# Effects of photoperiod on the timing of larval wandering in *Drosophila melanogaster*

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**Abstract:**

A rearing method is described that will allow the use of well-timed larvae of *Drosophila melanogaster* (Canton S) in physiological investigations. Using a LD 16:8 h cycle and uncrowded rearing conditions, it was shown that photoperiod influences the departure of third instar larvae from the food (i.e. post-feeding larvae or wandering behaviour). Larval wandering occurs during the scotophase, suggesting a gated event, and does not occur in larvae raised under constant light or in overcrowded cultures reared in a LD 16:8 h cycle. Although the data suggest, but do not prove, the existence of circadian gating, the rearing regimen described will provide Well-timed post-feeding larvae for future studies.

**Keywords:** Photoperiod, wandering, Drosophila.

**Article:**

## Introduction

The fruitfly, *Drosophila melanogaster,* has been the object of intense study by investigators in the fields of genetics, behaviour, biochemistry and molecular biology (see review by Oliver, 1976). Surprisingly, however, *Drosophila* larvae have not been utilized to any great extent for endocrinological and physiological studies, presumably because of their small size, but also because there has been no procedure for staging these larvae in a precise manner.

Because of the recent dramatic advances in the field of insect endocrinology (see Kerkut & Gilbert, 1985), particularly in the area of neuropeptide purification, mode of action, etc., and the wealth of information on the genetics of *Drosophila,* we turned our attention to dipteran neuroendocrinology. An assay for dipteran prothoracicotropic hormone (PITH) *in vitro* was developed (Roberts *et al.,* 1984), PTTH being the neuropeptide that triggers the moulting process by stimulating the synthesis of ecdysone by the prothoracic glands (a component of the dipteran ring gland). Ecdysone is, in turn, converted to 20-hydroxyecdysone, the principal moulting hormone. This led to the development of an analogous assay using the ring glands of *Drosophila* (V. Henrich and L. I. Gilbert, unpublished data; see also Redfern, 1983) and has provided the basis for the ultimate purification of *Drosophila* PTTH and the cloning of its gene. These assays *in vitro* are based on the original PIM assay utilizing the prothoracic glands of *Manduca sexta* (Bollenbacher *et a/.*. 1979; Gilbert *et al.,* 1981) and depend to a large extent on precise staging of larvae and prepupae. Although this has been accomplished for the large dipterans *Sarcophaga bullata* (Roberts *et al.,* 1984) and *S.argyrostoma* (Richard *et al.*. 1986), it has to our knowledge not been reported for *Drosophila.* The studies described here reveal that with a strict rearing programme and photoperiod regimen, one can obtain well-staged post-feeding larvae for endocrinological studies.

**Materials and Methods**

Breeding stocks of *Drosophila melanogaster* (Canton S) were initially reared at 25± under constant light. Both adults and larvae were cultured in 250 ml clear plastic vials containing *c. 50* ml of artificial diet. Uncrowded *Drosophila* stocks were taken from constant light conditions and reared under a long day photoperiod (LD 16:8 h) for at least five generations before being used in the experiments described below. An entrainment period is necessary for the reaction of the endocrine system to specific environmental conditions (Denlinger, 1971). Eggs were collected from 4-day-old females, because egg fecundity peaks at 3-5 days (Rensing & Hardeland. 1967; David *et al.*, 1974). Vials of breeding stock were transferred to walk-in constant temperature rooms maintained at 25±1°C with a LD 16:8 h cycle. The flies were cultured for at least five generations under these conditions. After this entrainment period, eggs were collected from females at 2-3 h after beginning of the photophase. the middle of the photophase and 2-3 h before the beginning of the scotophase.

In these studies, no more than twenty-five eggs were transferred to the food surface of the above-mentioned fresh culture vials. All experiments were conducted in triplicate and repeated at least twice. The vials were examined frequently for the presence of wandering (post-feeding larvae; larvae which have left the food permanently to seek a place to pupariate). During the scotophase all recordings were performed under a dim red light by using a Kodak Wratten 92 filter with a 15 W bulb.

