1936) and kiwifruit (Actinidia delicosa) (Stevens, 1988). It is the major leafroller pest in the South Island, especially Canterbury and Southland, with C. obliquana being more common in the North Island and E. postvittana in the central regions of the country (Thomas, 1975a).

Pest control measures include intensive spraying on kiwifruit (Stevens, 1988) and apples (Thomas, 1975b), and integrated pest control on apple orchard, combining spraying with diflurbenzuron and Baccillus thuringiensis (Wearing and Thomas, 1978). Parasitic flies and wasps were introduced in the 1960s to reduce migration into orchards by controlling migrant leafrollers from outside (Thomas, 1980).
CHAPTER TWO

MECHANISMS FOR TIME MEASUREMENT IN INSECT PHOTOPERIODISM

2.1. Introduction

Models for the photoperiodic mechanism in insects aim to increase our understanding of how photoperiod and temperature are utilised by insects to modify their physiology. According to Pavlidis (1978) a model is a hypothesis which has two essential properties. It must account for available experimental evidence and it must be able to predict further results under new conditions. In this chapter, published models of insect photoperiodism are reviewed and discussed in terms of these requirements.

Any model attempting to account for photoperiodism should be able to answer two questions on the photoperiodic mechanism. Firstly how does the insect measure the length of the days - the "clock", and secondly how are the number of long and short days summated - the "counter" (Saunders, 1981b).

2.2. The Photoperiodic Counter

In most insect species, a number of photoperiods are required to induce diapause in a population. This has led to the concept of a photoperiodic counter whereby the number of long and short day photoperiods experienced during the sensitive period are summated (Saunders, 1981b, 1982a); the end result depending on the number of long and short days experienced.

Based on the diapause response of Sarcophaga argyrostoma under different temperatures and photoperiods, Gibbs (1975) proposed that a diapause inducing substance is built up in short days.

In some species such as Tetranychus urticae (Veerman, 1977) diapause is inhibited in long days, or when transferred from short to long days, suggesting a breakdown of diapause inducing substance during long days (Vaz Nunes and Veerman, 1982b; Lewis and Saunders, 1987). In other species such as Nasonia vitripennis (Saunders, 1966) diapause can even occur during long day conditions if enough long
days are experienced, suggesting some build up of diapause substance even under long days.

The cumulative effect of photoperiod is a basic assumption for all models mentioned below.

2.3. Circadian Models for Photoperiodism

An assumption of circadian models is that photoperiodism is controlled by an internal clock similar to that controlling other rhythmical processes. Experiments with a number of organisms have shown that many rhythmical functions, in the absence of environmental cues repeat themselves with a frequency close to 24 hours. This free running period or $\tau$ is usually between 22 and 26 hours. The properties of biological clocks in insects and other organisms are reviewed by Saunders (1977, 1982a).

Circadian rhythms in insects (Saunders, 1982a) as well as in many other organisms (Saunders, 1977) can be reset if a light pulse is applied. This can cause a phase jump, so that the clock is advanced or delayed. It is this property that allows for entrainment of the clock to an external light cycle. If the period of this cycle is close to $\tau$, the advances or delays of the clock cause it to take on the period of the external cycle. Under natural conditions the internal or circadian clock is entrained to the natural 24 hour day-night cycle (Saunders, 1977, 1982a). This property of being reset by a light pulse allows the use of the experimental technique of skeleton photoperiods. These are short pulses of light placed a distance apart in a 24 hour cycle.

In the pupal eclosion rhythm of Drosophila pseudoobscura (Pittendrigh and Minis, 1964) and in the carbon dioxide rhythm of the aquatic plant Lemna perpusilla (Hillman, 1964) these short light pulses can simulate a complete photoperiod with one pulse acting as subjective dawn and the other as subjective dusk. The responses under short-day skeleton photoperiods produce good similarities with that under complete photoperiods. For example, a skeleton regime consisting of two one hour light pulses with 4 hours darkness between them gives the same response as a 6:18 hour photoperiod (Hillman, 1964; Pittendrigh and Minis, 1964).
When the skeleton pulses are close to 12 hours apart, there is a "bistability zone" (Pittendrigh, 1966) where the clock may be phase shifted so that either pulse can be interpreted as dawn. When the time between skeleton pulses becomes greater than 14 hours, the phase shift is stable, and the response is again similar to that of a short day complete photoperiod (Pittendrigh and Minis, 1964; Pittendrigh, 1966). During the "bistability zone", the response in L. perpusilla depends on the circadian time of the oscillator. The photoperiodic control of flowering depends on the number of hours of darkness experienced before the skeleton pulses are applied (Hillman, 1964).

The diapause response of Sarcophaga argyrostoma under skeleton pulses is also comparable to its response under complete photoperiods (Saunders, 1975), suggesting a circadian involvement in the photoperiodic response. The diapause incidence is high under all skeleton photoperiods except when the time between subjective dawn and dusk is close to 12 hours. This could correspond to the "bistability zone" in this species. When S. argyrostoma larvae were transferred from LL to skeleton photoperiods within the bistability zone, the ambiguity disappeared resulting in a long-day or short-day diapause response depending on which pulse was experienced first. This is analogous to the results for L. perpusilla (Hillman, 1964) and suggests a circadian response (Saunders, 1975). A similar bistability response has recently been documented in Calliphora vicina (Diptera) (Vaz Nunes et al., 1990).

Circadian rhythms are believed to be controlled by one or more chemicals inside a cell or organelle oscillating in concentration at regular intervals. Many ideas on circadian rhythmicity focus on the involvement of an oscillating chemical controlled by a negative time-delayed feedback loop (Johnsson and Karlsson, 1972; Christensen, 1978; Gander and Lewis, 1979; Christensen and Lewis, 1982).

The possible role of lipids in controlling the circadian oscillator was suggested by Njus et al., (1974), who put forward a theory involving transport of ions through lipid membranes. This action could explain the temperature compensation of characteristic of circadian clocks (Saunders, 1977, 1982a), since lipid viscosity and hence lipid transport remains constant throughout a range of biologically relevant temperatures (Hazel and Prosser, 1974).

A more specific theory based on protein synthesis has been proposed by Schweiger and Schweiger (1977) and further refined by Schweiger et al., (1986) following
experimental work on circadian rhythms in the unicellular alga *Acetabularia* sp. According to this theory, circadian concentration oscillations are regulated by feedback control of protein translation and incorporation of proteins into the lipid bilayer of a membrane. Mutagenic chemicals affecting protein synthesis (Cornelius and Rensing, 1982) and membrane-altering drugs (Enright, 1971; Sweeney, 1976) both alter rhythmical processes, providing support for the involvement of protein synthesis and membranes. There are also rhythms of membrane activity in plants and animals (Sweeney, 1976; Sweeney and Prezelin, 1978 for reviews).

Since the coupled-translation membrane model of Schweiger and Schweiger (1977) was first put forward, a protein with a circadian concentration oscillation has been discovered in *Acetabularia* (Schweiger et al., 1986) giving further support to the theory.

2.4. External Coincidence Models

Bunning (1936, cited Saunders, 1982a) first put forward the theory that the circadian system is involved in photoperiodism. His idea was that the circadian clock is divided into two parts, a photophil and a scotophil. When light falls in the photophil only, short-day responses occur, with long-day responses resulting when light falls in the scotophil.

If this model were valid, then light falling at any point in the scotophase would produce a long-day effect. Adkisson (1964), designed an experiment to test the diapause mechanism in *Pectinophora gossypiella* whereby the dark phase of a short day photoperiod was interrupted by a brief pulse of light at different times (see Fig. 6.1 for an example of this type of protocol). Results from these night interruption experiments showed that long day responses only occurred at two phases in the scotophase, at about 2 and 10 hours from lights off. Similar effects were found for diapause induction in *S. argyrostoma* (Saunders, 1975), *N. vitripennis* (Saunders, 1968) and *T. urticae* (Veerman, 1977).

Night interruption experiments show that the position of the light pulse and not the amount of light is important for diapause induction. Based on the night interruption results in *P. gossypiella* (Adkisson, 1964), Pittendrigh (1966) modified Bunning's original theory and proposed that a long-day response came about when the light pulse coincided with a certain part of the circadian oscillator, the photoinducible phase ($\phi_i$). According to this model, both long-day response peaks
correspond to the coincidence of the light with $\varphi_i$, with one peak coming about by a phase shift of the oscillator (See Chapter 6 for further discussion).

Night interruption experiments by themselves provide no evidence for a circadian mechanism; they can equally well be explained by an hourglass theory (Section 2.5).

Further evidence for an external coincidence mechanism comes from Nanda-Hamner and extended night experiments. The former involve a fixed short-day light cycle with the dark period varying to give a cycle from 16 to 22 hours (Nanda and Hamner, 1959) while in the latter a LD 12:60 cycle is interrupted by brief light pulses during different parts of the cycle (see Fig. 6.2 for an example of this type of protocol).

If the external coincidence model holds true, then these experiments would result in a long day effect occurring only during cycles when the light pulse coincides with $\varphi_i$ (Fig. 6.2). This phenomenon is known as resonance and has been observed in many insect species, including \textit{S. argyro stigma} (Saunders, 1973), \textit{N. vitripennis} (Saunders, 1970), \textit{Pieris brassicae} (Claret \textit{et al.}, 1981), the cricket \textit{Pteronemobius nigrofasciatus}, (Takeda, 1986) the spider mite \textit{T. urticae} (Veerman and Vaz Nunes, 1980) and the fruit fly \textit{Drosophila aurariae} (Pittendrigh, 1981).

Another test for the external coincidence model using a light-dark cycle (T) close to $\tau$ was designed by Pittendrigh and Minis (1964, 1971). When $\tau$ and T are close, the light phase of a day-night cycle falls at different phases of the oscillator, depending on the value of T. Using the eclosion rhythm as an indicator of the phase of the oscillator, these two workers entrained \textit{D. pseudoobscura} to different T periods but with limited success. The experiment was repeated more successfully by Hamner and Enright (1967) with finches and Elliott (1976) with hamsters. The photoperiodic response of these animals varied depending upon which part of the oscillator was illuminated, giving support for a photoinducible phase. In an insect, Saunders (1979) reported a similar effect for the diapause response in \textit{S. argyro stigma}. This species showed a reduced diapause incidence when the light pulse fell at a phase corresponding to the predicted position of $\varphi_i$, which supports an external coincidence mechanism.

The external coincidence model cannot account for all aspects of photoperiodic induction in all insects. Some insect species do not show any resonance response.
These include *Megoura viciae* (Aphididae) (Lees, 1973, 1986), *Pectinophora gossypiella* (Pittendrigh and Minis, 1971), *Diatraea grandiosella* (Takeda, cited Saunders, 1982a), *Plodia interpunctella* (Takeda and Masaki, cited Saunders, 1982a) and the cabbage whitefly *Aleyrodes proletella* (Adams, 1986b). The model also cannot account for aspects of the Type 1 (Fig. 1.1) diapause response, the drop off in diapause at short daylengths (Saunders, 1981a) and the increase in diapause during long days. Thermoperiodism is also very difficult to explain in terms of the model, unless we assume that $\psi_1$ is also sensitive to temperature pulses (Saunders, 1973b). When the T experiment described by Saunders (1979) was repeated for *T. urticae*, a high diapause incidence resulted during all T periods, contrary to predictions of the model (Vaz Nunes and Veerman, 1982a).

2.5. Hourglass Models

An hourglass hypothesis assumes that the circadian system is not involved in photoperiodism, but that the night-length is measured directly. Support for this model comes from the lack of resonance displayed by many insect species (Section 2.4).

The most explicit version of the hourglass model comes from Lees (1973) based on experiments with the aphid *M. viciae*. He proposed that the scotophase is divided into four distinct periods. A pulse of light near the beginning of the dark phase "resets" the hourglass, and it then starts running again from the start of the next dark cycle. The second part of the scotophase (up to about 5-6 hours) is insensitive to light. A light pulse here does not reset the hourglass, and a long-night response results. A pulse of light during the third part of the scotophase (up to the critical night length of 9.5 hours) "interrupts" the hourglass and the short-night (long-day) virginoparae are produced. Finally, interruptions after the critical period result in the long night oviparae. Since the hourglass is not affected by pulses in the middle of the night, the model predicts that some night interruption experiments will produce two peaks of long-day effects, and this is borne out experimentally in *M. viciae* (Lees, 1973) and *Aleyrodes proletella* (Homoptera) (Adams, 1986a).

The strongest support for an hourglass in *M. viciae* is the absence of a resonance response. Extended night experiments all produce a long-night effect, except when the light pulses are close enough together for one of the dark phases to be less than the critical nightlength (Lees, 1973). A similar response at three temperatures was found in *A. proletella* (Adams, 1986b).
As mentioned earlier (Section 2.3) the diapause response under short day skeleton photoperiods can approximate to a complete photoperiod in *S. argyrostroma* and *C. viciae* and this has been used as an argument for an external coincidence mechanism in these species (Saunders, 1975; Vaz Nunes *et al.*, 1990). In *M. viciae* on the other hand, a circadian response and bistability zone is absent and the photoperiodic responses are best interpreted as an hourglass (Hillman, 1973). A similar lack of bistability in response to skeleton photoperiods has been found in the aphid *Aphis fabae* (Vaz Nunes and Hardie, 1989).

Veerman and Vaz Nunes (1987) designed an experiment to distinguish between an hourglass and an oscillator mechanism in *T. urticae* based on the photoperiodic counter. In their experiment they found that the percentage of diapause during a 36 hour night was the same as that of a 12 hour night, suggesting that all long nights are counted as a single night until the hourglass is "reset" by a light pulse.

### 2.6. Two Oscillator Models

A two oscillator model for *Drosophila pseudoobscura* eclosion rhythms was put forward by Pittendrigh and Bruce (1959) and was applied to photoperiodism by Tyschenko (summarised by Danilevski *et al.*, 1970). In this model two oscillators are involved, one phase set by dawn and the other by dusk. Diapause results where the two oscillators overlap. This model accounts for the fall off in diapause in short photoperiods and can generate Type 3 and 4 PPRCs (Danilevski *et al.*, 1970). Further support for the model has come from a series of experiments by Saunders (1978 for review) on *N. vitripennis*. The amount of diapause in this species depends on both the night and day length, suggesting separate night and day oscillators (Saunders, 1968). In *S. argyrostroma* on the other hand, the night length assumes the most important role (Saunders, 1978). During long days, chilling in the daylight produces short day effects, suggesting that at least one oscillator starts from lights on (Saunders, 1968).

*N. vitripennis* shows a resonance response, but the ascending and descending peaks show different properties. The ascending slopes take their time cue from the dusk, whereas the descending peaks take theirs from the dawn. Saunders (1974) suggests that this supports the presence of separate dawn and dusk oscillators.
Saunders (1973b, 1978) also suggested that the presence of a thermoperiodic response provides support for an internal coincidence mechanism. Biological rhythms in insects can be entrained by temperature in the absence of light (Zimmerman et al., 1968; Rence and Loher, 1975; Gander, 1979). Since light is not required to illuminate \( \phi \), Saunders (1978) considered it a viable alternative to an external coincidence mechanism where thermoperiodic responses are found.

In the hamster, the locomotor rhythm in constant conditions split into two separate rhythms each with a different \( \tau \) value. To account for this, Pittendrigh and Daan (1976) suggested two oscillators were involved, phase set by sunset and sunrise. Christensen (1978) proposed a similar mechanism for locomotor rhythms in the weta Hemidiena thoracica (Orthoptera) after rhythm splitting was found in this species.

Another type of two oscillator model described by Pittendrigh and Bruce (1959) for D. pseudoobscura eclosion rhythms is the "pacemaker" and "slave" idea. The eclosion gates are controlled by a "slave" oscillator which is in turn entrained by a "master" clock. If the "slave" oscillator is temperature sensitive, then this model can account for light and temperature entrainment and the effects of combined thermophotoperiods (Pittendrigh, 1960, 1966, 1981).

The above studies show support for two oscillators controlling locomotor and eclosion rhythms. Pittendrigh (1972, 1981) used the multi-oscillator nature of insect biological rhythms to suggest a similar mechanism in photoperiodism. This assumes however that the photoperiodic clock is controlled by the same process which cannot be assumed to hold true. In S. argyrostroma for example, there are distinct differences in properties of the rhythm for larval wandering, pupal eclosion and diapause induction, suggesting the possibility of many separate clocks (Saunders, 1986). The presence of two oscillators controlling other rhythmical processes therefore does not necessarily provide support for an internal coincidence mechanism in photoperiodism.

2.7. The Dual System Model

This specific version of a two oscillator model was put forward by Beck (1974a,b). This was in response to work done by Truman (1971) on eclosion in the silkworm Antheraea pernyi. The model assumes that the clock is composed of two oscillators, an S system and a P system entrained by the S system.
The kinetics of the S system are based on Truman's (1971a) model for eclosion rhythms in *A. pernyi*. It acts as an oscillator in total darkness, but is stopped by photophases of four hours or more, and reset during the dark phase, essentially behaving like an hourglass. When the P system is between two values, a determination gate opens. The percentage of diapause then depends on the value of the S oscillator at that time (Beck, 1974b). Beck has tested the model and predicted the presence of Type 1-4 PPRCs (1974a), non-diel photoperiods (1975), skeleton photoperiods (1976a), night interruptions (1976b), thermoperiods and thermophotoperiods (1977).

The model has been modified and extended by Vaz Nunes and Veerman (1979a,b), to account for the sudden drop in diapause experienced by spider mites at short photoperiods (Veerman, 1977), compared with the more gradual decline in *O. nubilalis* (Beck, 1962). These workers changed the kinetics of the S and P systems so that both of them lost all circadian properties and behaved like hourglasses. Computer simulations on the PPRC and effects of skeleton photoperiods in *T. urticae* have been tested and compare well with real data (Vaz Nunes and Veerman, 1979a,b).

Objections to the dual system model have come from Skopik *et al.*, (1981) who compared simulations of the model with data on the oviposition rhythm in *O. nubilalis*. They found that the model could not account for circadian properties of the rhythm, including temperature compensation of \( \tau \), entrainment by non-diel photoperiods and transient cycles. Takeda (1985) also discovered that the Dual System theory cannot account for the different diapause responses in *D. grandiosella* brought about by night interruption pulses of different durations.

### 2.8. Resonance Models

Using Beck's (1962) data for diapause induction in *O. nubilalis*, Pittendrigh (1972) concluded that diapause was maximal when \( T \) was close to 24 hours and therefore in resonance with the circadian system. He then suggested that the circadian system may therefore change the photoperiodic response without being directly involved in time measurement.

An explicit resonance model, the "hourglass timer-oscillator counter" has been proposed by Vaz Nunes and Veerman (1982b) to account for photoperiodic effects in
The diapause response of *T. urticae* showed both rhythmical (Veerman and Vaz Nunes, 1980) and hourglass (Vaz Nunes and Veerman, 1982a) properties. To account for this discrepancy these authors proposed a model whereby the nightlength is measured by an hourglass, but also influenced by the phase relationship between lights-on and the circadian system (Vaz Nunes and Veerman, 1982b). Under some photoperiods, the light cycle is not in resonance with the circadian system, and build up of diapause inducing substance is inhibited. Computer simulations of this model successfully predicted the PPRC and the response of *T. urticae* to night interruption, resonance and skeleton photoperiods (Vaz Nunes and Veerman, 1982b, 1984, 1986a). It also accounted for results of a T experiment on this species which were contrary to the predictions of the external coincidence model (Vaz Nunes and Veerman, 1982b). Lees (1973) experiments with aphids were repeated with spider mites. The results were very similar and agreed with the resonance model (Vaz Nunes and Veerman, 1986b).

