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Embryonic diapause in the Australian plague locust relative to parental experience of cumulative photophase decline

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ABSTRACT

The Australian plague locust *Chortoicetes terminifera* (Walker) exhibits facultative embryonic diapause during autumn. To approximate natural photoperiod changes during late summer and autumn, locust nymphs were reared under different total declines in laboratory photophase (−0.5, −0.75, −1.0, −1.25, −1.5, −1.75, −2 h each lowered in 15 min steps) in a 24 h photoperiod to quantify any effect on the subsequent production of diapause eggs. Induction of diapause eggs was significantly affected by accumulated photoperiod decline experienced by the parental generation throughout all development stages from mid-instar nymph to fledgling adult. The incidence of embryonic diapause ranged from nil at −0.5 h to 86.6% diapause at −2 h. Continued declines in photoperiod for post-teneral locusts (transitioned from −1 h until fledging to −1.75 h) produced a further increase in the proportion of diapause eggs. The results were unaffected by time spent at any given photoperiod, despite a previously indicated maximal inductive photoperiod of 13.5 h being used as the mid-point of all treatments. Implications for the seasonal timing processes of photoperiodism in *C. terminifera*, which has a high migratory capacity and a latitudinal cline in the timing of diapause egg production across a broad geographic range, are discussed.

1. Introduction

Diapause in insects is a form of developmental dormancy controlled by a combination of environmental and molecular processes. It is a component of often complex physiological and behavioural syndromes that are adapted to environmental cycles (Bradshaw and Holzapfel, 2007; Saunders, 2010a; Tauber et al., 1986). The induction of diapause usually involves indirect environmental cues occurring during favourable conditions that result in the avoidance of later unfavourable conditions for potentially vulnerable life stages (Danks, 2007). Photoperiod-induced diapause is prominent in temperate and sub-polar regions, where seasonal conditions vary markedly (Meuti and Denlinger, 2013; Saunders, 2010a, 2011).

Diapause can occur in embryonic, larval, pupal or adult life stages, although it is commonly restricted to a single life stage within insect species (Denlinger, 2002). Experiments raising insects under different photoperiods, or subject to large changes in photoperiod, have categorised the diapause responses of species as either 'long-day' or 'short-day', usually depending on the season of development and feeding (Danks, 1987; Saunders, 2010a; Spieth and Sauer, 1991). In many species with embryonic or larval diapause, hormonal controls are transmitted to the subsequent generation through enzymatic pathways associated with reproduction (Bradshaw and Holzapfel, 2007).

Among the Orthoptera, embryonic diapause can occur at different or multiple developmental stages, even within a species (Dingle et al., 1990; Dingle and Mousseau, 1994; Hockam et al., 2001; Shim et al., 2013). Different levels of free ecdysone in the developing egg follicles and subsequent eggs appear to control diapause initiation (Gregg et al., 1987; Lagueux et al., 1979; Tawfik et al., 2002), through its role in embryonic development in what has been termed an 'ecdysteroid-deficiency syndrome' (Kidokoro et al., 2006).

Many insect species display geographic variation in diapause traits (Chen et al., 2013; Danilevski, 1965; Mousseau and Roff, 1989; Lankinen et al., 2013; Paolucci et al., 2013) and a trend of photo-responsive day-length increasing with latitude is seen as a general ecogeographic rule (Bradshaw and Holzapfel, 2010). Within a species there can also be genetic variation in diapause expression, phenotypic plasticity in photoperiodic response, or a distribution of diapause 'potential or intensity' (Masaki, 2002; Muraro et al., 2013; Schmidt and Conde, 2006). Temperature alone

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can control diapause induction in low latitude species or populations (Chen et al., 2013; Denlinger, 1986; Shim et al., 2013), but its common effect is through an interaction with photoperiod by reinforcement or limitation of the rate of entrainment and endocrine processes (Fantinou and Kagcow, 2000; Fantinou et al., 2002; Vaz Nunes, 1990).

Much research on photoperiod-induced diapause has focussed on the operation of the insect 'circadian clock' that detects day and night length, and the 'photoperiodic calendar' for seasonal changes in photoperiod (Saunders, 1981, 2008). Experiments based on extension, or light-pulse interruption, of scotophase established a circadian cycle with an approximate 24 h period and species-specific sensitive periods of response to night duration. Results of these and photoperiod change experiments have been related to several conceptual models of the operation of the circadian clock and calendar, with the responses of different species used to support different models (Kostal, 2011; Saunders, 2009, 2010a,b, 2013). Most models separate the internal interpretation processes of photoperiodism as an 'oscillator' that responds to daily changes in light/dark, and a 'counter' that detects the number of oscillations occurring within the photosensitive range (Saunders, 1981). Their extension to diapause in different insect species have identified a critical photoperiod (CPP or critical night length CNL) and required day number (RDN) experienced during photo-inducible life stages as thresholds to account for subsequent diapause proportions of the population (Beach, 1979; Denlinger, 1972; Saunders, 2013; Saunders and Bertossa, 2011).