Concurrent experiments were conducted using eggs from females reared under constant light, and the effects of overcrowding on the formation of post-feeding larvae from entrained cultures were also observed.

**Results**

To investigate the effects of photoperiod and overcrowding on development to the post-feeding larval stage. larvae were first raised under constant light in non-crowded conditions (i.e. up to twenty-five larvae per container). Fig. 1 reveals that larvae left the food 90-136 h after oviposition with a PFL50 of 116 h, the PFL50 being the time after oviposition when 50% of the larvae have left the food and are designated post-feeding larvae.

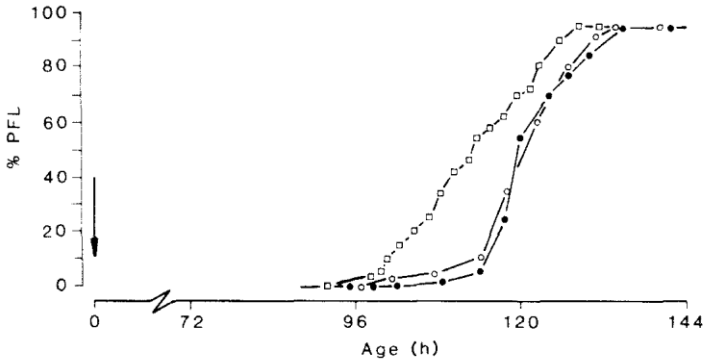


FIG. 1. The development of post-feeding (wandering) larvae reared under constant light at 25°C. Arrow denotes the time of egg collection. ○, ●, □ represent the results of three individual experiments in which the eggs were divided into at least three groups per experiment, each group not exceeding twenty-five eggs per vial.