In a later version of the model, the hourglass is replaced by an instantly damped oscillator and computer simulations of this model are compared with photoperiodic data for *Aphis fabae* (Vaz Nunes and Hardie, 1987). The model predicted the shape of the PPRC as well as most resonance, night interruption and skeleton photoperiod experiments (Vaz Nunes and Hardie, 1987, 1989).

### 2.9. Damped Oscillator Models

The involvement of a damped oscillator in photoperiodism was suggested by Saunders (1981a) to account for the low diapause response at short daylengths. Explicit models involving a damped circadian oscillator have been published by Vaz Nunes and Hardie (1987) and Lewis and Saunders (1987). The first of these has already been discussed in the previous section.

The Lewis and Saunders (1987) model is a modification of Pittendrigh's (1966) external coincidence idea (Section 2.4) and is based on results on diapause induction in *S. argyrostrona*. \( \varphi_i \) occurs when the concentration \( c \) of a hypothetical chemical falls through a threshold value. If this phase occurs in the dark, a hypothetical diapause inducing substance (INDSUM) is produced. This is destroyed if \( \varphi_i \) coincides with the light.

The model differs from the original external coincidence idea in that the oscillator damps out in short photoperiods and at low temperatures. When the oscillator...
The damped circadian oscillator, showing the threshold concentration and the position of the photoinducible phase ($\phi_i$). From Morris and Lewis (1990).
damps out below the threshold, there is no longer any photoinducible phase, and the clock is then essentially an hourglass (Fig. 2.1).

The model can successfully account for the drop off in diapause at short photoperiods (Lewis, and Saunders, 1987), since the photoperiodic counter is only summating one long night once the oscillator has damped below threshold. It can also simulate both resonance and hourglass responses by altering the degree of damping (Saunders and Lewis, 1987b). A sustained oscillator simulates a resonance response. As the oscillator damps out, resonance responses become smaller after the first peak, comparable with experimental results in *Pieris brassicae* (Claret *et al*., 1981). When the damping is increased further, the oscillations damp out after one cycle, effectively simulating hourglass responses (Saunders and Lewis, 1987b).

Some insect species show a resonance response at high temperatures and an hourglass response with a temperature drop (Saunders, 1982b; Pittendrigh 1981; Hardie, 1987) and as mentioned earlier (Section 1.1.4) some insects show a fall off in diapause at low temperatures. Both of these effects can be explained in terms of the oscillator damping out at low temperatures. Lowering the temperature causes firstly an hourglass effect (Saunders and Lewis, 1987b), then as the temperature drops still further, the oscillator fails to reach threshold concentration and no INDSUM is produced.

The model has been further refined so that the build up and breakdown of INDSUM is proportional to the total concentration of the oscillating chemical above the threshold (Saunders and Lewis, 1988; Morris and Lewis, 1990). Saunders and Lewis (1988) tested the diapause response of *S. argyrostoma* and *Calliphora vicina* under equal numbers of 12 hour days with 12 hour or 36 hour nights. The prediction of a damped oscillator is that the diapause response should be the same under these two regimes since each night is counted once. A sustained oscillator would give a greater diapause response under the longer nights since more phases have been summated in the night. Contrary to both these predictions, the diapause response in both species was less under the longer night. This effect can be explained by the modified model since the concentration of the oscillator over threshold was smaller under the 36 hour night regime (Saunders and Lewis, 1988).

The model can also account for both the presence and absence of a thermoperiodic response (Morris and Lewis, 1990). When the thermoperiod is short, the average
Fig. 2.2.

Computer simulations of the behaviour of the damped oscillator under three $18^\circ\text{C}/36^\circ\text{C}$ thermoperiods.

a: Thermoperiod = 2 h, b: Thermoperiod = 4 h, c: Thermoperiod = 14 h. The top of the figures show the position of the high temperature pulses (·) and the photoinducible phases (+). The bottom shows the pattern of the oscillator and the threshold concentration (from Morris and Lewis, 1990).
temperature is cooler so the oscillator never reaches threshold concentration (Fig. 2.2a) and no diapause results. As the thermoperiod gets longer, the amplitude of the oscillator increases and rises above the threshold (Fig. 2.2b). \( \phi_1 \) coincides with the cryophase resulting in a high INDSUM build up and thus a high rate of diapause. At longer thermoperiods \( \phi_1 \) occurs during the thermophase when the concentration of INDSUM inhibitor is high, so the diapause declines again (Fig. 2.2c). If the \( Q_{10} \) for INDSUM build up is high, the difference between \( \phi_1 \) falling in the cryophase or thermophase is very marked, so a thermoperiodic response curve is generated. If the \( Q_{10} \) is low, the difference is negligible, and no such response results (Morris and Lewis, 1990).

One assumption of the model is that INDSUM is not accumulated during long days. This is at variance with published data on \( S. argyrostoma \) which shows that some diapause can be induced by long days if enough photoperiodic cycles are experienced (Saunders, 1971).

The original Lewis and Saunders (1987) model cannot account for a discrepancy in the experimental results for \( T. urticae \). By comparing the effect of a 12 hour light cycle in a 12 hour or 36 hour night, Veerman and Vaz Nunes (1987) demonstrated that these night lengths both had the same effect on diapause induction, indicating an hourglass response. Extended night experiments on \( T. urticae \) however reveal four resonance peaks (Veerman and Vaz Nunes, 1980) suggesting a more sustained oscillator.

This discrepancy can be accounted for by an extension of the original damped oscillator idea, but incorporating the pace-maker slave model of Pittendrigh (1981) discussed in Section 2.6. This model, proposed by M. Vaz Nunes, D.S. Saunders and R.D. Lewis (unpubl. data) involves two damped circadian oscillators, a pacemaker and slave, both entrainable by light and temperature. The model successfully simulates a variety of experimental results, including PPRCs, night interruption experiments, resonance and skeleton photoperiods. It can account for the resonance effect in \( T. urticae \) by assuming that the coupling between the pacemaker and slave is weak, so the slave damps out after one cycle. If the circadian effect from the pacemaker is high, resonance peaks will still occur, despite the damping.
2.10. Conclusion

The photoperiodic models reviewed are summarised on Table 2.1, together with experimental results accounted or not accounted for by the models. The amount of experimental work on photoperiodism is vast, and the models have not all been tested and compared with all the experimental data.

With our present understanding of clocks; the damped oscillator, resonance and two oscillator ideas seem to explain most of the characteristics of photoperiodism. In terms of Pavlidis (1978) ideas on the importance of modelling, these go most of the way towards accounting for existing experimental data.

Two assumptions can be made concerning photoperiodic mechanisms in arthropods. The first of these is that the photoperiodic process is universal throughout all arthropod groups. This is supported by the extreme similarity in the photoperiodic response among diverse groups of organisms. The PPRCs of insects essentially resemble those of birds (Saunders, 1977), flowering plants (Vince-Prue, 1975) and mammals (Elliot, 1981) in their general shape. There are also similarities in the response to skeleton photoperiods in a plant (*L. purpusilla*) and an insect (*S. argyrostoma*) (Section 2.3), and evidence for a two oscillator mechanism in hamsters (Elliot, 1976) as well as in insects locomotor rhythms (Section 2.6). Aspects of biological rhythmicity in general, such as phase response curves and entrainment patterns are very similar across a diverse range of organisms (Saunders, 1977). Rhythm splitting in weta locomotor rhythms (Christensen, 1978; Christensen and Lewis, 1982) is duplicated in hamsters (Pittendrigh, 1960; Pittendrigh and Daan, 1976).

The second assumption backed up by the diversity of photoperiodic effects is that arthropods have evolved several distinct mechanisms for photoperiodic control. This is supported by the differences in photoperiodic effects and the number of models needed to account for them. Lepidoptera species are the only group so far where a Type 4 photoperiodic response has been demonstrated for larval diapause, and so far the dual system theory (Beck, 1974a) and the two oscillator model (Danilevski *et al.*, 1970) are the only models able to account for this shape. There could therefore be a separate mechanism for diapause induction in Lepidoptera.
Earlier models of the photoperiodic mechanism (e.g. Pittendrigh, 1966; Pittendrigh, 1972; Lees, 1973) were purely descriptive. However later models have become more explicit which allows them to be tested by computer simulations. Computer simulations of damped oscillator models (Saunders and Lewis, 1987a,b, 1988; Morris and Lewis, 1990) and the dual system model (Beck, 1975, 1976a,b, 1977; Skopik et al., 1981) have been performed. Further computer modelling could make it easier to differentiate between the various models and to pinpoint the mechanism(s) involved.
Table 2.1. Summary of photoperiodism models

<table>
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Account for

PPRC shape in *T. urticae*
Some T experiments
Resonance
Night interruptions
Hourglass responses
Non diel photoperiods

**Do not account for**

Some T experiments

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**Resonance Models**

Vaz Nunes and Veerman (1982b)
Vaz Nunes and Veerman (1982b)
Vaz Nunes and Veerman (1982b, 1986a)
Vaz Nunes and Veerman (1984, 1986b)
Vaz Nunes and Veerman (1986b)
Veerman and Vaz Nunes (1987)

---

**Accounts for**

The shape of PPRCs, Types 1 and 3
Hourglass responses
Resonance responses
Damping of resonance
Non diel photoperiods
Reduced diapause at low temperatures
Thermoperiodism
Hourglass and resonance effects in *T. urticae*
Night interruption experiments
Skeleton photoperiods

**Does not account for**

Build up of diapause in long days

---

**Damped Oscillator**

Saunders (1979)
Saunders and Lewis (1987a)
Saunders and Lewis (1987b)
Saunders and Lewis (1987b)
Saunders and Lewis (1988)
Saunders and Lewis (1987b)
Morris and Lewis (1990)
Vaz Nunes et al. (unpubl.)
Vaz Nunes et al. (unpubl.)
Vaz Nunes et al. (unpubl.)

Section 2.9
CHAPTER THREE

EFFECTS OF PHOTOPERIOD AND CONSTANT TEMPERATURE ON DEVELOPMENT IN P. OCTO

Note: Part of this chapter has been published (Morris, 1990a,b).

3.1. Introduction

Overwintering has been observed in field populations of P. octo (Section 1.4), but not in the laboratory. The first aim of this series of experiments is to determine whether overwintering can be induced by photoperiod. A photoperiodic effect on larval growth in the laboratory could indicate whether diapause is present and distinguish it from quiescence (Tauber et al., 1986).

Larval duration and instar number are both affected by photoperiod in a number of Lepidoptera species (Chippendale and Yin, 1973; Honek, 1979; Rock and Shaffer, 1983; Oku, 1984), and this raises the question of whether photoperiod/temperature interactions affect the physiology of larval growth and the initiation of pupation. In many insect species, pupation is initiated after the final instar larvae have reached a critical weight (Nijhout and Williams, 1974a,b; Lounibos, 1979; Ochieng-‘Odero, 1988; Palmer, 1982; Woodring, 1983); this weight is proportional to the size of the final instar (Nijhout, 1979, 1981; Jones et al., 1981). In Manduca sexta the attainment of the critical weight causes the corpora allata to stop secreting juvenile hormone. The titre of juvenile hormone declines, until it reaches a low enough level for PTTH to be secreted and pupation to be initiated. While the juvenile hormone level is declining there is a latent feeding period, during which larvae continue to feed and grow, reaching a prepupal threshold weight prior to pupation (Nijhout and Williams, 1974b). The competence of a larva to pupate depends on the attainment of a critical size, as measured by the head capsule width. Larvae which fail to reach this size moult to a supernumerary instar rather than to a pupa (Jones et al. 1981; Nijhout 1981).

Some of these larval growth parameters are influenced by photoperiod. Ochieng-‘Odero (1988) reports a photoperiodic effect on latent feeding period and prepupal threshold weight in the Tortricid 'Cnephasia' jactatana though
there was no variation in critical weight or pupal weight. Palmer (1982) reports a reduced critical weight in the beetle *Labidomera clivicollis* under short-day photoperiods.

In this chapter, the larval and pupal duration, number of instars, head capsule widths, critical head capsule size and pupal weight are monitored to determine the photoperiodic effects on these parameters in *P. octo*.

3.2. Materials and Methods

3.2.1. Insect Rearing and Experimental Conditions

Eggs were obtained from the DSIR Entomology Division, Auckland, New Zealand from a laboratory reared population described in Section 1.4.2. At the start of this study, eggs were kept in DD at 10°C until needed and then allowed to develop at 21°C+/-1°C and LD 18:6 until hatching.

First instar larvae were transferred onto plastic 6-well plates (45 mm diameter) to which about 3 g of general purpose diet (Singh, 1983) had been added. Insects were reared in separate growth cabinets at photophases of 0, 2, 6, 12, 14, 16, 18 and 24 h in a 24 h cycle at temperatures of 21 and 17+/-1°C. Lighting was provided by Thorn fluorescent tubes (Emission spectrum 87-88% at 510-610 nm) and the insects received a light intensity of 300-500 lux. The 21°C experiments at photophases 0, 6, 12, 16, 18 and 24 h were performed during mid 1988, and the 14 and 16 h photophases at the end of the year. The 17°C experiments and the 21°C two hour photophase treatment were run together during 1989.

Head capsules of each instar were measured with an ocular micrometer on an Olympus dissecting microscope at 40X magnification. Measurements were accurate to 0.012 mm. The instar number was determined from changes in head capsule measurements. These showed discrete differences between instars (Table 3.3) so that instar identification was simple. Pupae were sexed (Section 1.4) and weighed on a Mettler balance (accuracy +/- 2 mg).

Each cabinet contained 96 insects. Half of the larvae in each cabinet was not disturbed until pupation had started in order to check whether the disturbance due to measuring had any noticeable effect on development. For the 21°C
treatments each experiment was repeated with a separate batch of 96 insects in each cabinet.

3.2.2. Statistical Analyses

Statistical analyses were performed on an IBM PC using PROC GLM on the SAS package. All tests of significance were at \( P < 0.05 \). Multiple analyses of variance were first performed on all parameters. If the results were significant, a Tukeys test for a photoperiodic effect was performed to determine significant differences among individual treatments. Different letters on figures indicate significant treatment differences with variations due to sex, temperature, disturbance or instar grouping all taken into account.

3.3. Results

3.3.1. Larval Development

Larvae develop through 5-6 instars at \( 17^\circ C \) and 5-7 instars at \( 21^\circ C \), with significantly more females than males requiring the extra instar for development. The proportion of 6th instar larvae is displayed in Fig. 3.1.

Table 3.1 shows the effects of photophase, sex and number of instars on the duration of each instar and on total larval duration. Disturbance has no significant effect at either temperature. The total larval duration at each temperature is displayed on Fig. 3.2. Figs. 3.3 and 3.4 show the duration of each instar.

3.3.2. Head Capsule Widths

Head capsule widths of instars 1-6 were measured at \( 21^\circ C \) and 5th instar capsule widths were measured at both temperatures. The results of analysis of variance are displayed on Table 3.2. The head capsule widths of larvae developing through supernumerary instars are smaller; this difference is significant from the 3rd instar onwards (Table 3.2). The head capsule widths are displayed on Table 3.3. The capsule widths of instars 2, 4 and 5 showed significant differences among photophases (Table 3.2), and the 5th instar capsule widths at \( 17^\circ C \) were also significantly larger. The capsule widths of these instars are plotted on Fig. 3.5.
Table 3.1. Analysis of variance for larval duration, the duration of each instar and the number of supernumerary instars. One or more * shows the classification variable significantly influences the analysis variable.

n.s = Not significant, * = P < 0.05, ** = P < 0.01, *** = P < 0.001. L1-L6 = duration of instars 1-6

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Temperature = 21°C

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To determine the critical head size for pupation, the 5th instar capsule widths are plotted on Fig. 3.6, grouped according to temperature. This shows that there is a critical head size in *P. octo*, with all larvae smaller than 1.44 mm moulting to a sixth instar. This critical size does not vary with temperature (Fig. 3.6).
Fig. 3.1.

Percentage (+/- SEM) of larvae developing through more than 5 instars.

a = 17°C, each point represents 14-24 insects; b = 21°C, each point represents 18-38 insects. Squares = females, triangles = males. Closed symbols = more than 5 instars, open symbols = 7 instars. Letters indicate significant photophase differences.
Mean (+/-SEM) larval duration at two temperatures.

a = 17°C, each point represents 14-31 insects; b = 21°C, each point represents 18-51 insects. Squares = females, triangles = males. Open symbols = undisturbed, closed symbols = disturbed. Letters indicate significant photophase differences.
Fig 3.3.

Mean (+/-SEM) duration of instars 1-3.

1-3 = 1st - 3rd instar. a = 17°C, each treatment represents 29-44 insects; b = 21°C, each treatment represents 50-73 insects. Squares = 5 instar development, circles = 6 instar development, triangles = 7 instar development. Letters indicate significant photophase differences.
Fig. 3.4.

Mean (+/- SEM) duration of instars 4-6.

4-6 = 4th - 6th instars, a = 17°C, b = 21°C. Symbols and sample sizes as for Fig. 3.3.

Letters indicate significant photophase differences.
Mean (+/-SEM) head capsule width for instars 2 (a), 4 (b) and 5 (c).

Circles = 5 instar development, squares = 6-7 instar development. Open symbols = 17°C, closed symbols = 21°C. a = 2nd instar, b = 4th instar, c = 5th instar. Sample sizes as for Fig. 3.3. Letters indicate significant photophase differences.
Table 3.2. Analysis of variance for head capsule widths of instars 1-6. Symbols as in Table 3.1.

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<td>W6, 21°C</td>
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<td>n.s</td>
</tr>
</tbody>
</table>

Table 3.3. Mean (SEM) head capsule widths in mm, grouped according to the number of instars of development. Symbols as in Table 3.1. Five instar, n = 140-313; 6 instar, n = 48-124; 7 instar, n = 6.

<table>
<thead>
<tr>
<th></th>
<th>5 Instar</th>
<th>6 Instar</th>
<th>7 Instar</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1, 21°C</td>
<td>0.283 (0.000)</td>
<td>0.284 (0.000)</td>
<td>0.282 (0.003)</td>
</tr>
<tr>
<td>W2, 21°C</td>
<td>0.407 (0.000)</td>
<td>0.402 (0.001)</td>
<td>0.394 (0.007)</td>
</tr>
<tr>
<td>W3, 21°C</td>
<td>0.628 (0.002)</td>
<td>0.598 (0.003)</td>
<td>0.558 (0.020)</td>
</tr>
<tr>
<td>W4, 21°C</td>
<td>1.024 (0.003)</td>
<td>0.883 (0.007)</td>
<td>0.776 (0.026)</td>
</tr>
<tr>
<td>W5, 17°C</td>
<td>1.755 (0.012)</td>
<td>1.321 (0.011)</td>
<td></td>
</tr>
<tr>
<td>W5, 21°C</td>
<td>1.662 (0.006)</td>
<td>1.290 (0.010)</td>
<td>1.066 (0.035)</td>
</tr>
<tr>
<td>W6, 21°C</td>
<td>1.872 (0.013)</td>
<td>1.840 (0.063)</td>
<td></td>
</tr>
<tr>
<td>W7, 21°C</td>
<td></td>
<td></td>
<td>1.476 (0.073)</td>
</tr>
</tbody>
</table>

3.3.3. Pupal Weight

Pupal weights show significant differences with photophase, temperature, disturbance and sex (Table 3.4). Pupal weight is plotted on Fig. 3.7.

The correlation between pupal weight and the head capsule width of the final instar is significantly positive for 5 instar larvae at both temperatures, but not significant for 6 instar larvae at 21°C.

3.3.4. Pupal Duration

The pupal duration at both temperatures is displayed on Fig. 3.8. Disturbance and instar number have no effect on duration (Table 3.4), so these are pooled.
Fig. 3.6.

Distribution of 5th instar head capsule widths.

a = 17°C, b = 21°C. Black = 5 instar development, white = 6 instar development. Letters indicate significant photophase differences.
Fig. 3.7.