Chortoicetes terminifera (Walker) is a multivoltine acridid endemic to Australia, with 2–4 generations from spring to autumn and a high potential for migratory exchange across a wide latitudinal range (20–39°S) (Clark, 1971; Deveson and Walker, 2005; Farrow, 1979). Facultative autumn diapause occurs at pre-anatrepsis stage in embryos laid by adults of the second or third generation, depending on the latitude of origin (Wardhaugh, 1973). Diapause occurs almost exclusively in eggs laid during the southern hemisphere autumn months of March and April at temperate latitudes, preventing large numbers of eggs hatching prior to winter. However, a latitudinal gradient in the timing of diapause egg production has been observed, occurring later at lower latitudes (Wardhaugh, 1973, 1986). Diapause embryos are first produced in eggs laid in late February in locations >33°S and the proportion rises rapidly to >50% by mid-March, but similar proportions occur only from early April at 26°S.

Several experiments by Wardhaugh (1973, 1980a) over a range of constant photoperiods showed a small proportion of diapause eggs were produced within a range of day-lengths corresponding to those experienced in the field (15.5–11 h). For insects derived from field diapause eggs, <10% diapause eggs were laid by females raised at 15.5, 14.5, 13.5, 12.5, 11.5 and 11 h day-length, except 13.5 h where there was 23.7% diapause (Wardhaugh, 1973). Further trials involving a single change in day-length and temperature, established that a decline in day-length experienced by the parental generation was the principal inductive cue influencing the production of diapause eggs, with 50–80% resulting from a 3 h decline in day-length. Increasing day-length resulted in almost complete absence of diapause eggs (0.5%). The results of these and other trials involving 1 h photophase declines indicated a maximum diapause response around 13.5:10.5 h Light/Dark (LD) (Wardhaugh, 1980a). Temperature decline appeared to enhance diapause induction under certain photoperiod changes, but had a primary influence on subsequent embryos, just before anatrepsis. Temperatures below 15.5 °C prevented diapause initiation (Wardhaugh, 1980b) and in the range 26–38 °C caused a 'reversal' of diapause in a proportion of eggs (Gregg, 1985). Wardhaugh (1980a) also showed that each nymphal stage after second instar up to fledging was photoinducible. Nymphs transferred from long to short day-length at 3rd, 4th, 5th instar or at final moult subsequently laid >50% pods containing diapause eggs.

C. terminifera is a significant and frequent agricultural pest in Australia (Murray et al., 2013; Wright, 1986, 1987). Understanding photoperiodic and other factors involved in diapause in this species is important to improve simulation models of developmental timing and therefore for monitoring and management operations. In an attempt to approximate natural photoperiod change experienced during the life of locusts in late summer and autumn, we conducted laboratory trials exposing developing *C. terminifera* nymphs to different cumulative amounts of decline in day-length (from 0.5 h to 2 h, in 15 min steps) to test the effect on the proportion of diapause in subsequent embryos. This research aimed to test a hypothesis of accumulated day-length decline as the principal inductive cue to diapause. This could potentially reconcile both the photoinducibility throughout nymphal development and the latitudinal gradient in first production of diapause eggs, given sufficient gene flow through migration between populations from different latitudes to maintain a panmictic distribution (Chapuis et al., 2011). Additionally, continued declines of day-length (from 1 h to 1.75 h) after fledging and after first oviposition were included to test if adult life stages are also photoinducible.

2. Methods

2.1. Source population

C. terminifera eggs were sourced from a single swarm egg bed, laid in late March 2012 (collected in excavated soil in June 2012) near Burra, South Australia (33.50°S, 138.88°E). Random sampling showed all eggs were at the same embryonic stage (stage IVc of Wardhaugh, 1978), indicative of diapause. Egg pods were extracted from clods, placed with moist soil into 800 mL plastic food containers and incubated at 25 ± 2 °C until hatched.

2.2. Nymph husbandry

Within 24 h of hatching, nymphs were transferred into clean 800 mL containers with fibreglass gauze covers. Twelve containers, each with ~80 individuals, were used for each of the subsequent 9 photoperiod change treatments (including 2 designed to test the photoperiodic response of post-teneral adults), giving initially 800–900 nymphs per treatment.

Nymphs were fed *ad libitum* a daily diet of freshly cut wheat grass, occasionally supplemented by fresh annual ryegrass (*Lolium rigidum*), bran and wheatgerm, and were incubated at 35/25 °C LD temperature cycle under initial photoperiods of either 14:10 LD or 13.75:10.25 LD for different treatments (Table 1). The incubators used (TRIL-495-1SD and TRIL-250-1SD, Thermoline Scientific, Wetherill Park, Australia) were fitted with fluorescent light tubes (Stancoat T5-F54-W830 or Philips TLD-18W865) and relative humidity was maintained at 60–75% (Kestrel 4500, Nielsen-Kellerman, USA) by evaporation from open water pots. Locust containers were transferred to a refrigerator with internal lighting (Thermoline Scientific, TELR-1-440-GD) each morning and cooled for 10 min to allow for the provision of food and the removal of frass, uneaten food and dead nymphs, without nymphs escaping. Overall mortality of nymphs exceeded 70% at fledging, not dissimilar for field *C. terminifera* reared in preliminary experiments using the same incubators (data not shown).

When 25% of surviving nymphs reached third instar stage (9–10 days after hatching) a series of 15 min stepped reductions of photophase was commenced to produce total declines ranging from 0.5 h to 2.0 h during nymphal development (Table 1). The timing of each change was dependent on the number of equally

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