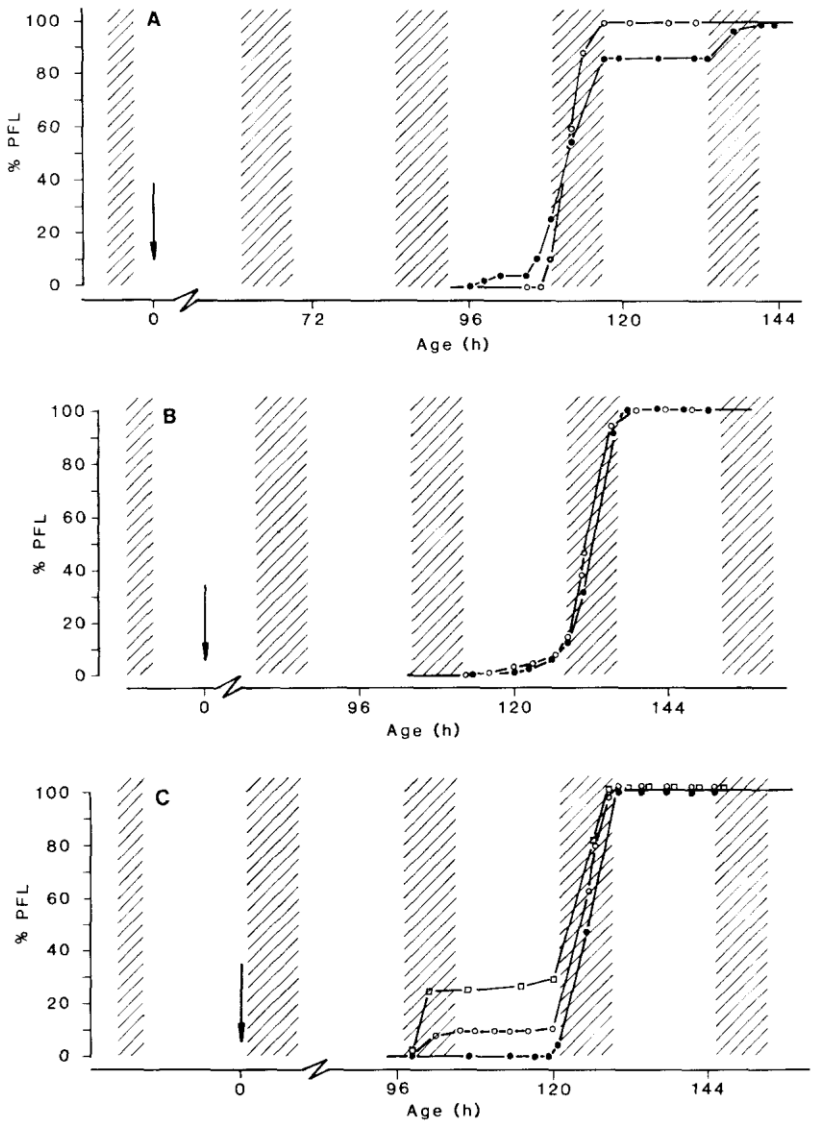


FIG. 2. The formation of post-feeding (wandering) larvae reared in a LD 16:8 h cycle at 25°C. Arrow denotes time of egg collection. (A) Eggs deposited 2–3 h after the beginning of the photophase ($n$=145); (B) eggs deposited midway through the photophase $n$=130); (C) eggs deposited 2–3 h prior to the end of the photophase ($n$=225). Each vial contained no more than twenty-five eggs.

When the strict LD 16:8 h cycle conditions were imposed on the progeny of entrained fly cultures, the resulting data suggested strongly that the photoperiod influenced the departure of the larvae from the food. Fig. 2 summarizes the result of studies in which eggs were collected soon after beginning of the scotophase, halfway through the photophase, and just before the beginning of the scotophase. In each group the formation of post-feeding lar-vae occurred during the scotophase. Indeed, in two cases (Figs. 2A, 2C) essentially no larvae left the food during the photophase. These were larvae raised from eggs deposited and collected within 2-3 h of the beginning of the photophase (PFL50=114 h), and larvae raised from eggs deposited 2-3 h before the end of the photophase (PFL50=124 h). In the case of larvae from eggs oviposited midway through the photophase (Fig. 2B), about 10% of the larvae left the food during the end of the scotophase (PFL50=130 h).

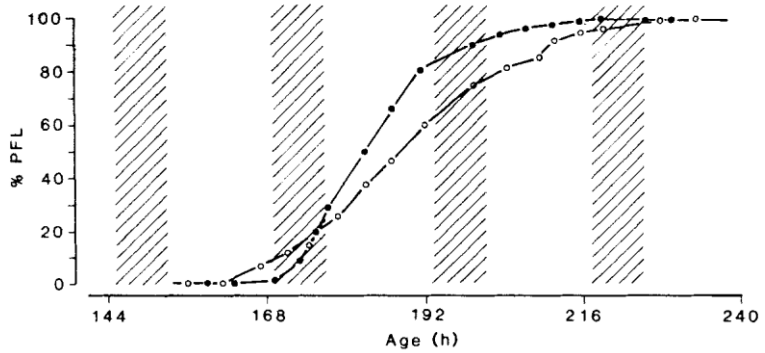


FIG. 3. The effects of overcrowding on the formation of post-feeding (wandering) larvae reared in LD 16:8 h at 25°C. ○ and ● represent the results of two individual trials. In all cases a minimum of fifty, and up to 150, larvae were placed in individual vials ($n$=1225).

The apparent environmental photoperiodic cue controlling the initiation of wandering during the scotophase was no longer effective when cultures were overcrowded. Fig. 3 shows that more than 50% of the larvae left the food during the scotophase when larvae were exposed to a LD 16:8 h cycle photoperiod under crowded conditions. In addition, there was a delay in development in that the PFL50 was almost 190 h under these conditions.