Mean (+/-SEM) pupal weight.

a = males at 17°C, b = males at 21°C, c = females at 17°C, d = females at 21°C. Closed symbols = undisturbed, open symbols = disturbed. Each point represents 14-51 insects. Letters indicate significant photophase differences.
Mean (+/-SEM) pupal duration.

a = 17°C, each point represents 29-48 insects; b = 21°C, each point represents 35-63 insects. Squares = females, triangles = males. Letters indicate significant photophase differences.
Table 3.4. Analysis of variance for pupal weight and pupal duration. Symbols as for Table 3.1.

<table>
<thead>
<tr>
<th>Analysis variable</th>
<th>Temperature = 17°C</th>
<th>Classification variable</th>
<th>Temperature = 21°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Photophase</td>
<td>Sex</td>
<td>Photophase</td>
</tr>
<tr>
<td>Pupal weight</td>
<td>***</td>
<td>***</td>
<td>Pupal weight</td>
</tr>
<tr>
<td>Pupal duration</td>
<td>***</td>
<td>n.s</td>
<td>Pupal duration</td>
</tr>
<tr>
<td></td>
<td>Photophase</td>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Pupal weight</td>
<td>***</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>Pupal duration</td>
<td>***</td>
<td>***</td>
<td></td>
</tr>
</tbody>
</table>

Temperature, sex and photophase all have a significant effect (Table 3.4), with males eclosing later than females (Fig. 3.8). There are also significant sex-photophase, temperature-photophase and sex-temperature-photophase interactions, and this is reflected in the different effects of photophase at each temperature. At 21°C there is a pronounced decline in pupal duration at intermediate photophases. While this is also present to a lesser extent at 17°C, it is overshadowed by a strong negative correlation between development rate and photophase.
3.4. Discussion

3.4.1. Larval Parameters

Previous workers on the life history of *P. octo* in the field (Green, 1984) and in the laboratory (G. Clare, pers. comm.) have shown 5 or 6 instar development. This is the first indication of a 7th instar in this species.

Average head capsule sizes of instars 1-3 at 21°C are larger than those found by Green (1984) in field collections of *P. octo*. This might be due to dietary differences, or it may suggest that the field population had a high proportion of the smaller larvae developing through six or more instars.

Larval and pupal growth parameters are similar to those found in other New Zealand Tortricidae. Larvae of *C. jactatana* (Ochieng-'Odero, 1988), *Epalkiphora axenana* (Clare and Singh, 1988a) and *Ctenopseutis obliquana* (Clare and Singh, 1988b) have been reared on a modified general purpose diet. Females showed a longer larval duration, shorter pupation time and heavier pupae in all three species. *C. obliquana* developed through 5 or 6 instars, with more 6th instar individuals among females (Clare and Singh, 1988b).

The larval duration of *P. octo* on GPD at 17°C and a photophase of 18 h varies between 25-33 days (Fig. 3.2). This compares with 48-54 days on a raspberry diet under identical daylength conditions at 18°C (Hobson and Singh, 1987). The variation in pupal duration with diet is not so marked, being 14-16 days on GPD at 17°C (Fig. 3.8), 18 days on a raspberry diet at 18°C (Hobson and Singh, 1987) and 2-3 weeks in the field (Clark, 1936). Pupal weight at 17°C and LD 18:6 varies between 63-70 mg for males and 95-110 mg for females (Fig. 3.7a,c). This compares with 40 mg and 48 mg for males and females respectively on a raspberry diet (Hobson and Singh, 1987).

3.4.2. Photoperiodic Response Curves

Larval duration and instar number (Figs. 3.1, 3.2) both show a Type 1 photoperiodic response (Fig. 1.1) typical of long-day diapausing insects (Danilevski 1965; Beck, 1980; Saunders, 1982a). The critical photoperiod from
Figs. 3.1 and 3.2 is about 14 h at both temperatures. The strongest photoperiodic response is that of the instar number, but Table 3.1 shows that the photoperiod has an effect on larval duration which is not merely due to the difference in instar number. The PPRCs for instars 2-6 (Fig. 3.3, 3.4) also resemble the shape of Fig. 3.2, showing that photoperiod controls development rate. The PPRC for 1st instar duration at 21°C (Fig. 3.3 1b) shows a different response to all other instars (Fig. 3.3, 3.4) and to the total larval duration (Fig. 3.2) since the duration is significantly longer at photophases of 14 and 16 h. Since these treatments were performed later than the others (Section 3.2), these differences could possibly be due to slight variations in insect batches or experimental conditions. Since first instar larvae have not yet experienced many photoperiodic cycles this difference could outweigh any photoperiodic effect, in contrast with the later instars when the photoperiodic effect is well established.

The PPRC for pupal duration is closer to a Type 4 curve with a critical photoperiod of around 14 h (Fig. 3.8). A photoperiodic effect on larval and pupal development therefore appears quite likely.

Superimposed upon this photoperiodic response is a tendency for the duration to decline with increased photophase. This is especially marked for pupal duration (Fig. 3.8) but can also be seen with the decline in larval duration as the photophase increases from 2 to 12 h (Fig. 3.2). A possible explanation could be that P. octo larvae feed during the day, so that longer photophases would result in a longer feeding period and therefore a faster growth rate.

3.4.3. Critical Head Capsule Width and Instar Number

The PPRC for instar number (Fig. 3.1) shows a stronger Type 1 response compared to that for larval development, suggesting a stronger photoperiodic influence.

The head capsule widths of five instar larvae, for instars 2,4 and 5 show a Type 4 (Fig 1.1) response and are larger at the lower temperature (Fig. 3.5). Raising the rearing temperature also gave smaller head capsule widths in the tortricid Platynota stultana (Zenner-Polania and Helgesen, 1973). These authors suggested that the high-temperature larvae were metabolising and therefore moulting much faster, but without a correspondingly high increase in growth.
rate. This would result in smaller larvae and could explain the variation with temperature in *P. octo*.

A possible explanation for the photoperiodic effect could be found in terms of the critical capsule size and the growth rate. Under short days (LD 2:22 and LD 6:18) the critical head capsule size may be larger. This would result in larger five instar larvae and explain the high proportion of supernumerary instars (Fig. 3.1). Close to the critical daylength (LD 14:10), the critical size is smaller, giving a corresponding decrease in supernumerary instar proportions (Fig. 2.1) and head capsule sizes. As the photophase increases, the larval growth rate may also increase, again resulting in larger larvae.

### 3.4.4. Pupal Weights

Since pupation depends on final instar larvae reaching a critical weight, and since this weight is size dependent (Nijhout 1979, 1981; Jones *et al.*, 1981), then it would be safe to assume that pupal weight is also size dependent. In the related *C. obliquana*, the larger six instar larvae developed into heavier pupae than five instar larvae (Clare and Singh, 1990), supporting this assumption.

In *P. octo* the head capsule widths show that larvae are larger at 17°C (Fig. 3.5e), and these develop into heavier pupae (Fig. 3.7). The correlation between larval size and pupal weight however does not hold true for photoperiodic effects within each temperature as shown by the shapes of the curves on Fig. 3.7. If the heavier pupae came from larger 6th instar larvae then the curves for pupal weight should show the same Type 1 PPRCs shown in Fig. 3.1 and 3.2. Only one curve approximates to this shape, that of the disturbed males at 21°C (Fig. 3.7b).

The shape of these curves and the lack of correlation between head capsule size and pupal weight especially for 6th instar larvae, indicate that other factors may more strongly influence photoperiodic effects on pupal weight. Jones *et al.* (1981) found a smaller ratio between critical weight and head capsule widths for *Trichoplusia ni* with an extra instar. Possible explanations he suggested for this are firstly that in the supernumerary sixth instar, PTTH is released earlier, causing pupation at a lower weight. The second and more likely explanation is that in the faster growing five instar larvae, the critical weights are reached.
well in advance of the gate period of PTTH release, allowing a longer latent feeding period and thus more weight gain.

Another explanation could be a variation in critical weight or latent feeding period with photoperiod. Photoperiodic control of latent feeding period has been documented for *C. jactatana* (Ochieng-'Odero, 1988). This could easily be brought about by differences in juvenile hormone titre. Since pupation is not initiated until the juvenile hormone titre has been reduced (Nijhout and Williams, 1974a,b), a higher JH titre before the critical weight is reached would result in a delay in PTTH secretion and a longer latent feeding period.

The photoperiodic control of pupal weight therefore appears to be influenced by many factors. A closer examination of photoperiodic effect of critical weight, latent feeding period and juvenile hormone titre should be looked at before any conclusions can be made.
CHAPTER FOUR
EFFECTS OF THERMOPERIODS AND THERMOPHOTOPERIODS ON DEVELOPMENT IN P. OCTO

4.1. Introduction

As mentioned in Section 1.1.5, fluctuating temperature cycles can take the place of light cycles in some insect species. Thermophotoperiods also increase or decrease diapause incidence depending on the relationship between the thermophase and the photophase. Goryshin (1964) published photoperiodic response curves for thermophotoperiods and photoperiods in three Lepidoptera species. These graphs showed clearly how diapause and critical daylength increase when the thermophase and photophase coincide.

The thermoperiodic mechanism of diapause induction in Ostrinia nubilalis has been examined extensively by Beck (1982, 1984, 1985, 1987) who reared it under several different thermoperiods and thermophotoperiods. When the cryophase is above \(10^\circ\)C, diapause induction is mainly influenced by the cryophase temperature, with thermophase temperature having very little effect. When the cryophase is below \(10^\circ\)C, the thermophase then has more influence on diapause incidence (Beck, 1982, 1984). The addition of \(0^\circ\)C cold pulses during the scotophase of a diapause inducing photoperiod causes diapause to be completely averted (Beck, 1985a). Beck (1985, 1987) suggests this is brought about because of a slowing down or stoppage of the biological clock controlling diapause induction.

Thermoperiods can also affect insect development rates. In most cases a fluctuating temperature causes an acceleration of development, but in a few cases the opposite can occur (see reviews by Cloudsley-Thompson, 1953; Hagstrum and Hagstrum, 1970; Beck, 1983b; Ratte, 1985).

Beck (1983a) discovered that larvae of O. nubilalis were larger and grew more rapidly under thermoperiods than constant temperatures, although larval duration remained the same. An increase in both larval duration and growth rate is reported in Agrotis ipsilon (Lepidoptera: Noctuidae) (Beck, 1986). In the
midge *Chaoborus crystallinus* thermoperiods caused a lower body weight, and thermophotoperiods with a warmer scotophase caused faster development (Ratte, 1979).

The similarities between photoperiodic and thermoperiodic responses led Beck (1983b) and Ratte (1985) to propose that temperature alters development rate not only by a direct effect on metabolism but also by influencing the biological clock through the neuroendocrine system and acting as a zeitgeber or timekeeper.

In *P. octo*, the photophase has an effect on larval and pupal duration, head capsule width and pupal weight (Morris, 1990a,b; Chapter 3). The photoperiodic response curves for larval duration and instar number are similar to those for diapause in a long-day species. This could suggest that diapause incidence, larval duration and instar number are all controlled by photoperiod through a similar hormonal mechanism.

The effects of thermoperiods and thermophotoperiods are examined in this chapter and compared with photoperiodic effects to further test the photoperiodic response in this species.

### 4.2. Materials and Methods

*P. octo* eggs were obtained from the DSIR Entomology Division as described in Section 1.4.2 and reared under fluctuating temperatures in Honeywell growth cabinets. Temperature changes of 8°C were accomplished in 30 minutes, and temperatures were accurate to within 1°C. Lighting was provided by tungsten bulbs at an intensity of 500 lux.

Insects were reared at thermoperiods and thermophotoperiods with an average temperature of 18°C and an amplitude of 8°C. The developmental zero for growth in *P. octo* is around 8°C (G. Clare, pers. comm.) so the temperatures in all treatments were kept above this value. The thermophotoperiod and thermoperiod treatments were compared with some of the Chapter 3 experiments in order to compare the effects of constant and fluctuating temperatures on development. The rearing conditions used in subsequent analyses were:
1/. 12 h light, 22°C: 12 h dark, 14°C: thermoperiod in phase

2/. 12 h light, 14°C, 12 h dark, 22°C: thermoperiod out of phase

3/. 12 h light, 12 h dark, 17°C (from Chapter 3)

4/. Total darkness, 16 h at 20.5°C: 8 h at 12.5°C: long-day thermoperiod

5/. Total darkness, 8 h at 23.5°C: 16 h at 15.5°C: short-day thermoperiod.

6/. Total darkness, 17°C (from Chapter 3)

7/. Total darkness, 21°C (from Chapter 3)

Insects were reared from first instar to adults as described in Section 3.2. Larvae and pupae were examined every two days and instar number determined by the changes in head capsule width. Head capsule widths of the fifth instar and pupal weight were measured (Section 3.2). Half of the larvae in each treatment were left undisturbed until pupation to monitor the effect of disturbance. A total of 96 disturbed and 96 undisturbed insects were used for each treatment.

Analyses of variance and Tukeys tests were performed on the data as described in Section 3.2.

4.3. Results

4.3.1. Thermophotoperiods

The data for the two thermophotoperiod treatments (1 and 2) were compared with data for a 12 hour photophase at 17°C from Chapter 3. Larval development was through 5 or 6 instars; the percentage of each instar is shown in Fig. 4.1a.
Fig. 4.1.

Larval development under different thermophotoperiodic regimes.

\[ a = \text{Percentage ( +/- SEM) developing through 6 instars, } b = \text{mean ( +/- SEM) larval duration.} \]

Dense stripes = Disturbed males, large stripes = undisturbed males, dense stipples = disturbed females, light stipples = undisturbed females. Each bar represents 14-41 insects.

Letters indicate significant treatment differences.
Fig. 4.2.

Mean (+/-SEM) duration for each instar under thermophotoperiodic regimes.

a-f = 1st-6th instar. Dense stripes = 5 instar males, large stripes = 6 instar males, dense stipple = 5 instar females, light stipple = 6 instar females, diagonal stripes = 6 instar development with sexes pooled, black = sexes and instars pooled. Each treatment represents 31-78 insects (a-e) and 9-50 insects (f).

Letters indicate significant treatment differences.
Larval development under different thermoperiodic regimes.

a = Percentage (+/- SEM) developing through more than five instars, b = Mean (+/- SEM) larval duration.

White = 7 instar females, all other shading as in Fig. 4.1. Each bar represents 21-43 insects. Letters indicate significant treatment differences.
Table 4.1. Analysis of variance for thermophotoperiods and photoperiods. Symbols as for Table 3.1.

<table>
<thead>
<tr>
<th>Analysis variable</th>
<th>Treatment</th>
<th>Classification variable</th>
<th>Instar no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>L1</td>
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<td>n.s</td>
</tr>
<tr>
<td>L2</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>L3</td>
<td>*</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>L4</td>
<td>*</td>
<td>*</td>
<td>n.s</td>
</tr>
<tr>
<td>L5</td>
<td>***</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>L6</td>
<td>**</td>
<td>n.s</td>
<td>***</td>
</tr>
<tr>
<td>W5</td>
<td>n.s</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td>Larval duration</td>
<td>***</td>
<td>n.s</td>
<td>***</td>
</tr>
<tr>
<td>Pupal weight</td>
<td>*</td>
<td>***</td>
<td>n.s</td>
</tr>
<tr>
<td>Pupal duration</td>
<td>*</td>
<td>***</td>
<td>n.s</td>
</tr>
<tr>
<td>Instar no.</td>
<td>***</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of variance for thermophotoperiods and the 17°C photoperiod is displayed on Table 4.1. The data for larval duration is plotted on Fig. 4.1b and the duration of each instar is displayed on Fig. 4.2. Since there is no significant difference between instar groups (Table 4.1), these are pooled on Fig. 4.2a-d and f.

The pupal weights for the 5 instar group were compared with the head capsule widths of the final instar. There was no significant correlation.

4.3.2. Thermoperiods

The thermoperiod treatments are compared with data from insects kept at constant temperatures of 17°C and 21°C (Chapter 3). The former temperature is close to the average temperature of the thermoperiod; the latter is close to the thermophase of the long-day thermoperiod.

Larval development was mostly through 5 or 6 instars, though two females under the long-day thermoperiod developed through 7 instars. The proportion
Mean (+/-SEM) duration for each instar under thermoperiodic regimes. a-f = 1st - 6th instar.

White dots on black = 5 instar development with sexes pooled, all other shading as in Fig. 4.2. Each treatment represents 29-79 insects (a-d) and 10-46 insects (e-f).
Letters indicate significant treatment differences.
Table 4.2. Analysis of variance for thermoperiods and constant temperatures. Symbols as for Table 3.1

<table>
<thead>
<tr>
<th>Analysis variable</th>
<th>Treatment</th>
<th>Classification variable</th>
<th>Instar no</th>
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<tr>
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<td>n.s</td>
<td>n.s</td>
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<td>L5</td>
<td>**</td>
<td>n.s</td>
<td>***</td>
</tr>
<tr>
<td>L6</td>
<td>**</td>
<td>n.s</td>
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</tr>
<tr>
<td>w5</td>
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<td>n.s</td>
<td>***</td>
</tr>
<tr>
<td>Larval duration</td>
<td>***</td>
<td>*</td>
<td>n.s</td>
</tr>
<tr>
<td>Pupal weight</td>
<td>***</td>
<td>*</td>
<td>n.s</td>
</tr>
<tr>
<td>Pupal duration</td>
<td>***</td>
<td>**</td>
<td>n.s</td>
</tr>
<tr>
<td>Instar no.</td>
<td>***</td>
<td>***</td>
<td>n.s</td>
</tr>
</tbody>
</table>

Disturbance had no effect on any variables.

of 6th and 7th instars is shown on Fig. 4.3a and total larval duration on Fig. 4.3b.

The analysis of variance for thermoperiods and constant temperatures is displayed on Table 4.2. Disturbance had no effect, but there was a significant disturbance-treatment interaction for larval duration and pupal weight. The duration of each instar is displayed on Fig. 4.4. Results for pupal weight and pupal duration are displayed on Fig. 4.5.

The pupal weight and the final instar head capsule width were compared in the five instar group. There was a significant correlation for males but not for females.

4.4. Discussion

4.4.1. Effects of Thermophotoperiod on Larval Duration

The in-phase treatment produced the greatest number of supernumerary 6th instar larvae, with the out-of-phase treatment producing the least (Fig. 4.1a).
Pupal duration (a) and pupal weight (b) under thermoperiodic regimes.

Shading as for Fig. 4.1. Each bar represents 13-45 insects. Letters indicate significant treatment differences.
The larval duration is also longer during the in-phase treatment (Fig. 4.1b) as is the duration of all instars except the first (Fig. 4.2).

This follows the pattern observed in photoperiodic diapause induction whereby diapause incidence is higher during in-phase thermophotoperiods (Goryshin, 1964; Section 1.1.3). The thermophotoperiodic effects on development rate and its similarities to the diapause response in other species supports the theory put forward in Chapter 3 that there is a photoperiodic mechanism for larval development in *P. octo* similar to the diapause mechanism in other insects.

**4.4.2. Effects of Thermoperiod on Larval and Pupal Duration**

The number of supernumerary instars, the total larval duration and the duration of each instar all increase under long-day thermoperiods (Fig. 4.3, 4.4). If thermoperiod exerts a similar effect as photoperiod on larval and pupal development, then duration and instar number should be higher under short-day thermoperiods. In contrast, these are higher under the long-day thermoperiod, making a thermoperiodic response in this species unlikely.

A useful non-clock model for calculating development rates under fluctuating temperatures is the rate summing concept, summarised by Ratte (1985). According to this theory, the development rate at fluctuating temperatures can be calculated by averaging the rate at both the thermophase and the cryophase. In some insects the development rate is directly proportional to the temperature, so if the developmental duration is multiplied by the temperature, the resulting day-degrees value (Hagstrum and Hagstrum, 1970) is a constant. In these species, development rate under fluctuating temperatures is equivalent to that under the constant average temperature. In other insect species, the development rate is disproportionately slower under low temperatures. The day-degree value is therefore higher in cold conditions, making development rate slower under fluctuating temperatures (Ratte, 1985). This corresponds well to the *P. octo* results whereby the duration is increased in the treatment with the colder cryophase (Fig. 4.3 to 4.5a).

It appears as if two mechanisms could be involved in thermoperiodic control of development in *P. octo*. Under thermoperiods alone, the development rate appears to follow the rate summing concept. If a photoperiod is added however,
this factor becomes overshadowed by the photoperiodic effect, with the
thermoperiod influencing the photoperiodic mechanism.

4.4.3. Pupal Weights

The pupal weight shows little variation under thermophotoperiods (Table 4.1)
and there is no correlation between head capsule width and pupal weight. This
is contrary to expectations, since heavier pupae should result from larger larvae
(Chapter 3). One possible explanation discussed in Section 3.4.4 is that pupal
weight, prepupal threshold or latent feeding period could be influenced by a
photoperiodic mechanism. Since the action of thermophotoperiods on
development rate is consistent with a photoperiodic mechanism, a similar effect
could be exerted by thermophotoperiods.

The correlation between larval size and pupal weight is stronger for larvae
reared under thermoperiods, and this again is consistent with the action of
thermoperiods on development rate. Since the photoperiodic effect has little or
no influence under thermoperiods alone, the influence of larval size on pupal
weight is likely to be stronger.

4.4.4. Effects of Disturbance

Disturbance has a major effect on larvae reared under thermophotoperiods but
no significant effect on larvae reared under thermoperiods alone (Tables 4.1,
4.2). Disturbance had no significant effect overall on larvae reared under
photoperiods, but at 21°C and LD 12:12, disturbed larvae grow significantly
more slowly (Fig 3.2). This increased sensitivity to disturbance may have been
brought about because the photophase was close to the critical photoperiod. The
effect of disturbance on thermophotoperiodic treatments is consistent with this
hypothesis. The critical daylength at 17°C is about 14 h (Chapter 3). Since
critical daylength decreases during out-of-phase thermophotoperiods (Goryshin,
1964) it is likely that the critical daylength for this treatment would be closer to
12 h, which is the thermophotoperiod used.

If the photoperiodic effect on thermoperiod treatments is very slight as
suggested earlier, this would account for disturbance having very little effect on
the thermoperiod treatments.
CHAPTER FIVE

LIPID CONTENTS OF P. OCTO UNDER LONG AND SHORT DAY PHOTOPERIODS: FURTHER EVIDENCE FOR DIAPAUSE

5.1. Introduction

An increase in total lipid content is common among diapausing and overwintering insects. Migratory insects also accumulate lipid reserves prior to migration, and in both cases this is believed to be an adaptation which allows greater energy reserves (Downer and Matthews, 1976).

Since migratory behaviour and diapause can both be photoperiodically induced (Section 1.1), photoperiod could play some part in lipid accumulation. Laboratory studies reveal that photoperiodically induced diapause increases lipid content in larval *Pectinophora gossypiella* (Lepidoptera) (Adkisson et al., 1963), larval *Sarcophaga crassipalpis* (Diptera) (Adeokum and Denlinger, 1985) and adult *Anthonomus grandis* (Coleoptera) (Lambremont et al., 1964).

The relative amounts of saturated and unsaturated fatty acids also differ among diapause or reproductive individuals. An increase in unsaturated fatty acid composition in short days during photoperiodically induced diapause is documented by Harwood and Takata (1965), Lambremont et al., (1964) and Goldson et al. (1981). The adult female mosquito *Culex tarsalis* overwinters during short days and also shows accumulation of unsaturated body fats under short day photoperiods in contrast with the non-overwintering male (Harwood and Takata, 1965).

An increase in saturated fatty acid composition as the ambient temperature increases is a common occurrence, not only among insects (Fast, 1964) but in a variety of other organisms (Sinensky, 1974; Hazel and Prosser, 1974; Morris, 1985). The increased saturation increases the viscosity of the lipid membranes, and Sinensky (1974) suggested that this allows a constant lipid fluidity to be maintained within the range of environmental temperatures.
Among winter diapausing species, the build up of unsaturated fatty acids prior to diapause could be explained in terms of the lipid fluidity hypothesis discussed in the above paragraph. This would provide another example of photoperiod acting as a token stimulus in preparation for adverse conditions. The build up of polyunsaturated fatty acids in the summer-diapausing weevil *H. postica* (Tombes, 1966) however suggests that this hypothesis cannot always hold. Goldson *et al.* (1981) put forward an alternate hypothesis suggesting that unsaturated fatty acids are preferentially oxidised during periods of high metabolic activity.

This chapter examines the effect of long and short days on lipid content in larval and pupal *P. octo* to further determine whether a diapause is indicated and what stage of the life cycle is involved.

### 5.2. Materials and Methods

*P. octo* were reared from first instar larvae under long-day (LD 18:6) and short-day (LD 6:18) conditions as described in Section 3.2.

From the 4th instar onwards larvae and pupae were collected for lipid analysis. All larvae had moulted less than 24 h previously. Fifth instar larvae were separated into two groups depending on whether they would moult to a sixth instar before pupation. These could be distinguished by the head capsule width. Head capsules below 1.38 mm all moult again before pupating (Fig. 3.6). The head capsules of penultimate fifth instar larvae also started to slip after the first day.

Lipids were extracted using a modification of the method of Folch *et al.* (1957) described by Christie (1982). Between 70 and 140 mg of tissue was ground up in a hand homogenizer. Insects were either homogenized fresh or stored at -80°C prior to use. Insects were firstly homogenized in 2.5 ml ethanol for one minute. Chloroform (5 ml) was added and the procedure repeated for another two minutes. The mixture was filtered, then washed once with chloroform (5 ml) and once with methanol (2.5 ml). The combined filtrates were transferred to a measuring cylinder, and 25% of the filtrate volume of 0.88% potassium chloride was added. The mixture was shaken thoroughly for three minutes and allowed to settle for 20 minutes. The upper layer was removed by aspiration, 25% of the volume of the remaining solution of methanol:water 1:1 was added, the mixture was shaken for a further three minutes, and transferred to a separating funnel. After 30 minutes the bottom layer was poured into a pre-weighed vial. The solvent was evaporated
in a stream of air under ice. The vial was placed in a desiccator for two days at 35°C and the remaining residue containing the lipids was weighed on a Mettler balance (accuracy +/- 0.1 mg).

This extraction yields approximately 95-99% of lipids (Christie, 1982). All chemicals were Analar or Univar grade. Between uses all glassware was washed with "Pyroneg" detergent then thoroughly rinsed under running water then twice with distilled water, methanol and chloroform.

Eleven controls were run using all the extraction steps but without the insect tissue. This yielded 0.4 mg (0.4 mg S.E.M) of impurities. This value was therefore subtracted from all final weights before calculating the percentage of lipids.

Statistical analysis was performed on an IBM PC using the SAS package.

5.3. Results

The percentages of lipids for each life cycle stage are displayed on Table 5.1. Analysis of variance shows that insects reared under short days have a significantly higher percentage of fats. A Tukeys test on each life cycle stage showed three significant groupings. Female pupae showed the highest percentage, followed by male pupae and all larval stages (Table 5.1).

A t-test was performed to compare results for long and short day treatments at each life cycle stage. Among 4th instar larvae, the amount of body lipids does not differ significantly between treatments. At the start of the 5th instar, the amount of lipids declines significantly in long-day individuals. By the 6th instar, short-day larvae accumulated significantly more lipids whereas the lipid content of long day larvae remained the same.
Table 5.1. Percentage lipids found in different life cycle stages of *P. octo* under long and short day conditions. Different letters by the life cycle stages and daylength conditions indicate significant differences.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Daylength</th>
<th>n</th>
<th>% (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>long&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11</td>
<td>5.05 (0.56)</td>
</tr>
<tr>
<td>L5 penultimate&lt;sup&gt;a&lt;/sup&gt;</td>
<td>short&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10</td>
<td>5.62 (0.30)</td>
</tr>
<tr>
<td>L5 final&lt;sup&gt;a&lt;/sup&gt;</td>
<td>long&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9</td>
<td>3.29 (0.36)</td>
</tr>
<tr>
<td>L6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>short&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10</td>
<td>5.95 (0.38)</td>
</tr>
<tr>
<td>Pupa, female&lt;sup&gt;b&lt;/sup&gt;</td>
<td>long&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11</td>
<td>3.86 (0.35)</td>
</tr>
<tr>
<td>Pupa, male&lt;sup&gt;c&lt;/sup&gt;</td>
<td>short&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9</td>
<td>5.34 (0.31)</td>
</tr>
<tr>
<td></td>
<td>short&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12</td>
<td>3.87 (0.53)</td>
</tr>
<tr>
<td></td>
<td>short&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9</td>
<td>7.19 (0.70)</td>
</tr>
<tr>
<td></td>
<td>long&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16</td>
<td>12.43 (1.06)</td>
</tr>
<tr>
<td></td>
<td>short&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9</td>
<td>12.97 (0.58)</td>
</tr>
<tr>
<td></td>
<td>long&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11</td>
<td>8.67 (0.72)</td>
</tr>
<tr>
<td></td>
<td>short&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10</td>
<td>9.15 (0.84)</td>
</tr>
</tbody>
</table>

5.4. Discussion

The results suggest that lipids start to accumulate during the 5th instar in short-days and that the 6th instar is an overwintering stage with some overwintering in the 5th instar. This is in contrast with the account of the life cycle published by Thomas (1979) in which he describes the 2nd-4th instars as the overwintering stages. A photoperiodic response for pupal duration has been documented in *P. octo* (Morris, 1990b; Fig. 3.8), but the similarities in pupal lipid reserves suggest that there is no overwintering in the pupal stage. One characteristic of diapause is that it is often confined to one specific life cycle stage, and larval diapause often occurs in the final instar (Danks, 1987).

A build up of lipid reserves in the last instar in response to short days therefore gives support for the presence of a larval diapause response in *P. octo*. An analysis of the amount of saturated and unsaturated fatty acids under long and short days may give further insight into the photoperiodic control of larval development rate in *P. octo*.
CHAPTER SIX
LARVAL AND PUPAL DEVELOPMENT OF P. OCTO
UNDER NIGHT INTERRUPTION AND SKELETON
PHOTOPERIODS.

6.1. Introduction

Photoperiodic control of insect diapause is well documented in a number of species (Saunders, 1982a), and ideas on the photoperiodic mechanism involved are reviewed in Chapter 2. Speculation on insect photoperiodic measurement has led to two major hypotheses. The first of these is that nightlength is measured directly by an "hourglass" mechanism and has found support in the photoperiodic response of aphids (Lees, 1973, 1986). The second hypothesis is that the circadian system is involved. One specific circadian mechanism proposed by Pittendrigh (1966) and later extended and quantified by Lewis and Saunders (1987), Saunders and Lewis (1988) and Morris and Lewis (1990), is an external coincidence model whereby diapause is inhibited if light falls during a photoinducible phase ($q_1$) of the circadian oscillator.

Hourglass and circadian mechanisms can be distinguished by two types of extended night experiments. "Nanda-Hamner" or "resonance" experiments (Nanda and Hamner, 1958) involve a constant light phase coupled to a variable dark phase. In the second type of experiments, the dark phase of a 48 or 72 hour light cycle is interrupted by short light pulses. It is the latter type of experiments which will be used for monitoring $P$. octo development rate (Fig. 6.2). If the photoperiod is measured by an hourglass then long night responses should occur at all photoperiodic regimes. A circadian mechanism however would result in peaks and troughs of diapause induction, corresponding to non-illumination or illumination respectively of $q_1$ (Saunders, 1981a). Results from resonance experiments support an hourglass hypothesis in some species (Lees, 1987; Adams, 1986b) but in many others a circadian mechanism is supported (Saunders, 1982a; Section 2.4, 2.5).

The possible position of $q_1$ can be initially determined by night interruption experiments in which a normal short-day photoperiod such as LD 6:18 is interrupted by a light pulse during different parts of the night (Fig. 6.1). These
Fig. 6.1.

Night interruption experiments used for determining the position of \( \phi_i \). The * show the position of a \( \phi_i \) phase occurring on the oscillator 13 h after sunset.

Numbers at the left indicate treatments, and at the right show the LDLD cycle used. For further information see text.
commonly result in one or two troughs of low diapause with the smaller trough resulting from a phase shift (Section 2.3).

When the short pulse is close to the end of the long pulse, the end of the short pulse is interpreted as sunset by the circadian oscillator. As the pulses become further apart, the oscillator phase shifts so that the end of the long pulse is equivalent to sunset. According to this theory, based on the diapause response in Sarcophaga argyrostoma, a \( \phi_1 \) phase 13 h after sunset coincides with the long light pulse during treatments 2 and 3 and the short light pulse during treatment 6. This results in troughs of low diapause incidence during these treatments (Fig. 6.1a; Saunders, 1981a). The presence of one or two peaks during night interruption photoperiods does not in itself support a circadian hypothesis since it can be equally well explained by an hourglass model (Section 2.5), but by showing a possible position for \( \phi_1 \) it allows further extended night experiments to be carried out to confirm it.

Chapters 3 to 5 provide possible evidence for a photoperiodic diapause response in larval and pupal development. In this chapter, night interruption and extended night experiments are carried out to determine the photoperiodic mechanism involved.

6.2. Materials and Methods

First instar caterpillars were reared under 13 different photoperiodic regimes as shown in Figs. 6.1 and 6.2. Night interruption experiments were set up first to determine possible positions of \( \phi_1 \). Extended night experiments were then performed to provide further information based on results of night interruption treatments.

The night interruption experiments involved a two hour light pulse interrupting the dark phase of a 6 h photoperiod (Fig. 6.1). For extended night experiments a 3 day cycle with a six hour light period was interrupted by a 4 h light pulse (Fig. 6.2).

Other rearing conditions, methods of measurement and statistical analyses are described in Section 3.2.
Fig. 6.2.

The extended night treatments used in rearing *P. octo*.

The circadian oscillator is shown, and the positions of $\varphi$ predicted by the night interruption experiment results are marked with a *. Numbers on the left indicate the treatment and on the right the LDLD cycle used. For further information see text.
Larval development under night interruption experiments.

a = percentage (+/-SEM) developing through 6 or more instars. b = mean (+/-SEM) larval duration. Squares = females, triangles = males. Each point represents 26-38 insects. Arrows show significant troughs of low percentage or duration. Letters indicate significant photophase differences.
6.3. Results

6.3.1. Night Interruption Experiments

Most larvae developed through 5 or 6 instars. A few larvae developed through 7 instars and for the first time one 8th instar was recorded.

There are significant differences between photoperiodic regimes and instar groups for all parameters (Table 6.1). The number of instars and larval duration is displayed on Fig. 6.3; duration of each instar on Fig. 6.4, and pupal weight and duration on Fig. 6.5. The photophases on the abscissa correspond to the skeleton photophase, i.e. the time between the start of the long light pulse and the end of the short pulse.

The graphs of larval and pupal duration all show a trough of faster development (Figs. 6.3 to 6.5). These troughs occur at photophases of 12-14 (mean 13) and 18-20 (mean 19.5) h. According to Saunders (1981a) these results would correspond to a $\phi_1$ position 13 h after sunset (Fig. 6.1).

6.3.2. Extended Night Experiments

Extended night experiments were set up to distinguish between an hourglass and circadian mechanism. Under the extended night treatments, the short light pulse coincides with different phases of the oscillator. The oscillator is drawn in Fig. 6.2, and the time (in hours) from the start of the oscillation to the end of the short light pulse is shown to the left of the figure. This is known as the zeitgeber time (Zt) (Pittendrigh, 1960). This assumes that the oscillator free-runs during the dark, is damped out by the light pulses and has a $\tau$ value of about 24 h; these assumptions being supported by theories on the oscillator (Pittendrigh, 1966; Truman, 1971; Saunders, 1981a; Lewis and Saunders, 1987). If the oscillator has a $\phi_1$ phase at Zt = 13 h as suggested by the extended night protocols, then this phase will coincide with the light pulse during treatments 3,6 and 9 (Fig. 6.2).
Fig. 6.4.

Mean (+/ SEM) duration of each instar under night interruption experiments.

a-f = 1st to 6th instar. Squares = females, triangles = males, circles = pooled sexes. Closed symbols = 5 instar development, open symbols = 6 or more instar development. Each treatment represents 59-72 insects (a-e) and 13-33 insects (f). Arrows show significant troughs of low duration. Letters indicate significant photophase differences.
Fig. 6.6.

Mean (+/- SEM) pupal duration (a) and pupal weight (b) for night interruption experiments.

Squares = females, triangles = males. Closed symbols = 5 instar development, open symbols = 6 instar development. Each treatment represents 25-55 insects. The arrow shows a significant trough of shorter duration. Letters indicate significant photophase differences.
Fig. 6.6.

Mean (+/- SEM) larval duration for extended night experiments.

Squares = females, triangles = males. The arrow shows a significant trough of shorter duration. Letters indicate significant treatment differences.
Table 6.1. Analysis of variance for night interruption experiments. Symbols as for Table 3.1.

<table>
<thead>
<tr>
<th>Analysis Variable</th>
<th>Classification Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Sex</td>
</tr>
<tr>
<td>L1</td>
<td>***</td>
</tr>
<tr>
<td>L2</td>
<td>**</td>
</tr>
<tr>
<td>L3</td>
<td>**</td>
</tr>
<tr>
<td>L4</td>
<td>***</td>
</tr>
<tr>
<td>L5</td>
<td>*</td>
</tr>
<tr>
<td>L6</td>
<td>***</td>
</tr>
<tr>
<td>W5</td>
<td>***</td>
</tr>
<tr>
<td>Pupal Weight</td>
<td>***</td>
</tr>
<tr>
<td>Larval Duration</td>
<td>***</td>
</tr>
<tr>
<td>Pupal Duration</td>
<td>***</td>
</tr>
<tr>
<td>Instar Number</td>
<td>***</td>
</tr>
</tbody>
</table>

Table 6.2. Analysis of variance for extended night experiments. Symbols as in Table 3.1.

<table>
<thead>
<tr>
<th>Analysis variable</th>
<th>Classification variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Sex</td>
</tr>
<tr>
<td>Larval Duration</td>
<td>*</td>
</tr>
<tr>
<td>Pupal Duration</td>
<td>n.s</td>
</tr>
<tr>
<td>Pupal Weight</td>
<td>n.s</td>
</tr>
<tr>
<td>W5</td>
<td>n.s</td>
</tr>
<tr>
<td>Instar Number</td>
<td>n.s</td>
</tr>
<tr>
<td>Group</td>
<td>Sex</td>
</tr>
<tr>
<td>Larval Duration</td>
<td>n.s</td>
</tr>
<tr>
<td>Pupal Duration</td>
<td>n.s</td>
</tr>
<tr>
<td>Pupal Weight</td>
<td>n.s</td>
</tr>
<tr>
<td>W5</td>
<td>n.s</td>
</tr>
<tr>
<td>Instar Number</td>
<td>n.s</td>
</tr>
</tbody>
</table>

Treatments are grouped according to the predicted position of $\varphi_i$, with treatments 3, 6 and 9 in one group and all other treatments in the other.

The analysis of variance for extended night experiments is displayed on Table 6.2. The nine treatments and the two groupings described above were analysed. The larval duration is the only variable showing any significant differences among treatments. This is displayed in Fig. 6.6.
6.4. Discussion

As discussed in Section 6.3.1, night interruption experiments for larval and pupal duration, instar number and pupal weight all show two troughs (Figs. 6.3 to 6.5). This confirms that the position of the light pulse is important in determining development rate - it is not merely dependant on the amount of light experienced.