**Discussion**

*Drosophila* shows distinct behavioural rhythms in oviposition (Rensing & Hardeland, 1967), locomotion and mating (Hardeland & Strange, 1971). and adult eclosion (Pittendrigh, 1954; Brett, 1955). In addition, several physiological functions also show rhythmicity, e.g. daily rhythms of oxygen consumption (Rensing, 1964; Belcher & Brett, 1973), cyclic changes in brain cerebral neurosecretory cell, corpus allaturn, and prothoracic gland staining properties (Rensing, 1964; Rensing *et al.*, 1965), rhythmic activity of enzyme systems (Tauber & Hardeland, 1977), daily patterns of RNA synthesis (Probeck & Rensing, .1974), etc. Indeed, the clock gene has been cloned recently (Jackson *et al.*, 1986). In all of these studies it appears that photoperiodic cues elicit or modulate the rhythmic response (see Saunders, 1982).

One could extrapolate from the above that specific developmental events such as moulting, wandering and pupariation would also be under environmental (photoperiodic) control. Indeed, with *Manduca sexta,* Truman (1972; Truman & Riddiford, 1974) has presented convincing evidence that similar developmental events (e.g. PTTH release) are regulated by a circadian oscillation which is entrained by photoperiodic inputs, a finding that has led to the general use of gated, well-timed insects, and is one reason that *Manduca* has become an insect of choice for endocrinological experiments (e.g. Gilbert *et al.*, 1981).

Up to now it has been generally accepted that puparium formation in *Drosophila melanogaster* does not appear to occur on a rhythmic basis but is more or less randomly distributed within the diel cycle, even in the presence of a defined photoperiod (Harker, 1965). The closely related event of larval wandering in another dipteran, *Sarcophaga* is, however, under strict photoperiodic control *(S. bullata,* Roberts, 1984; *S. argyrostoma,* Richard *et al.,* 1986). The data presented here reveal that when *Drosophila* larvae are reared under a strict photoperiodic (long day) regimen at low population density, they behave in a way similar to *Sarcophaga.* In the latter, larvae feed voraciously during the first two stadia and approximately halfway through the third stadium, and when raised in a LD 16:8 h cycle at 25 ± 1°C they leave their food source only during the scotophase and seek a place for pupariation (Roberts & Warren, 1976; Roberts, 1984). When reared under similar conditions, *Drosophila* larvae feed for a relatively longer time during the final larval stadium but react similarly to photoperiod in that they wander from the food during the scotophase.

Our use of twenty-five or less larvae per container is in accord with past data on the effects of population density on the development of *Drosophila* (Sang, 1949). Both sets of observations indicate that *Drosophila* larvae require an excess of food since they demonstrate behavioural territorialism of their food source. This is in

contrast to the situation in *Sarcophaga* species where larvae appear to cooperate in devouring their food source (Robert, unpublished observation).

*Drosophila* reared under the strict conditions described here appear to display gated behaviour resulting, as in the case of *Sarcophaga* (Roberts, 1984; see Pittendrigh, 1966), in well-timed post-feeding larvae. However, if reared under crowded conditions (i,e. over twenty-five eggs per vial) this behaviour is not recognizable, This suggests that overcrowding affects the neuroendocrine system, because Roberts (1984) showed that the development of post-feeding larvae is controlled by the brain and associated endocrine glands, a situation similar to that in *Manduca* (Truman, 1972; Truman & Riddiford, 1974). Further evidence for this supposition is the extended period of larval life associated with ungated animals (i.e. longer PFL50). Although the possible endocrine implications of these data (e.g. gated release of **PTTH** followed by an ecdysteroid surge) remains conjectural in fhe case of *Drosophila*, these studies suggest that when grown under a specific photoperiodic regimen in uncrowded conditions, larval wandering is a gated event in *Drosophila*. The data do not distinguish between endogenous control (circadian gating), exogenous driving or a mixture of both (D. S. Saunders, personal communication), because no free run studies were conducted under constant darkness. However, the purpose of this study was not to prove the existence of circadian gating but rather to provide a regimen for obtaining well-timed post-feeding larvae. Our findings offer an improved method for the staging of immature *Drosophila*, suggest a variety of endocrinological experiments as in the case of *Manduca* (Oilbert *et al.*, 1981), and may provide a method for assaying putative mutations affecting gating behaviour.

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