Two possible explanations for the influence of photoperiod on these parameters are hourglass or external coincidence mechanisms. The presence of either of these could result in one or two troughs during night interruption experiments (Chapter 2). If an external coincidence mechanism applied, a photoinducible phase is likely to be present 13 h after sunset (Fig. 6.1; Saunders, 1981a).

Extended night experiments were designed so that some pulses would coincide with possible positions of \( \phi_1 \). This assumes that \( \tau \) for the oscillator is close to 24 h. If an external coincidence mechanism is responsible for photoperiodic control, then extended night experiment should result in a clear resonance pattern showing cycles of peaks and troughs at intervals of around 24 h (Saunders, 1981a; Saunders and Lewis, 1987a). In insects showing an hourglass response, extended night protocols produce a short day response under most photophases. When the pulses are close together however, a long-day response can result (Lees, 1973; Adams, 1986b). According to the hourglass theory of Lees (1973), the shorter of the two dark periods is interpreted as a short night by the hourglass.

Larval duration (Fig. 6.6) shows some differences among photophases (Table 6.2), and the drop in duration under treatment 9 (a long-day response) suggests the involvement of an hourglass mechanism, since the shorter of the dark pulses is less than the critical night of 10 h (Chapter 3) for this species. The trough at treatment 9 could also be due to a coincidence of light with \( \phi_1 \) on the third cycle (Fig. 6.2) but this is unlikely since there are no indications of troughs corresponding to the second cycle and treatments corresponding to the possible position of \( \phi_1 \) show no significant effect on duration (Table 6.2).

In conclusion, the night interruption experiments show the involvement of a photoperiodic mechanism, and although extended night experiments show some evidence for an hourglass response, this is far from conclusive. Further
extended night experiments with all parts of the night illuminated would cover all possible positions of $\varphi_1$ and provide a more conclusive test of the mechanism involved.
CHAPTER SEVEN

EFFECTS OF LARVAL TRANSFER FROM LONG TO SHORT AND SHORT TO LONG DAYS

7.1. Introduction

As mentioned in Chapter two, any explanation of a photoperiodic mechanism in insects should incorporate both the way in which daylength is measured - the "clock" and the way the number of long and short days are accumulated - the "counter" (Saunders, 1981b). In Chapter 5 I concentrated on possible clock mechanisms responsible for photoperiodic induction in P. octo. In this chapter the counter mechanism is examined.

Most insects are not responsive to photoperiod throughout the whole of development, but have a specific life cycle stage which is sensitive to photoperiodic stimuli. In a few insects with larval diapause, the sensitive stage is the same instar in which diapause occurs, but in most species the earlier instars, embryos or the adults of the previous generation are sensitive to photoperiod (Saunders, 1982a; Section 1.1.2).

In some species, the middle instars are most sensitive to photoperiod. In Hyphantria cunea (Lepidoptera), the 5th - 15th day of larval development is the most sensitive (Park and Kyung Saeng Boo, 1989). The spider mite Tetranychus urticae shows the greatest sensitivity during the proynymph or third immature stage (Veerman, 1977), and in Limenitis archippus (Lepidoptera), the sensitive stage is the 2nd-3rd instar (Platt and Harrison, 1989).

In P. octo all larval instars show a response to photoperiods (Chapter 3) and thermophotoperiods (Chapter 4). Lipids accumulate during the fifth and sixth instars (Chapter 5), suggesting that these instars are the most affected by photoperiod. The stages most sensitive to photoperiod are therefore likely to be the earlier instars. In this chapter the sensitive period and cumulative effects of photoperiods are determined by rearing different instars under long or short day conditions.
Larval development under transfer experiments.

$a =$ Percentage (+/-SEM) developing through 6 or more instars, $b =$ mean (+/-SEM) larval duration, $c =$ mean (+/- SEM) duration of instars 3-7. Stippled = female; striped = male; white = female, 7 instar development. Each bar represents 18-34 insects. Letters indicate significant treatment differences.
7.2. Materials and Methods

P. octo eggs were obtained from the DSIR Insect Rearing Division and reared to pupation as described in Section 3.2. Some larvae were reared under short days (LD 6:18) until they moulted to the third instar when they were transferred to fresh diet under long day (LD 18:6) conditions. Other larvae were reared in long days and transferred to fresh diet under short days at the start of the third instar.

Larvae were reared at 21°C +/- 1°C. Forty-eight insects were used for each treatment. The treatments were then repeated with a further 48 insects. Other rearing conditions and statistical analyses are described in Section 3.2.

7.3. Results

The head capsule widths, duration of each instar and total larval duration were recorded. The combined duration of the later instars was also recorded since this shows the larval duration after the transfer. Results from long-short and short-long day transfers were compared with results from LD 6:18 and LD 18:6 from Chapter 3.

The analysis of variance is shown on Table 7.1. Larvae developed through 5 or 6 instars. Instar number and larval duration is displayed on Fig. 7.1; duration of instars 3-5 on Fig. 7.2; pupal weight on Fig. 7.3 and head capsule width of the 4th and 5th instars on Fig. 7.4.

7.4. Discussion

7.4.1. Larval Development

The number of supernumerary 6th instars, larval duration (Fig. 7.1) and duration of the later instars (Fig. 7.2) are all significantly greater under short days than under all other treatments. This suggests that the presence of long days in either the earlier or later instars shortens development time and reduces the proportion of supernumerary instars.

The action of long days therefore appears to have more influence on larval development than the short day effect. Similar results for transfer experiments
Mean (+/- SEM) duration for the later instars under transfer experiments.

a - c = 3rd to 5th instars, each treatment represents 52-66 insects. White dots = 5 instar development, stripes = 6 instar development, white = 7 instar development, black = instar groups pooled. Letters indicate significant treatment differences.
Fig. 7.3.

Mean (± SEM) pupal weight.

Stipples = females, stripes = males. Each bar represents 17-86 insects.

Letters indicate significant treatment differences.
Mean +/-SEM head capsule widths of instars 3 and 4.

a = 4th instar, b = 5th instar, each treatment represents 53-74 insects. Shading as in Fig. 7.2. Letters indicate significant treatment differences.
Table 7.1. Analysis of variance for transfer experiments. Symbols as for Table 3.1.

<table>
<thead>
<tr>
<th>Analysis variable</th>
<th>Treatment</th>
<th>Classification variable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sex</td>
</tr>
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<tr>
<td>L4</td>
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<td>n.s</td>
</tr>
<tr>
<td>L5</td>
<td>***</td>
<td>n.s</td>
</tr>
<tr>
<td>L6</td>
<td>n.s</td>
<td>*</td>
</tr>
<tr>
<td>L3-final</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>Larval Duration</td>
<td>***</td>
<td>n.s</td>
</tr>
<tr>
<td>W3</td>
<td>***</td>
<td>n.s</td>
</tr>
<tr>
<td>W4</td>
<td>**</td>
<td>n.s</td>
</tr>
<tr>
<td>W5</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>W6</td>
<td>n.s.</td>
<td>**</td>
</tr>
<tr>
<td>Pupal weight</td>
<td>***</td>
<td>**</td>
</tr>
</tbody>
</table>

have been found in *Acronycta rumicis* (Tyschenko et al., 1972, cited Saunders, 1982). In this species, the diapause inhibition by long days is stronger than diapause promotion by short days. In terms of the photoperiodic counter idea (Gibbs, 1975; Saunders, 1981b) this means that the build up of diapause inducing titre during short days is slow, whereas its breakdown during long days is much faster.

There is little difference in larval duration or instar number between insects exposed to long days during early or late instars (Fig. 7.1 to 7.2). This indicates that there is little difference in sensitivity to photoperiod between early and late instars in *P. octo*.

### 7.4.2. Pupal Weight

Fig. 7.3 shows that male pupae are heavier under the short-day photophase than under any of the long-day treatments. The same is not true for the female pupae however, and the pupal weight does not show the same kind of treatment effect as the larval duration. The relationship between photoperiodic conditions and pupal weight is complex and cannot be determined from Fig. 7.3. Possible explanations are discussed in Section 3.4.4.
7.4.3. Head Capsule Widths

Treatments involving transfers have the narrowest head capsule widths (Fig. 7.4). This could be a result of disturbing larvae during the transfer, or it could correspond to the results in Chapter 3, where the narrowest head width occurred during intermediate length photophases. As discussed in Section 3.4.3, the reason for these differences is uncertain, but could be due to different growth rates and critical head size under different conditions.
CHAPTER EIGHT

INSENSITIVITY OF THE GALLERIA WAX TEST IN MEASURING JUVENILE HORMONE TITRES IN P. OCTO

8.1. Introduction

As mentioned in Section 1.3, juvenile hormone has been implicated in photoperiodically induced diapause in Lepidoptera. In this chapter, the role of juvenile hormone is evaluated by comparing the titres of larvae reared under long-day and short-day photoperiods.

Juvenile hormone is found in three homologues in Lepidoptera; JH I, JH II and JH III. These vary in biological activity as measured by their effect on larval and pupal development. The activity of JH I and JH II is about 100 times greater than JH III (Dahm et al., 1976).

Three common methods used for JH assays are radioimmunoassay (RIA), (Granger and Goodman, 1988), gas chromatography with mass spectroscopy (GC-MS) (Mauchamp et al., 1985; Rembold and Lackner, 1985) and various forms of bioassays (Slama, 1974).

GC-MS and RIA are both extremely sensitive, but both require expensive equipment and chemicals and experienced operators (N. Granger; P.S. Woodgate, pers. comm.). Bioassays are cheaper and easier, so a bioassay was used for JH analysis. Bioassays involve measuring the biological activity of JH on insect larvae or pupae, but have the disadvantage that only relative concentrations of JH can be determined (Slama, 1974). The Galleria wax test is one of the most sensitive for determining JH activity in crude extracts (Slama, 1974), so this method was used for P. octo analysis.

8.2. Materials and Methods

8.2.1. Preparation of JH Crude Extract

P. octo eggs were reared under long (LD 18:6) and short (LD 6:18) days as described in Section 3.2. Fourth to 6th instars and pupae were selected for
Fig. 8.1.

The percentage response (+/- SEM) for different concentrations of JH analogue ZR 619 using the Galleria bioassay.

Each point represents 21-32 Galleria pupae.
analysis and stored at -80°C until use. Final and penultimate 5th instar larvae were determined as described in Section 5.2.

JH crude extract was prepared according to the method of DeWilde et al., (1968) for the *Galleria* wax test. Whole animals were homogenised in 2.5 ml/g tissue ether:ethanol (6:1 v/v) with a third of their weight of celite, following suggestions of Granger and Goodman (1988) for whole animal extractions. The homogenate was filtered, and the celite cake rehomogenised in ether:ethanol as before. The extract was collected into a centrifuge tube, mixed with 2 ml water and centrifuged for 10 minutes at 2000 r.p.m. The ether layer was collected and the water layer and interjacent layer was centrifuged as before with 2.5 ml ether:ethanol then 2.5 ml ether. The pooled ether extracts were evaporated in a stream of air under ice and stored at -80°C prior to analysis.

All glassware was precoated with 10% polypropylene glycol solution (MW 20 000), rinsed and dried (Goodman et al., 1976). This prevents JH from binding to the glass (Giese et al., 1977).

8.2.2. The *Galleria* Bioassay

From DeWilde et al., (1968).

Final instar *Galleria mellonella* larvae were obtained from Biosuppliers Ltd., Auckland and left at 28°C in the dark to pupate. Pupae less than 1 day old were used for the analysis. A cuticle fragment of 1 mm square was cut from the dorsal surface of the median crest on the thorax of anaesthetised pupae, and a little powdered Ampicillin was added to the wound. A mixture of olive oil (0.1 ml = 0.042 g) and 0.042 g paraffin wax was added to the JH extract. The extract was heated to melt the wax, and the wound sealed with the wax mixture.

The pupae were left in the dark at 28°C for 6-8 days and the pharate adult was examined. If the JH titre is high, the developing epidermis forms pupal cuticle instead of adult tissue and this is scored as a positive response. The pupal tissue can be easily distinguished from adult cuticle by its browner appearance and rugose structure (Schneiderman and Gilbert, 1958).
Table 8.1. Results of *Galleria* bioassay for larvae and pupae at different developmental stages.

<table>
<thead>
<tr>
<th>Tissue Weight</th>
<th>No of Galleria</th>
<th>% response (SEM)</th>
<th>GU</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Long day</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L5, final</td>
<td>1.54g</td>
<td>12</td>
<td>8.3 (14.3)</td>
</tr>
<tr>
<td>0-1 day</td>
<td>1.31g</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>3 days</td>
<td>1.28g</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>4 day</td>
<td>0.59g</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>L6, 4 day</td>
<td>0.76g</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Pupa, male</td>
<td>0.45g</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td><strong>Short day</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L4, 0-2 day</td>
<td>0.19g</td>
<td>9</td>
<td>11.1 (13.6)</td>
</tr>
<tr>
<td>L5, 7 day</td>
<td>0.37g</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>L5 penultimate</td>
<td>1.35g</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>3-4 day</td>
<td>0.92g</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>L6, 2 day</td>
<td>0.59g</td>
<td>9</td>
<td>22.2 (17.5)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long day</td>
<td>6.83g</td>
<td>61</td>
<td>1.6 (2.6)</td>
</tr>
<tr>
<td>Short day</td>
<td>3.42g</td>
<td>50</td>
<td>6(4.7)</td>
</tr>
</tbody>
</table>

8.3. Results

Before testing with *P. octo* JH sample, known concentrations of a JH analogue (Zoecon ZR 619) were used as a standard (Fig. 8.1). The percentage response for ZR 619 concentration would only correspond to the response JH concentration in *P. octo* if the biological activities of the two hormones were the same. However, the standard curve computed from the responses at known concentration would be useful for correlating relative concentration to response. For example, one *Galleria* unit (GU) is the amount of JH needed to elicit a response in 50% of *G. mellonella* pupae (DeWilde et al., 1968). From Fig. 8.1 this corresponds to a ZR 619 concentration of 1 in 3 X 10⁻⁵. Two GU therefore corresponds to a concentration of 1 in 150, or a response of 70%, and half a GU gives a response of 35%.

The results from the *P. octo* assays are displayed on Table 8.1. The responses from Fig. 8.1 are used for calculating GU concentrations.
8.4. Discussion

None of the positive results differ significantly from zero (Table 8.1). This indicates that the test was not sensitive enough to show the juvenile hormone titres.

The Galleria wax test is normally extremely sensitive, being able to detect $1.35 \times 10^{-12}$ g of Cecropia JH (Hsaio and Hsaio, 1977). If 1 µl of wax mixture is used to seal the wound this means the limit of sensitivity corresponds to $2.7 \times 10^{-10}$ g per sample.

This value can be compared with the JH titres found in other immature Lepidoptera. Using gas chromatography, Schooley et al., (1976) reported a concentration of $1.6 \times 10^{-9}$ g combined JH1 and JH11 per g of insect tissue in 4th instar Manduca sexta. Rembold and Lackner (1985) and Rembold and Sehnal (1987) report $2.8 \times 10^{-9}$ g/g and $7.5 \times 10^{-10}$ g/g of JHII in G. mellonella larvae.

These values are inside the detection limit for the Galleria bioassay. The Galleria wax test has been used successfully to determine JH titres in larval G. mellonella (Hsaio and Hsaio, 1977), so it is likely that the extraction method is at fault. DeWilde et al., (1968) used the extraction method described in Section 7.2, but it was used on haemolymph, not whole animals. A more thorough extraction procedure on whole animals was followed by Hsaio and Hsaio (1977). A successful bioassay for P. octo would therefore involve either using more whole animal tissue, extracting from haemolymph or using an improved extraction method.
CHAPTER NINE

GENERAL DISCUSSION

9.1. Evidence for Diapause in *Planotortrix octo*

The photoperiodic response curves for both larval duration and instar number (Chapter 3; Morris 1990a) are typical of a diapause response in long-day insects (Beck, 1980; Masaki, 1984). Beck (1980) mentions a suppressed development rate as one criterion for distinguishing diapause, and Oku (1966) reviews the diapause response in three Tortricidae in which an extra stationary instar is part of diapause. In *Diatraea grandiosella* (Chippendale and Yin, 1973; Yin and Chippendale, 1973) and in *Chilo suppressalis* (Yagi and Fukaya, 1974), diapause larvae undergo one or more supernumerary instars in which the diapause occurs.

The build up of lipids in the 5th and 6th instars provides further confirmation of a photoperiodic diapause response (Chapter 5). Larval diapause is often confined to a specific instar, generally the final one (Saunders, 1982; Danks, 1987).

As mentioned earlier (Section 1.2, also Morris, 1989) diapause in New Zealand insects is rare, and undocumented in phyllophagous species. This is thought to be the result of a year round food supply and a warmer climate while diapause was evolving in the northern hemisphere (Dumbleton, 1967). The results presented in this thesis are therefore significant in that they show the first documented case of a photoperiodically controlled diapause in a New Zealand leaf-eating insect.

In northern hemisphere species, diapause is often a clear cut all or nothing response (Saunders, 1982a). A sharply distinct diapause response is present in the New Zealand moth *Stathmopoda aposema* (Muggleston, 1988). This species has a seasonally limited food supply, so a winter diapause could have some selective advantage.

In contrast, the diapause response in *P. octo* is graded. Larvae still grow under short day conditions, albeit more slowly, the number of instars increases and there is a build up of lipid reserves. This could represent the beginning of a diapause response evolving in this species. *P. octo* is one of a group of cryptic species, originally all labelled as *P. excessana*, but recently reclassified by Dugdale (1990).
based on their mate recognition systems. The "P. excessana" complex described by Foster et al. (1987) and Foster and Dugdale (1988) have been renamed as P. excessana, P. octo, P. octoides and P. avicenniae (Dugdale, 1990). These species differ in their geographical distribution, and therefore in the climatic conditions experienced. The Northern P. avicenniae for example is found on mangroves in Auckland and the Coromandel Peninsula, and would experience far less variation in temperature and photoperiod than the Southern P. octoides, found in the Chatham Islands (Foster et al., 1987; Dugdale, 1990). A comparison of the overwintering response between the group of Planotortrix cryptic species could provide further information about the evolutionary relationships between the species.

Other authors have shown some indication of a diapause response in other New Zealand phytophagous species, though this has not been so thoroughly investigated as the P. octo response. Laboratory reared 'Cnephasia' jactatana show a shorter final instar duration under short days (Ochieng'Odero, 1988) and field studies on Ovocrambus vitellus and O. flexuosus revealed a vastly lengthened final instar under winter conditions (Gaskin, 1974). This experiment could not differentiate between quiescence and diapause, but in view of the P. octo results further experiments on these two species could be well worthwhile.

9.2. The Photoperiodic Mechanism in P. octo

9.2.1. Introduction

Having established that a photoperiodic diapause response in P. octo is likely, the next step is to determine the photoperiodic mechanism involved. The night interruption experiments (Chapter 6) provide further evidence for a photoperiodic response but by themselves cannot differentiate between a circadian or hourglass response. The action of thermophotoperiods (Chapter 4), suggests a circadian mechanism whereas extended night responses suggest an hourglass could be involved (Chapter 6).

To test how well the diapause response in P. octo fits in with a circadian mechanism, simulations of the diapause response will be run using a circadian computer model.
9.2.2. Description of the Model

Simulations will be based on the damped oscillator model of Lewis and Saunders (1987), as modified by Morris and Lewis (1990). This model was chosen for a variety of reasons. Firstly, simulations using this model have reproduced the shapes of the PPRC curves and resonance results in other species (Saunders and Lewis, 1987a,b). It also explains a variety of other photoperiodic and thermoperiodic effects (Section 2.9). Finally it is an explicit model which can easily be tested by running computer simulations.

The original Lewis and Saunders (1987) model assumed diapause is due to a diapause inducing substance (INDSUM), produced when a damped oscillator drops through a threshold value. This was later modified so that the INDSUM build up also depends on the value of the oscillator above threshold (Saunders and Lewis, 1988; Morris and Lewis, 1990; Fig. 1.2). This modified version can simulate both the presence and absence of a thermoperiodic response (Morris and Lewis, 1990), and since thermophotoperiods have an influence on larval duration (Chapter 4), this version will be used here. Further details of the model are explained in Section 1.3.8.

Lewis and Saunders (1987) based the model on diapause induction in the fleshfly Sarcophaga argyrostoma. The parameters used in their paper reflect their knowledge of the biology of this species. The original parameters were altered in a later paper to reflect the biology of D. grandiosella. Simulations using these values reproduced the thermoperiodic response curve found in this species. When the original Sarcophaga values were used, no thermoperiodic response was generated (Morris and Lewis, 1990). This demonstrates the importance of basing models on what is known of the species under scrutiny. The parameters of the model are therefore altered to reflect the biology of P. octo.

Lipid reserves in P. octo are built up during the 5th and 6th instars (Chapter 5), so diapause is assumed to occur during these stages. The presence of a 6th instar and the duration of the 5th to 7th instars are assumed to be indicative of a diapause response. Since the size of the 5th instar determines whether there will be another larval moult (Fig. 3.6), it is assumed that the larva is already committed to a diapause or non-diapause response by the end of the 4th instar.
Experiments transferring larvae from long to short days (Chapter 7) show that sensitivity to photoperiod continues past the third instar. The model therefore assumes that the 1st to 4th instars are all equally sensitive to photoperiod. The effect of long days is also stronger than that of short days (Chapter 7). This can be reflected in simulations by giving the light intensity a value of 2, so that INDSUM breakdown in the light is twice as fast as its synthesis in the dark.

The average development time for the first four instars of females at 17°C under total darkness is 25 days (Chapter 3). The duration at 21°C is 17 days giving a $Q_{10}$ value of 2.62 for larval development. These parameters are used in the model. In *S. argyrostonTa*, diapause is a distinct response (Saunders, 1971). The amount of INDSUM was therefore compared with the percentage diapause response in a population (Lewis and Saunders, 1987). In *P. octo* the intensity of diapause depends on the length of the 5th to final instars, and it is assumed that this duration is influenced by the INDSUM titre. The duration of the earlier non-diapausing instars is also affected by photoperiod (Figs. 3.3 and 3.4), so the duration of these instars is also lengthened by INDSUM build-up, giving a slight positive feedback effect in short days. A longer sensitive period increases INDSUM build-up which in turn increases larval duration.

The $Q_{10}$ for INDSUM synthesis is 2 in the original Saunders and Lewis (1987) model, and by keeping it at this value, simulations mimic the absence of a thermoperiodic response in this species. If the $Q_{10}$ value is increased, a thermoperiodic response is generated (Morris and Lewis, 1990; Fig. 1.3). Since *P. octo* shows no thermoperiodic response in total darkness (Chapter 4), the low value of 2 is used.

All simulations are run on an IBM PC using TURBO PASCAL and TURBO GRAPHIX software. All other parameters not mentioned above are kept the same as in the Lewis and Saunders (1987) model. The PASCAL program is printed in Appendix 1.

### 9.2.3. Simulations Using the Damped Oscillator Model

The simulated PPRCs at 17°C and 21°C (Fig. 9.1a) show the drop off in DD and the slight increase under long photophases. This corresponds to the experimental results for both larval duration and instar number (Figs. 3.1 and 3.2). This shape has also been simulated for other insect species (Saunders and Lewis, 1987a).
What Fig. 9.1 fails to show is the very long larval duration during a 2 hour photophase (Fig. 3.2). A later version of the damped oscillator model (M. Vaz Nunes, D.S. Saunders, R.D. Lewis, unpubl. data) can simulate this effect. As mentioned in Section 3.4, the PPRCs for larval and pupal duration show some indication of a direct photoperiodic effect. This could influence the expression of the circadian mechanism and explain the discrepancy between observed and predicted duration.

Night interruption simulations (Fig. 9.1b) show one trough for larval duration at a photophase of 20 hours. This corresponds to one of the troughs for experimental data shown in Fig. 6.3 and 6.4 though the simulated response is much more intense than the real data. The simulation failed to find the second trough since the simulated light pulses did not entrain the oscillator. In contrast, the new model of Vaz Nunes et al., (unpubl. data) can simulate both troughs.

With simulations of the extended night experiments (Fig. 9.1c) the oscillator only reaches threshold concentration every third cycle. It therefore essentially behaves like an hourglass in that INDSUM is only accumulated once during each light:dark cycle. However the simulations show the resonance troughs very clearly. The first trough at Treatment 3 (Fig. 9.1c) is caused by the coincidence of $\phi_l$ with the light. The second and third troughs come about because of differences in the oscillator values. Fig. 9.1c corresponds to experimental results on Tetranychus urticae. This mite shows hourglass properties when reared under a 72 hour LD cycle (Veeran and Vaz Nunes, 1987) but shows a resonance pattern during extended night protocols (Veeran and Vaz Nunes, 1980). According to Vaz Nunes et al. (unpubl. data) this discrepancy between an hourglass timer and circadian resonance could not be explained by the damped oscillator model of Lewis and Saunders (1987). The modified model described here however can account for both these effects.

As far as the P. octo results go, the comparison between simulated and experimental data is poor. P. octo did not demonstrate any resonance effect and instead showed a response better explained in terms of an hourglass (Chapter 6).

Fig. 9.1d shows simulations of $14^\circ$C:$22^\circ$C thermophotoperiods and thermoperiods. From this figure it can be seen that the INDSUM value is increased if the thermophase and photophase are in phase and reduced if the photophase falls in the dark. This corresponds to published results for other insect species (Beck, 1983b; Section 4.1) and to the thermophotoperiodic response curves generated by
Simulations of photoperiodic response curves for larval development of *P. octo* using a damped oscillator model.

a = Photoperiodic response curves, open circles = 17°C, closed circles = 21°C. b = Night interruption experiments. c = Extended night experiments. d = thermoperiods and thermophotoperiods; closed circles = thermophotoperiod, dark = 14°C, light = 22°C; open circles = thermophotoperiod, dark = 22°C, light = 14°C; squares = Thermoperiod, 14°C: 22°C.
duration (Fig. 4.2) and instar number (Fig. 4.3) are also consistent with the simulations, showing a longer duration and higher instar number during in-phase thermophotoperiods.

The simulated data for thermoperiods alone (Fig. 9.1d) successfully predict the absence of a thermoperiodic response in *P. octo*. A possible explanation for this in terms of the rate summing concept (Ratte, 1985) is discussed in Section 4.3.2.

In conclusion, the action of thermophotoperiods and skeleton photoperiods and the shape of the photoperiodic response curves provide evidence for a damped circadian oscillator mechanism in *P. octo*. Results from extended night experiments suggest a photoperiodic response, but provide no evidence for a damped circadian mechanism, and the results for resonance experiments are contrary to predictions of a damped circadian oscillator. The photoperiodic mechanism in *P. octo* therefore remains uncertain.

9.2.4. Possible Identity of INDSUM and the Oscillator

As mentioned in Chapter 2, current circadian models for photoperiodism involve two separate mechanisms, the "clock" and the "counter" (Saunders, 1981b). These assumes the presence of two chemicals, the oscillator (c) and the diapause inducing agent (INDSUM) the identity of which still remains speculative.

In insects, JH is a likely contender for INDSUM. The effect of raised JH levels in larval diapause induction has already been discussed (Section 1.3). In pupal diapause also, the role of JH cannot be discounted (Denlinger, 1985). In *Sarcophaga crassipalpis* (Walker and Denlinger, 1980) and *Mamestra brassicae* (Lepidoptera) (Yagi, 1976) the level of JH is higher in larvae destined for pupal diapause than in non-diapause destined larvae. Although experiments on JH titres in *P. octo* have so far been inconclusive (Chapter 8), further studies on JH titres under different photoperiodic conditions in this species could prove worthwhile.

N-Acetyl transferase (NAT) has been implicated in vertebrate photoperiodism. The concentration of this enzyme oscillates with a circadian frequency in constant darkness (Kasal *et al*., 1979; Hamm and Menaker, 1980). NAT catalyses the reaction of a precursor to melatonin, the hormone which influences vertebrate circadian rhythmicity (Klein, 1985). An oscillating enzyme could also be responsible
for c in insects. If JH is equated with INDSUM, then an enzyme catalysing JH production could correspond to the oscillator.

9.3. Other Possible Photoperiodic Effects in *P. octo*

A photoperiodic effect on larval and pupal development has been demonstrated in *P. octo* (Chapter 3). However, development and diapause are only one of many factors controlled by photoperiod. As discussed in Section 3.4 there could be some indication of a photoperiodic influence on critical head capsule width, critical weight for pupation and pre-pupal threshold. A larger sample size could provide further information on the effect on head capsule width. Critical weight can be measured by starving final instar larvae, weighing them and determining the minimum weight at which pupation occurs (Palmer, 1982; Jones et al., 1982; Ochieng-'Odero, 1988). When larvae have reached a critical weight, they keep on eating and growing until the JH levels reach a critically low level. This pre-pupal threshold, or increase above the critical weight can be calculated by taking the highest larval weight before pupation (Ochieng-'Odero, 1988).

As mentioned in Section 1.1, physiological and behavioural responses in preparation for migration are also under photoperiodic control. In *P. octo*, preliminary studies have shown that females start sexual calling behaviour slightly later than the related *Ctenopseutis obliquana* (J. Clearwater, pers. comm.). This phenomenon is well documented in the migratory army worm *Pseudaletia unipuncta* and is believed to be an adaptation to delay mating until the moth has reached a favourable climate (Section 1.1.1). The delay of calling in *P. octo* might therefore suggest some migratory tendencies in this species.

The related tortricid *Epiphyas postvittana* shows some wing polymorphism. In field studies Danthanarayana (1976) showed that under unfavourable conditions of temperature and humidity adult moths had larger wing and lighter bodies. He suggested that this was a response allowing more rapid migration from unfavourable environments. Whether wing polymorphism can be induced by photoperiod remains to be seen, but if *P. octo* is a migratory species as suggested by the sexual calling results, then experiments on photoperiod and polymorphism will be worth looking at.

The photoperiodic responses for larval duration (Fig. 3.2) and especially for pupal duration (Fig. 3.8) suggests that the duration of the photophase may have a direct
influence on development. It is possible that this is brought about by larvae feeding during daylight. An examination of the feeding rhythms could therefore give further insight into the photoperiodic response in *P. octo*. 
REFERENCES

Note: A * indicates that the abstract only has been read.


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HONEK, A. (1979) Regulation of diapause, number of instars, and body growth in the moth species Amanthes c-nigrum (Lepidoptera: Noctuidae). Entomologia Generalis 5: 221-229.


Appendix One

A printout of the PASCAL damped oscillator computer program used for simulations (Section 9.2).

```pascal
{$I typedef.sys}
{$I graphix.sys}
{$I kernel.sys}
{$I polygon.hgh}

{last revision: 22/4/90, compiled version}
{This version synthesises and destroys INDSUM proportionally to the area plus time above threshold}

type str = string[50];
  str2 = string[2];

var cycle, symbol, int, day, delay, iter, llength1, llength2, dlength, len, state, phase, ybase, i, last, ymax, wht,
  choice, ext, tempcycle, pulses, tempphase, thermoperiod: integer;

indsyn, conc, weaklight, hours, slope, ub, light, lux, synthesis,
difference, threshold, previous, dia, days, height, temperature,
thermophase, cryophase, lasttemp, buildup, diapause: real;

numtext, strlux, strtemp, strperdia, strdays, strlen, strtherm, strcryo,
strpulse: wrkstring;

cr: array[-40..1500] of real;

a: plotarray;
ans: str;
next: str2;

const start = 42;

{**************************LIGHT CHANGE**************************}

procedure lightchange;
begin
writeln('Do you want extended night?');
readln(ans);
if ans <> 'y' then ext := 1
else begin
  writeln('How many days in a cycle?');
  readln(ext);
end;
writeln('Duration of 1st light pulse in hours? ');
readln(hours);
llength1 := round(hours*3);
writeln('Duration of 2nd light pulse in hours');
readln(hours);
```

llength2 := round(hours*3);
writeln('Time between pulses in hours');
readln(hours);
dlength := round(hours*3);
writeln('Light intensity. Try 0.5 to 3 "lux"');
readln(lux);
end;

{**********************TEMPERATURE CHANGE**********************}

procedure tempchange;
begin
writeln('Do you want temperature pulses?');
readln(ans);
if ans='y' then begin
writeln('New temperature? ');
readln(temperature);
pulses:=0;
end
else begin
writeln('Thermoperiod? (0-24 hours)');
readln(hours);
themoperiod:=round(hours*3);
writeln('Temperature of cryophase?');
readln(cryophase);
writeln('Temperature of thermophase?');
readln(thermophase);
writeln('Phase between thermophase and start of photoperiod?');
readln(hours);
tempphase:=(round(hours) mod 24)*3;
tempphase:=start+tempphase;
if tempphase>=72 then tempphase:=tempphase-72;
tempcycle:=1;
pulses:=1;
end;
end;

{***********************GET VALUES***********************}

procedure getvalues;
begin
clrscr;
writeln('Do you want light changes? y/n ');
readln(ans);
if ans='y' then lightchange;
writeln('Do you want to set the temperature? y/n ');
readln(ans);
if ans='y' then tempchange;
end;

{***********************SET VALUES***********************}

procedure setvalues;
begin
for int:=-40 to 1500 do ct[int]:= 0;
slope:=0.1;
threshold:=21;
delay:=28;
distsyn:=0.6;
light:=0;
llength1:=0;
llength2:=0;
dlength:=0;
cycle:=1;
lux:=0;
ub:=10;
dia:=0;previous:=100;state:=0;
phase:=-4;
thermophase:=0;
cryophase:=0;
thermoperiod:=0;
pulses:=0;
crsr;
end;

{--------------------------LIGHT ON--------------------------}

procedure lighton;
begin
if int=start+(ext*72*cycle) then cycle:=cycle+1;
if (((int>=((cycle-1)*72*ext+start)) and
(int<((cycle-1)*72*ext+start+llength1))) or
((int>=((cycle-1)*72*ext+start+llength1+dlength)) and
(int<((cycle-1)*72*ext+start+llength1+dlength+llength2))))
then light:= lux
else light:=0;
end;

{-----------------------------TEMPERATURE COMPENSATE-----------------------------}

procedure tempcompensate;
var
avtemp:real;
begin
avtemp:=(thermophase*thermoperiod+cryophase*(72-thermoperiod))/72;
ub:=10*(exp(((temperature-17)/10)*0.1823));
slope:=0.1*(exp(((temperature-17)/10)*0.1823));
distsyn:=0.6*(exp(((temperature-17)/10)*-0.956));
if pulses=0 then days:=round(25*(exp(((temperature-17)/10)*-0.875)));
if pulses=1 then days:=round(25*(exp(((avtemp -17)/10)*-0.875)));
if days=0 then days:=1;
end;

{-----------------------------TEMPERATURE ON-----------------------------}

procedure tempon;
begin
lasttemp:=temperature;
if int=tempphase+(72*tempcycle) then tempcycle:=tempcycle+1;
if (int>=((tempcycle-1)*72+tempphase)) and (int<((tempcycle-1)*72+tempphase+thermperiod))
then temperature:=thermophase else temperature:=cryophase;
if lasttemp<>temperature then tempcompensate;
end;

***************PRINT VALUES***************

procedure printvalues;
begin
  definewindow(1,1,ybase+5,73,ybase+15);
  selectwindow(1);
  str(lux:3:2,strlux);
  str(len:3:1 div 3,strlen);
  str(temperature:3:2,strtemp);
  str(days:3:2,strdays);
  dia:=dia/80+days+(1.271*exp((temperature-17)/10)*-0.21);
  str(dia:3:2,strperdia);
  str(thermophase:3:2,strtherm);
  str(cryophase:3:2,strtcr); 
  drawtext(2,ybase+10,1;'Light'+strlux+'Pulselenth'+strlen);
  if pulses=0 then drawtext(240,ybase+10,1,'Temp' +strtemp+'Percent diapause '+strperdia)
  else drawtext(240,ybase+10,1,'cryophase' +strcryo+'thermophase '+strtherm+'Percent Diapause '+strperdia);
end;

***************DRAW TEMPERATURE PULSES***************

procedure tempulse;
begin
  definewindow(1,iter,wht,iter+1,wht+7);
  selectwindow(1);
  if light>0 then begin
    setcolorblack;
    drawtext(iter*8,wht+2,1,'-');
    setcolorwhite;
  end
  else drawtext(iter*8,wht+2,2,'-');
end;

***************CHANGE STATE***************

procedure changestate;
begin
  symbol:=43;
  state:=0;
  phase:=int;
end;
{***************CALCULATE DIAPAUSE***************}

procedure calculatediapause;
var lght,c:real;
begin
    int:=(day-1)*72+iter;
    if length1<>0 then lghton;
    if pulses<>0 then tempOn;
    difference:=30 -ct[int-delay];
    synthesis:= difference*slope;
    if synthesis>ub then synthesis:=ub;
    if synthesis<0 then synthesis:=0;
    ct[int]:=ct[int-1]+synthesis;
    ct[int]:=(ct[int]-ct[int]*0.1);
    ct[int]:=ct[int]-light;
    if ct[int]<1 then ct[int]:=1;
    conc:=ct[int];
    symbol:=32;
    if ((conc/previous)<1) and (conc <= threshold)
    and (state=1)) then changestate;
    if ((conc/previous>1.0) and (conc > threshold)) then state:=1;
    previous:=conc;
    if ((light>0) and (symbol=43)) then symbol:=42;
    lght:=lux;
    if lght=1 then lght=1;
    if state=1 then buildup:=buildup+indsyn*conc*1.5/13;
    if symbol=42 then begin
        dia:=dia-light*buildup*2;
        days:=days-light*buildup/80;
        end;
    if symbol=43 then begin
        dia:=dia+buildup;
        days:=days+buildup/80;
        end;
    if symbol>40 then buildup:=0;
end;

{***************DRAW PHOTOPERIOD***************}

procedure drawphotoperiod;
begin
    wht:=5*day;     {for each day, height of strip=5 pixels}
    if light>0 then begin
        definewindow(1,iter,wht,iter+1,wht+7);
        selectwindow(1);
        setbackground(255);
        if symbol=42 then begin
            setcolorblack;
            drawtext(iter*8,wht+3,1,'*');
            setcolorwhite;
            end;
        end
        else begin
            definewindow(1,iter,wht,iter+1,wht+7);
            selectwindow(1);
            end
end
setbackground(0);
if symbol=43 then begin
  drawtext(iter*8,wht+3,2,');
end;
end;
if (pulses=1) and (temperature=thermophase) then temppulse;
end;

***************PRINT DIAPAUSE***************

procedure printdiapause;
begin
  definewindow(1,75,wht,78,wht+7);
  selectwindow(1);
  str(dia:3.0,numtext);
  drawtext(608,wht+4,1,numtext);
end;

***************DRAW OSCILLATOR***************

procedure drawoscillator;
begin
  ybase:=wht+13;
  drawline(2.5,2,ybase);
  drawline(596.5,596,ybase);
  drawline(2,ybase,596,ybase);
  setwindowmodeon;
  ymax:=40;
  defineword(1.0,ymax,1800,0);
  defineword(1.0,280,80,330);
  selectworld(1);
  selectwindow(1);
  last:=round(18*days);
  for i:=1 to last do begin;
    int:=(i-1)*4;
    a[i,1]:=int;
    a[i,2]:=ct[int];
  end;
  drawpolygon(a,1,last,0,1,0);
  drawline(0,ymax-threshold,2*last,ymax-threshold);
  drawtextw(2*last+10,ymax-threshold,1,'THRESHOLD');
end;

***************SIMULATE OSCILLATOR***************

procedure simulate;
begin
  initgraphic;
  clearscreen;
  setwindowmodeoff;
  setlinestyle(0);
  Setbreakoff;
  drawline(2.4,596.4);
  tempcompensate;
  day:=1;
repeat;
  for iter:=1 to 72 do begin;
    calculatediapause;
    drawphotoperiod;
    end;
  printdiapause;
  day:=day+1;
  until day > days;
end;

{***************************MAIN PROGRAM***************************}

begin
  setvalues;
  getvalues;
  simulate;
  diapause:=dia/80+day+(12.71*exp(((temperature-17)/10)*-0.21));
  drawoscillator;
  read(kbd,next);
  leavegraphic;
  writeln('INDSUM = ',dia);
  writeln('Percentage diapause = ', diapause);
  writeln('Days = ', days);
  writeln('Temperature = ',temperature);
end.
APPENDIX TWO

Further results not included in the main text.

Chapter Three

3.1 Mean (SEM) head capsule widths in μm, sorted by the number of instars of development.

<table>
<thead>
<tr>
<th>Photophase (hours)</th>
<th>Instar no.</th>
<th>1st Instar</th>
<th>3rd Instar</th>
<th>6th Instar</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>285 (1) n=47</td>
<td>637 (4) n=45</td>
<td>1918 (22) n=12</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>287 (1) n=12</td>
<td>593 (13) n=12</td>
<td>1602 (234) n=2</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>282 (6) n=2</td>
<td>564 (36) n=2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>283 (2) n=16</td>
<td>635 (8) n=16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>285 (1) n=42</td>
<td>607 (4) n=42</td>
<td>1893 (20) n=38</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>288 (0) n=2</td>
<td>570 (18) n=2</td>
<td>1422 (54) n=2</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>281 (1) n=27</td>
<td>618 (5) n=27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>285 (1) n=21</td>
<td>605 (6) n=21</td>
<td>1834 (42) n=21</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>276 (0) n=2</td>
<td>540 (60) n=2</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>5</td>
<td>283 (2) n=18</td>
<td>601 (2) n=18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>284 (4) n=3</td>
<td>584 (7) n=11</td>
<td>1842 (27) n=10</td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>280 (1) n=28</td>
<td>610 (5) n=54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>276 (0) n=4</td>
<td>588 (34) n=4</td>
<td>1898 (43) n=5</td>
</tr>
<tr>
<td>18</td>
<td>5</td>
<td>285 (1) n=50</td>
<td>646 (3) n=54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>286 (4) n=6</td>
<td>574 (20) n=6</td>
<td>1778 (66) n=5</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>283 (1) n=50</td>
<td>643 (5) n=52</td>
<td>1887 (43) n=9</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>285 (3) n=8</td>
<td>595 (16) n=9</td>
<td></td>
</tr>
</tbody>
</table>
3.2. Frequency distribution of head capsule widths grouped by the number of instars of development. w2-w4 = width in μm of instars 2-4.

<table>
<thead>
<tr>
<th>w2</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Instar</th>
<th>w3</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Instar</th>
<th>w4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
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<td>336</td>
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<td>0</td>
<td>480</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>666</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<td>0</td>
<td>0</td>
<td>492</td>
<td>0</td>
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<td>0</td>
<td>690</td>
<td>0</td>
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<td>0</td>
<td></td>
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</tr>
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<td>1</td>
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<td>714</td>
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<td>0</td>
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<td>516</td>
<td>0</td>
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<td>0</td>
<td>738</td>
<td>0</td>
<td>2</td>
<td>0</td>
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<td>8</td>
<td>0</td>
<td>528</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>762</td>
<td>0</td>
<td>4</td>
<td>1</td>
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<td>36</td>
<td>3</td>
<td>540</td>
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<td>2</td>
<td>0</td>
<td>786</td>
<td>0</td>
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<td>1</td>
<td></td>
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<td>408</td>
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<td>31</td>
<td>2</td>
<td>552</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>810</td>
<td>0</td>
<td>11</td>
<td>2</td>
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<tr>
<td>420</td>
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<td>0</td>
<td>0</td>
<td>564</td>
<td>10</td>
<td>8</td>
<td>0</td>
<td>834</td>
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<td>9</td>
<td>1</td>
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<td>432</td>
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<td>6</td>
<td>0</td>
<td>576</td>
<td>26</td>
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<td>0</td>
<td>858</td>
<td>1</td>
<td>14</td>
<td>0</td>
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<tr>
<td>444</td>
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<td>0</td>
<td>0</td>
<td>588</td>
<td>18</td>
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<td>12</td>
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<td>34</td>
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<td>606</td>
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<td>0</td>
<td>678</td>
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<td>0</td>
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</tr>
</tbody>
</table>

Chapter Four

4.1. Mean (SEM) 5th instar head capsule width in μm under thermoperiods. Grouped by number of instars of development.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 instar</th>
<th>6 instar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short day</td>
<td>1789 (13.2) n=42</td>
<td>1346 (11.3) n=14</td>
</tr>
<tr>
<td>Long day</td>
<td>1649 (42.9) n=9</td>
<td>1328 (22.3) n=17</td>
</tr>
</tbody>
</table>
4.2. Mean (SEM) pupal weight in mg under thermophotoperiods.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sex</th>
<th>Disturbed</th>
<th>Undisturbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>In phase</td>
<td>Male</td>
<td>62.2 (1.3) n=35</td>
<td>67.7 (1.1) n=38</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>88.0 (2.4) n=21</td>
<td>104.4 (3.9) n=20</td>
</tr>
<tr>
<td>Out of phase</td>
<td>Male</td>
<td>58.4 (1.0) n=37</td>
<td>64.7 (1.6) n=33</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>88.8 (1.6) n=24</td>
<td>102.9 (3.2) n=21</td>
</tr>
</tbody>
</table>

4.3. Mean (SEM) pupal duration in days under thermophotoperiods.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sex</th>
<th>Disturbed</th>
<th>Undisturbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>In phase</td>
<td>Male</td>
<td>18.2 (1.3) n=18</td>
<td>15.8 (0.2) n=37</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>16.5 (0.3) n=91</td>
<td>5.2 (0.3) n=18</td>
</tr>
<tr>
<td>Out of phase</td>
<td>Male</td>
<td>17.0 (0.2) n=22</td>
<td>15.9 (0.2) n=39</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>16.6 (0.3) n=20</td>
<td>14.4 (0.4) n=22</td>
</tr>
</tbody>
</table>

Chapter Six

6.1. Mean (SEM) 5th instar head capsule width in μm under night interruption experiments grouped by number of instars of development.

<table>
<thead>
<tr>
<th>Photophase (hours)</th>
<th>Instar no.</th>
<th>Capsule width</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>5</td>
<td>1,772 (34) n=7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1,286 (64) n=50</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1,176 (0) n=1</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>1,668 (38) n=12</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1,299 (13) n=49</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>960 (0) n=1</td>
</tr>
<tr>
<td>14</td>
<td>5</td>
<td>1,675 (20) n=12</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1,296 (11) n=40</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1,664 (22) n=15</td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>1,293 (9) n=42</td>
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<td>6</td>
<td>1,671 (19) n=10</td>
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<td></td>
<td>7</td>
<td>1,288 (10) n=46</td>
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<td>18</td>
<td>5</td>
<td>1,652 (18) n=16</td>
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<tr>
<td></td>
<td>6</td>
<td>1,298 (11) n=37</td>
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<tr>
<td></td>
<td>7</td>
<td>1,602 (26) n=8</td>
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<td>20</td>
<td>5</td>
<td>1,254 (12) n=22</td>
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<tr>
<td></td>
<td>6</td>
<td>1,120 (0) n=1</td>
</tr>
<tr>
<td>22</td>
<td>5</td>
<td>1,120 (0) n=1</td>
</tr>
</tbody>
</table>
6.2. Mean (SEM) pupal duration in days, pupal weight in mg and percentage of 6th instars under extended nights.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sex</th>
<th>Duration</th>
<th>Weight</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>m</td>
<td>14.6 (0.3) n=14</td>
<td>87.6 (2.1) n=14</td>
<td>21.4 (14) n=14</td>
</tr>
<tr>
<td>f</td>
<td>13.9 (0.3) n=19</td>
<td>92.5 (3.7) n=16</td>
<td>95.0 (8) n=20</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>m</td>
<td>14.3 (0.3) n=15</td>
<td>63.6 (1.7) n=19</td>
<td>42.1 (11) n=19</td>
</tr>
<tr>
<td>f</td>
<td>13.1 (0.3) n=15</td>
<td>90.6 (4.2) n=20</td>
<td>100 (5) n=20</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>m</td>
<td>13.8 (0.2) n=19</td>
<td>65.4 (1.6) n=19</td>
<td>38.9 (11) n=18</td>
</tr>
<tr>
<td>f</td>
<td>13.3 (0.2) n=21</td>
<td>91.3 (2.2) n=21</td>
<td>95.0 (6) n=24</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>m</td>
<td>13.8 (0.1) n=16</td>
<td>66.7 (1.6) n=20</td>
<td>30.0 (11) n=20</td>
</tr>
<tr>
<td>f</td>
<td>13.3 (0.2) n=20</td>
<td>85.3 (3.0) n=22</td>
<td>87.0 (9) n=23</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>m</td>
<td>13.8 (0.2) n=26</td>
<td>66.6 (1.4) n=27</td>
<td>51.9 (11) n=27</td>
</tr>
<tr>
<td>f</td>
<td>13.2 (0.3) n=13</td>
<td>89.5 (3.4) n=13</td>
<td>85.7 (14) n=14</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>m</td>
<td>13.5 (0.2) n=20</td>
<td>66.5 (1.2) n=21</td>
<td>51.9 (11) n=21</td>
</tr>
<tr>
<td>f</td>
<td>13.0 (0.3) n=20</td>
<td>91.5 (2.5) n=22</td>
<td>90.9 (8) n=22</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>m</td>
<td>14.0 (0.2) n=21</td>
<td>63.9 (1.3) n=19</td>
<td>33.3 (11) n=21</td>
</tr>
<tr>
<td>f</td>
<td>13.1 (0.2) n=16</td>
<td>87.1 (3.9) n=18</td>
<td>94.4 (8) n=18</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>m</td>
<td>14.1 (0.3) n=17</td>
<td>67.8 (1.4) n=18</td>
<td>38.9 (14) n=18</td>
</tr>
<tr>
<td>f</td>
<td>12.8 (0.3) n=15</td>
<td>89.5 (2.1) n=17</td>
<td>88.9 (8) n=18</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>m</td>
<td>14.3 (0.2) n=13</td>
<td>65.6 (2.0) n=24</td>
<td>25.0 (10) n=24</td>
</tr>
<tr>
<td>f</td>
<td>13.2 (0.2) n=12</td>
<td>97.8 (3.4) n=16</td>
<td>100 (6) n=16</td>
<td></td>
</tr>
</tbody>
</table>

6.3 Mean (SEM) 5th instar head capsule width in µm under extended nights.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sex</th>
<th>5 Instar</th>
<th>6 Instar</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>m</td>
<td>1672 (18) n=91</td>
<td>276 (39) n=3</td>
</tr>
<tr>
<td>f</td>
<td>1636 (20) n=9</td>
<td>1307 (11) n=9</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>m</td>
<td>1680 (0) n=1</td>
<td>1272 (19) n=6</td>
</tr>
<tr>
<td>f</td>
<td>1644 (23) n=10</td>
<td>1284 (13) n=11</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>m</td>
<td>1664 (21) n=11</td>
<td>1326 (32) n=4</td>
</tr>
<tr>
<td>f</td>
<td>1700 (29) n=11</td>
<td>1310 (19) n=14</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>m</td>
<td>1746 (30) n=2</td>
<td>1212 (60) n=2</td>
</tr>
<tr>
<td>f</td>
<td>1642 (27) n=7</td>
<td>1318 (17) n=17</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>m</td>
<td>1800 (0) n=1</td>
<td>1271 (8) n=9</td>
</tr>
<tr>
<td>f</td>
<td>1670 (25) n=12</td>
<td>1272 (34) n=5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>m</td>
<td>1716 (0) n=1</td>
<td>1277 (20) n=9</td>
</tr>
<tr>
<td>f</td>
<td>1733 (25) n=7</td>
<td>1319 (23) n=12</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>m</td>
<td>1692 (36) n=2</td>
<td>1266 (35) n=6</td>
</tr>
<tr>
<td>f</td>
<td>1674 (18) n=15</td>
<td>1304 (22) n=12</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>m</td>
<td>1329 (19) n=5</td>
<td>1242 (17) n=6</td>
</tr>
<tr>
<td>f</td>
<td>1300 (16) n=13</td>
<td>1320 (28) n=9</td>
<td></td>
</tr>
</tbody>
</table>
Chapter Seven

7.1. Mean (SEM) larval duration in days under transfer experiments. Grouped according to number of instars of development.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Instar</th>
<th>L1</th>
<th>L2</th>
<th>L6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long-short</td>
<td>5</td>
<td>6.1 (0.2) n=42</td>
<td>3.3 (0.1) n=39</td>
<td>10.8 (1.1) n=8</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6.5 (0.5) n=11</td>
<td>4.1 (0.3) n=11</td>
<td>3.0 (0) n=1</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>6.0 (0) n=1</td>
<td>4.0 (0) n=1</td>
<td></td>
</tr>
<tr>
<td>Short-long</td>
<td>5</td>
<td>6.3 (0.2) n=39</td>
<td>4.4 (0.1) n=39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7.5 (0.4) n=14</td>
<td>3.8 (0.2) n=14</td>
<td>9.6 (1.2) n=10</td>
</tr>
</tbody>
</table>

7.2. Mean (SEM) head capsule widths in μm under transfer experiments. Grouped according to number of instars of development.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Instar</th>
<th>W1</th>
<th>W2</th>
<th>W5</th>
<th>W6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long-short</td>
<td>5</td>
<td>282 (2) n=26</td>
<td>394 (6) n=26</td>
<td>587 (7) n=40</td>
<td>1584 (55) n=5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>282 (3) n=8</td>
<td>385 (6) n=7</td>
<td>560 (10) n=11</td>
<td>1075 (113) n=8</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>287 (0) n=1</td>
<td>396 (0) n=1</td>
<td>516 (0) n=1</td>
<td>1128 (0) n=1</td>
</tr>
<tr>
<td>Short-long</td>
<td>5</td>
<td>282 (1) n=31</td>
<td>396 (3) n=21</td>
<td>583 (5) n=38</td>
<td>1590 (22n=41)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>280 (2) n=8</td>
<td>372 (6) n=5</td>
<td>555 (8) n=12</td>
<td>1263 (20) n=10</td>
</tr>
</tbody>
</table>

Chapters, Three, Four, Six, Seven.

Mean (SEM) seventh instar head capsule width in μm and larval duration in days, grouped by the number of instars of development.

<table>
<thead>
<tr>
<th>Instar</th>
<th>Width</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1868 (32) n=17</td>
<td>12.5 (1.6) n=13</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>14.0 (0) n=1</td>
</tr>
</tbody>
</table>
Evidence for diapause in indigenous New Zealand insects: A review

MICHAEL MORRIS
Department of Zoology
University of Auckland
Private Bag, Auckland, New Zealand

Abstract Recent experimental work suggesting the presence of diapause in indigenous New Zealand phytophagous insects is reviewed. This reopens the question of the frequency of true diapause in New Zealand insects; this is discussed in relation to other cases of diapause and overwintering in New Zealand.

Keywords Diapause; overwintering; Insecta

INTRODUCTION
Diapause is a common strategy adopted by many Northern Hemisphere insects to survive adverse conditions. It has been defined as a dormancy brought about by token stimuli which trigger a neurohromonal event (Tauber et al. 1986). Diapause induced dormancy often leads to a suppressed development rate. Other characteristics include accumulation of food reserves, reduced metabolic rate, increase in instar number, and raised levels of juvenile hormone (Danilevski 1965; Beck 1980; Saunders 1982; Danks 1987). A specific stimulus such as chilling or photoperiod is often required to terminate diapause (Tauber & Tauber 1976). Diapause can be contrasted with quiescence in which dormancy is directly controlled by adverse conditions (Tauber et al. 1986). Saunders (1982) listed over 300 species in 15 orders in which photoperiodic induction of diapause has been demonstrated or reasonably inferred. Many of these are laboratory studies in which diapause can be easily distinguished from quiescence by providing diapause inducing stimuli in conditions which are suitable for continued growth.

Overwintering strategies in indigenous New Zealand insects were first reviewed by Roberts (1977, 1978) who found temperature-induced torpor common but true diapause appeared rare. This author cited only two instances where true diapause appeared to have been accurately inferred; both involved non-phytophagous insects. Roberts (1978) went on to suggest that its rarity in phytophagous insects was a result of the availability of a year round food supply from the predominantly evergreen native flora. The predominance of non-diapause insects and evergreen plant species was attributed by Dumbleton (1967) to the warmer climate in New Zealand during the Pleistocene when diapause was evolving in the Northern Hemisphere.

In support of this hypothesis, Ramsay (1978) and Watt (1978) reported vastly reduced incidences of seasonality in New Zealand Orthoptera and Coleoptera, respectively, when compared with Northern Hemisphere species. Gibbs (1980) also noted that diapause is absent in the 11 species of New Zealand butterflies. Winterbourne (1978) and Towns (1981) looked at the life cycles of New Zealand freshwater insects and noted the lack of seasonal synchrony compared with similar habitats in the Northern Hemisphere, suggesting a lack of diapause.

Studies on New Zealand populations of the Argentinian Stem Weevil *Listronotus bonariensis* Kuschel (Curculionidae) (Goldson & Emmberson 1980) demonstrated the existence of a true diapause, but no variation in critical daylength with latitude. The authors suggest that this diapause is a "relict" response which has not evolved further since the species arrived in New Zealand, as it confers no adaptive advantage for this species under New Zealand conditions.

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EVIDENCE FOR DIAPAUSE

The above published reports support Dumbleton’s (1967) hypothesis that diapause is rare in New Zealand insects. However, recent experimental work on three species of Lepidoptera suggest that another review of the literature and of the applicability of this theory may be warranted. Evidence for diapause in New Zealand insects will be discussed below.

Lepidoptera

Recent laboratory work on two phytophagous native moths has revealed the influence of photoperiod on length of larval development and number of instars. Ochieng-Odero (1988) tested the duration of the final instar in Cephalasia juctataana Walker (Tortricidae) under five photoperiods. In this species, the duration is longer in total darkness and total light. In other photoperiods the duration decreases with increasing daylength. This is a similar response to a photoperiodic response curve for a short day species (Saunders 1982) and the authors suggest the circadian system is involved. A similar photoperiodic response for larval duration and number of instars has been found in Planotortrix excessana Walker (Tortricidae). This lepidopteran shows a photoperiodic response curve of a long day species with a critical day-length of 14-15 h (Morris 1990).

Although presence of diapause in the above two species is far from conclusive, the evidence points to a diapause mechanism being involved. Danks (1987) cited several instances where larval number and larval duration are influenced by photoperiod, with the comment that this is normally associated with diapausing species. Rock & Shaffer (1983) suggested that lengthened larval durations can be regarded as part of diapause. The presence of a photoperiodic response curve also suggests a diapause mechanism with the involvement of the circadian system (Saunders 1982) and not merely a direct effect of photoperiod.

Field observations on the endemic species Ovocrambus vitellus Doubleday (Pyralidae) and O. flexuosus Butler revealed a vastly lengthened final instar in winter populations (Gaskin 1975). Roberts (1977) suggested that this is not true diapause since some retardation is also found in other instars, but in light of the recent findings in C. juctataana and P. excessana it may be necessary to revise this opinion. Laboratory studies on these species would clarify the nature of the overwintering mechanism.


A photoperiodically induced diapause has recently been reported in laboratory studies of the native moth Sitthmopoda aposema Meyrick (Oecophoridae). This species has a lengthened prepupal stage and diapausas over a narrow range of photoperiods. Its photoperiodic response curve is that of a long-short day species, rare among Lepidoptera (Muggleston 1989).

The introduced codlin moth Cydia pomonella L. (Tortricidae) exhibits diapause, a trait that it evolved in the Northern Hemisphere. In contrast with the Argentinian stem weevil mentioned earlier, different populations in New Zealand show a variation in critical day-length with latitude (Roberts 1978). This could suggest that diapause has continued to evolve in this species, and that this strategy can be advantageous in New Zealand conditions.

Coleoptera

The indigenous weevil Praelaepra uniformis Marshall (Curculionidae) is one record of diapause mentioned by Roberts (1977). P. uniformis exists in close synchrony with its host plant, diapausing inside mature fruit and emerging 16-18 months later.

Freshwater insects

As mentioned earlier, field studies of New Zealand freshwater insects show a lack of synchrony of life cycles compared with Northern Hemisphere species. Exceptions have been found in Zelandobius confusus (Hare) (Plecoptera: Grippopertygidae), Z. furnicilatus Tillyard, and in Zelandopsyche ingenstra Tillyard (Trichoptera: Oecosini), all of which show synchrony of life cycle stages. Z. ingenstra is also reported to overwinter in the final instar (Winterbourne 1978). Diapause is a major factor in achieving synchronisation in insects (Tauber et al. 1986; Danks 1987) so the presence of seasonality or synchronisation of life cycle stage in field studies could point to the presence of diapause.

Among native Odonata, diapause has been reported in the field in the final and penultimate instars of Xanthochenum zealandia McLachlan (Coenagroniidae), and in the egg stages of Austrolestes colonensis White (Lestidae) and Procordula smithii White (Corduliidae) (Rowe 1987). The indigenous Sigara arguata White (Hemiptera: Corixidae), Anisops assimilis White (Heteroptera: Notonectidae), and A. wakefieldi White show ovarian regression similar to their British counterparts, except that spring ovarian development takes place earlier in New Zealand (Young 1978).
Hymenoptera

Harris (1974) found a prepupal diapause in the native spider-eating wasps *Spictostethus (=Priocnemis) nitidus* (Fabricus) (Pompilidae) and *Priocnemis carbonarius* (Smith). These were mentioned by Roberts (1977) as one of the two records of diapause in New Zealand. The prepupal stage of the native bee *Leioproctus boltoni* Cockerell (Colletidae) has also been found to overwinter. Pupation does not occur until October, even when the temperatures were raised (Donovan 1968), suggesting that diapause could be involved.

A similar lengthened prepupal stage has been observed in Auckland populations of the Mason wasp *Pison spinola* Shuckard (Sphecidae). Prepupae in the winter generation pass into a stage of arrested development as early as February. The onset of dormancy when the weather is still warm suggests a true diapause and not a winter quiescence, especially as diapause is terminated in August with subsequent development during cooler conditions (Cowley 1959). The author noted that *P. spinola* is well established in New Zealand, but also found in the Eastern region of Australia. Its endemism is therefore in doubt.

Orthoptera

A facultative egg diapause has been found in the native grasshopper *Phaulacridium imaginale* Walker (Acrididae) (Ramsay 1978) and in the native phytophagous crickets *Pteronemobius nigrovus* Swan (Gryllidae) and *P. bigelowii* Swan (McIntyre 1978). Parkes (1972) also compared two populations of *Pteronemobius* spp. The inland population exhibited seasonal synchrony and had an egg diapause. The population inhabiting the warmer coastal environment showed no synchrony or diapause. From these observations it appears that diapause in *Pteronemobius* species is influenced by temperature. The role of photoperiod has not been studied.

CONCLUSION

Diapause in insects can come about as a protection against climatic conditions or shortage of food (Roberts 1978). According to Dumbleton’s (1967) hypothesis, adaptations to the former should be rare, whereas the presence of a year round food supply would make the latter adaptation unnecessary in phytophagous species (Roberts 1978). Most published reports support this hypothesis. However, the presence of diapause in native phytophagous species, and especially photoperiodic control of development in phytophagous Lepidoptera, reopens the question of the frequency of diapause in indigenous species and the value of diapause in New Zealand conditions. One possible reason for diapause in some New Zealand species could be the advantage of synchronising life cycle stages. This would ensure that adults emerge over a short period and would facilitate reproduction.

Many observations of overwintering in New Zealand come from field studies where it is difficult to distinguish between diapause and quiescence. A closer look at these species under laboratory conditions would give further insight into the nature of the diapause response in New Zealand and the relative frequency of diapause in New Zealand insects compared with overseas species.

ACKNOWLEDGMENTS

I am grateful to Mere Roberts for her review of the manuscript.

REFERENCES


Photoperiodic control of larval development rate in Planotortrix excessana (Lepidoptera: Tortricidae): evidence for larval diapause

Michael Morris
Department of Zoology
University of Auckland
Private Bag, Auckland, New Zealand

Abstract The larval development rate and pupal weights of Planotortrix excessana Walker were monitored under seven different 24 h photoperiods. Larvae developed through 5–7 instars with the extra instars being most common in females. Photoperiod had a significant effect on the duration of all instars, total larval period, and number of instars.

The response for both larval duration and instar number was typical of insects in which diapause occurs during long days. Evidence for the presence of a photoperiodically induced diapause is discussed. Disturbance of larvae during head-capsule measurement significantly reduced pupal weight in both sexes but had no effect on larval duration.

Keywords Diapause; development; photoperiod; Lepidoptera; Tortricidae; Planotortrix excessana

INTRODUCTION

Planotortrix excessana Walker is an endemic New Zealand tortricid moth. It is a serious pest on apples but also feeds on a wide range of native evergreen plants (Green 1984). It overwinters in the larval stage (Thomas 1979) though no diapause has been reported. In this study, the development rate and growth of larvae were monitored at different photoperiods to determine whether there is a photoperiodic response and whether any larval diapause is present.

MATERIALS AND METHODS

Eggs were obtained from the DSIR Entomology Division, Auckland, New Zealand. Original larvae had been collected from Canterbury, New Zealand and were reared in the laboratory since 1982 at 20°C and at a photoperiodic regime of LD18:6 (Hobson & Singh 1987). At the start of this study, eggs were kept in DD at 10°C until needed and then allowed to develop at 21°C ± 1°C and LD 18:6 until hatching. First instar larvae were transferred onto plastic 6-well plates (45 mm diameter) to which about 3 g of general purpose diet (Singh 1983) had been added. Insects were reared in separate growth cabinets at photophases of 0, 6, 12, 14, 16, 18, and 24 h in a 24 h photoperiod at a temperature of 21°C ± 1°C. Lighting was provided by Thorn fluorescent bulbs (Emission spectrum 87–88% at 510–610 nm) and the insects were positioned so that they received a light intensity of 300–500 lux. Ninety-six insects were used in each cabinet.

Each day the head capsules of the same 48 insects from each cabinet were measured using an ocular micrometer on an Olympus dissecting microscope at ×40 magnification. Measurement was accurate to 0.012 mm. The instar number was determined from changes in head-capsule measurements. These showed discrete differences between instars (Fig. 4) so that instar identification was simple. The average head-capsule measurement over the period of development was taken for each
instar of every caterpillar. The other 48 larvae in each cabinet were not disturbed until pupation had started, in order to check whether disturbance caused by measuring had any major effect on development. The experiment was then repeated with a further 96 insects per cabinet. Different cabinets were used for each treatment during the second experiment. Pupae were sexed and then weighed on a Mettler balance (accuracy ±2 mg) within 1 day of pupation.

Statistical analysis was performed on an IBM-PC using the SAS package. All tests of significance were at $P < 0.05$.

RESULTS

Survival to pupae ranged from 47–60% in disturbed and 73–84% in undisturbed treatments. Pupal to adult survival varied between 67% and 90% for each treatment. There was no significant difference in survival because of photoperiod. Since insects were sexed by examining pupae, all results in which the sexes were separated used only larvae surviving to pupation. Total numbers used in the analysis are shown on Table 1.

Larval development

Larvae developed through five to seven instars. Sixth instar development was most common in females with some showing a seventh instar (Fig. 1). Larvae were divided into two instar groups depending on whether development was through five instars or more. Analysis of variance was performed for durations of all instars and for total larval period. Photoperiod had a significant effect on duration of all instars, as well as on the total larval period (Fig. 2, 3).

Significantly more females required extra instars to complete larval development (Fig. 1). Females also took significantly longer to develop through instars 5 and 6 (Fig. 2) though there was no significant difference between sexes for the duration of earlier instars.

Larvae requiring the extra instars took significantly longer to develop during the first three instars and the final instar. Fifth instars that were to moult again were of significantly shorter duration than those which would pupate (Fig. 2). Disturbance had a significant effect on larval development at photophases of 12–16 h but no significant effect overall (Fig. 3).

There were significant differences in head capsule widths between instar groups from the 2nd instar onwards (Fig. 4), with sex and photoperiod having no direct effect on growth rate.

Pupal weight

Pupal weight is influenced by temperature, photoperiod, disturbance, and sex. The heaviest pupae are undisturbed females reared at 17°C. There is also a significant temperature-sex and temperature-photoperiod interaction. The pupal weight is plotted on Fig. 5.
Fig. 2  Mean (± SEM) duration of each larval instar under different photophases. Solid line = five instar development, broken line = six or seven instar development. ● = males, ▲ = females, ■ = Sexes grouped. a – f = First to sixth instars.
DISCUSSION

Previous workers on the life history of *P. excessana* in the field have reported five or six instar development (Green 1984; Thomas 1979). This is the first indication of a seventh instar in this species. Average head capsule sizes are similar to those found by Green (1984) in field collections of *P. excessana*, except that his fifth and sixth instar head capsules were much smaller (1.36 and 1.87 mm). This might be because of dietary differences or it may suggest that the field population had a high proportion of the smaller larvae developing through six or more instars.

Evidence for diapause in *P. excessana*

Diapause has been defined as a dormancy brought about in response to token stimuli such as photoperiod (Tauber et al. 1986), one of the characteristics of dormancy being a suppressed development rate (Beck 1980). Although larval diapause often only occurs at one specific instar (Danks 1987), exceptions to this rule are fairly common. Periods of diapause over more than one instar have been reported by Danilevski (1965), while Rock & Shaffer (1983) suggested that a prolonging of larval instars could be regarded as part of diapause.

The appearance of extra instars in diapausing individuals is also common in Lepidoptera (e.g., Chippendale & Yin 1973; Rock & Shaffer 1983; Oku 1984).

The duration of instars two to six, the total larval period and the number of instars all show a sudden drop off during long days and another decline in total darkness (Fig. 1–3). This shape corresponds to a type 1 photoperiodic response curve for diapause induction (Beck 1980) with a critical daylength of around 14–15 h. This similarity in the shape of the photoperiodic response curve as well as the increase in larval duration and number of moults required for development could point to a larval diapause being present in *P. excessana*.
Diapause in New Zealand insects

Incidence of overwintering in New Zealand insects have been reviewed by Roberts (1978) and Morris (1989). While overwintering was found in a great many species, few conclusive cases of diapause were found. Lack of diapause in New Zealand insects and native plants are both thought to be caused by the milder climate during the Pleistocene when diapause was evolving (Dumbleton 1967). Recently diapause has been documented in the New Zealand moth *Stathmopoda aposema* Meyrick (Muggleston 1988). The photoperiodic responses of *P. excessana* and *S. aposema* suggest that the possibility of diapause in New Zealand phytophagous insects should be looked at more closely.

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Simulation of Insect Thermoperiodic Response Curves Using a Damped Circadian Oscillator Model

by

M.C. Morris and R.D. Lewis

ABSTRACT

Simulations of thermoperiodic response curves for the insects Sarcophaga argyrostoma and Diaatraea grandiosella are made, using a damped circadian oscillator model. Results compare well with experimental data for these species and demonstrate how an external coincidence model can account for thermoperiodic effects.

INTRODUCTION

Circadian models for insect seasonality

Circadian mechanisms for photoperiodic induction in insects can be divided into two main categories, depending on the role of light.

The "internal coincidence" model (Tyschenko, 1966 cited Danilevski et al., 1970; Pittendrigh, 1972) assumes that light is only involved as an entraining agent. Photoperiodic induction comes about as a result of interactions between two oscillators, one phase set by dawn and the other by dusk.

The "external coincidence" model involves only one oscillator. Light serves both for entrainment and to illuminate a photoinducible phase ($\theta_i$) of the oscillator. Long day responses are effected by the illumination of $\theta_i$, with short day responses resulting if $\theta_i$ is not illuminated.

In most insect species a number of photoperiodic cycles are necessary to induce seasonal effects. This has led to the concept of the photoperiodic counter whereby a hypothetical diapause regulatory substance is built up in diapause inducing photoperiods. The photoperiodic counter is incorporated into both internal and external coincidence models (Saunders, 1981).

A damped circadian oscillator model has been put forward by Lewis and Saunders (1987) to account for diapause induction in the fleshfly Sarcophaga.
argyrostoma. This is a modification of Pittendrigh's (1966) external coincidence model and includes both a "clock" and a "counter" mechanism.

The clock is based on a chemical feedback oscillator developed earlier (Christensen et al., 1984) with the parameters set to give a damped oscillation. The concentration of chemical (c) oscillates around a threshold value, the phase \( \phi_1 \) occurring when the concentration falls through this value (Fig. 1). If \( \phi_1 \) occurs in the dark, a hypothetical diapause inducing substance (INDSUM) is built up. If \( \phi_1 \) coincides with the light, INDSUM is broken down.

The concentration of INDSUM is accumulated over a period of days, corresponding to the duration of larval growth in S. argyrostoma. The production and breakdown of INDSUM both diminish with increased larval age. At the end of this time, the total INDSUM concentration is assumed to be directly related to the percentage diapause in a population of insects.

Elevated temperatures reduce INDSUM in two ways. Firstly by speeding up the production of an INDSUM inhibitor and secondly by accelerating larval development and thus reducing the number of days that the insect is sensitive to photoperiod.

The model was later modified by Saunders and Lewis (1988) so that the rate of INDSUM production depends on the value of the oscillator all the time it is above threshold. For these thermoperiodism simulations the model is further refined so that the rate of INDSUM breakdown is also dependent on this value.

**Thermoperiodism in insects**

Thermoperiodism in insects has been recorded in a number of species (Beck, 1983). Conversely, thermoperiodic responses have been tested for Platyncta

![Graph showing concentration over days](image)

Fig. 1. The damped oscillator, showing the threshold concentration and position of the photoinducible phase (\( \phi_1 \)).
Simulations of thermoperiodic diapause induction in *Sarcophaga argyrostoma* and *Diatraea grandiosella* were run and compared with experimental data. These two species were chosen because their biology is well documented and because they differ in their thermoperiodic response. A thermoperiodic response curve has been generated for *D. grandiosella* (Chippendale et al., 1976) but was absent in *S. argyrostoma* (Saunders, 1984).

Parameters for the *S. argyrostoma* simulations were kept the same as the original model (Lewis and Saunders, 1987). These are: synthesis rate of $c = 0.1$, $Q_{10}$ for synthesis $= 1.2$, $Q_{10}$ for larval growth $= 2.6$, $Q_{10}$ for INDSUM inhibitor $= 2.0$, time delay value $= 28$, light intensity $= 2$. The cryophase and thermophase values of $15^\circ C$ and $25^\circ C$ correspond to temperatures used by Saunders (1984) in his thermoperiodism experiments on this species.

For *D. grandiosella* simulations the parameters were altered to reflect the biology of this species. The diapause response in *D. grandiosella* is sensitive to temperature, with diapause only occurring between $20^\circ C$ and $30^\circ C$ (Chippendale and Reddy, 1973). $Q_{10}$ values for synthesis rate of $c$ and INDSUM inhibitor were set at 1.6 and 64 respectively. Simulations with these values agreed with experimental data showing a diapause response between $20^\circ C$ and $30^\circ C$ (Fig. 2). The $Q_{10}$ for INDSUM inhibitor is high, but not beyond the bounds of possibility if a protein is involved. $Q_{10}$ values as high as 635 have been recorded for denaturation of proteins (Geise, 1968).

The larval development time at $30^\circ C$ was also altered to 16 days. This corresponds to the development time of non-diapause larvae reared at this temperature (Jacob and Chippendale, 1971). The thermophase and cryophase values of $36^\circ C$ and $18^\circ C$ correspond to temperatures for which a thermoperiodic response has been demonstrated (Chippendale et al., 1973). In the absence of further information, all other parameters were kept the same as for *S. argyrostoma* simulations.

All simulations were carried out on an IBM PC using TURBO PASCAL and TURBO GRAPHIX software.
RESULTS

Fig. 3. shows the simulated thermoperiodic response curves for *S. argyrogestoma* compared with experimental results at the same temperature. Similarly, simulated and real thermoperiodic data for *D. grandiosella* is shown in Fig. 4. Thermoperiodic response curves are compared with simulated responses at constant temperatures equivalent to the average temperature during the cycle (Figs. 2-4).

A comparison between these responses for *S. argyrogestoma* shows that thermoperiod exerts very little effect. This corresponds with the data of Saunders
Fig. 3. Simulated thermoperiodic response curve for S. argyrostroma, compared with simulated diapause values at constant temperatures equal to the average temperature during one cycle. Triangles = 15°C/25°C thermoperiod Squares = constant temperature.

(1984). In contrast, a comparison between Fig. 2 and Fig. 4 shows that thermoperiods had a marked effect in D. grandiosella simulations, and the response curve has the same shape as experimental results for this species (Chippendale et al., 1976).
DISCUSSION

The mechanism by which thermoperiodic induction can come about in *D. grandiosella* can be seen by examining the behaviour of the oscillator under different thermoperiods. When the thermoperiod is short, the average temperature is cooler, so the oscillator never reaches threshold (Fig. 5a) and no diapause results. As the thermoperiod gets longer, the amplitude of the oscillator increases and rises above threshold (Fig. 5b) coincides with the cryophase, resulting in a high build up of INDSUM and thus high diapause rate. At longer thermoperiods,
Fig. 5. The damped oscillator under different thermoperiods
a: Thermoperiod = 2 hours
b: Thermoperiod = 4 hours
c: Thermoperiod = 14 hours
The top of the figures show the position of the high temperature pulses (−) and the photoinducible phases (+). The bottom shows the pattern of the oscillator and the threshold concentration.

$\phi_f$ falls during the thermophase when the concentration of INDSUM inhibitor is high, so that diapause declines again (Fig. 5c).

A similar mechanism applies to S. argyrostoma simulations, but since the temperature sensitivity is not so great, the difference between $\phi_f$ falling in the thermophase or cryophase is not so marked, so the effect of thermoperiod is slight. The lower temperature sensitivity also means that the oscillator does not remain below threshold at the low temperatures (Lewis and Saunders, 1987) so that the INDSUM values remain high even during a short thermophase (cf Fig. 5a).

The above simulations have shown that both the presence and the absence of a thermoperiodic response can be explained in terms of the damped oscillator model depending on the choice of parameter values. As discussed earlier, thermoperiodism can be accounted for by the internal coincidence mechanism, and Saunders (1978) used thermoperiodism as one criteria for distinguishing internal from external coincidence mechanisms in insects. However, the above results
have demonstrated that a specific model for external coincidence with temperature can account for thermoperiodic responses.

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