Author/s:
Cheng, X;Hoffmann, AA;Maino, JL;Umina, PA

Title:
A cryptic diapause strategy in Halotydeus destructor (Tucker) (Trombidiformes: Penthaleidae) induced by multiple cues

Date:
2018-11-01

Citation:
Cheng, X., Hoffmann, A. A., Maino, J. L. & Umina, P. A. (2018). A cryptic diapause strategy in Halotydeus destructor (Tucker) (Trombidiformes: Penthaleidae) induced by multiple cues. Pest Management Science, 74 (11), pp.2618-2625. https://doi.org/10.1002/ps.5053.

Persistent Link:
https://hdl.handle.net/11343/283890

Umina Paul (Orcid ID: 0000-0002-1835-3571)
Cheng Xuan (Orcid ID: 0000-0002-7548-9299)

Title: A cryptic diapause strategy in *Halotydeus destructor* (Tucker) (Trombidiformes: Penthaleidae) induced by multiple cues

Running title: Unraveling summer diapause in redlegged earth mites

Xuan Cheng*[1], Ary A. Hoffmann[1], James L. Maino[1,2], and Paul A. Umina[1,2]

[1] School of BioSciences, The University of Melbourne, Victoria 3010, Australia

[2] cesar, 293 Royal Parade, Parkville, Victoria 3052, Australia

* Corresponding author: xuan@student.unimelb.edu.au

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ps.5053

Abstract

BACKGROUND: The polyphagous mite pest, *Halotydeus destructor*, typically has three generations during the cool moist season in Australia and produces over-summering diapause eggs in spring. Diapause eggs have a distinct thick and dark chorion and can survive heat, desiccation and the applications of pesticides. Farmers suppress the mite generation producing diapause eggs by a carefully-timed spring pesticide application using Timerite®, which predicts the onset of diapause egg production based largely on daylength. We investigated the association between diapause induction and other environmental factors which may complicate diapause predictions.

RESULTS: Diapause in *H. destructor* induction was influenced by three interacting environmental factors, namely daylength, temperature, and soil moisture. A cryptic type of diapause egg that lacked a thick chorion and was instead morphologically similar to non-diapause eggs was also discovered. Like diapause eggs, this newly discovered egg-type could also survive hot and dry summer conditions.

CONCLUSIONS: There is an opportunity to refine the Timerite® spring spray by incorporating knowledge of other environmental factors inducing diapause in *H. destructor*. Compared with typical diapause eggs, the production of cryptic diapause eggs could reflect a strategy to deal with diversifying environmental stresses and/or represent a bet-hedging strategy to adapt to unpredictable environments.

Keywords

Halotydeus destructor, summer diapause induction, heat and desiccation resistance,

bet-hedging strategy, phenotypic plasticity, ovoviviparity

1 Introduction

The regulation of active and dormant stages in the lifecycle of arthropods is critical for surviving unfavorable seasonal conditions, and maximizing activity under favorable conditions[1,2]. From an eco-physiological point of view, dormancy is defined as two principal forms: quiescence and diapause. In quiescence, activity or development is arrested due to adverse environmental factors (e.g. thermal stress) and/or the deficiency of essential factors (e.g. moisture and food), but is rapidly recovered after elimination of these stresses[3]. In contrast, diapause is a prospective dormancy antedating environmental stress and therefore reactivation requires species-specific mechanisms[4]. Although a few species enter obligatory diapause at a specific stage in the life cycle regardless of environmental cues[2], facultative diapause is induced in most species by abiotic factors (e.g. daylength[5,6], temperature[7], soil moisture[8,9]) and/or biotic factors (e.g. maturity of host plants[9–11], predator-prey interactions[12], scarcity of food[13]).

Facultative diapause can be further classified as winter and summer diapause according to the seasonal destination[14]. Winter and summer diapause are sometimes referred to as hibernation and aestivation according to seasons[15,16]; hibernation refers to the dormancy period that is terminated by exposure to cold temperatures over winter, while aestivation is the dormancy period allowing species to survive desiccation over summer[14]. The relationship between diapause mechanisms, abiotic factors and biotic factors is highly species-specific. Abiotic factors such as shorter daylength and cold temperatures are common diapause-inducing factors for many

winter-diapause species[17–19], but are diapause-inhibiting factors for some summer-diapause species[14]. Biotic factors such as increasing maturity of host plants can induce diapause in both winter-diapause[10,20–22] and summer-diapause species[8,9,11]. Much is known about the interactions between multiple factors affecting winter diapause[7,17,22–24], however comparatively speaking, few published studies have focused on species undergoing summer diapause[14]

Diapause can be categorized according to life stage, which in Acari can include egg, larva, nymph and adult diapause[25]. Winter diapause of eggs in Acari has been reported in seven species from the Tetranychoidea superfamily. Summer diapause is known in at least five species from the Eupodoidea superfamily[16], including *Halotydeus destructor* (Tucker) (Trombidiformes: Penthaleidae), an invasive pest from South Africa introduced into Australia, which attacks pastures, grains and vegetables[26–28]. Although legumes are preferred hosts of this polyphagous species, broad-leaf weeds and microflora also provide moist habitat and supplementary nutrition[29–32]. In Australia, this pest is active in the cool and wet months, typically between April and November, completing at least three generations a year[33,34]. The female typically lays 'winter' non-diapause eggs in the first two generations, but retains summer diapause eggs in the body in the third generation[5,33,35,36]. Although active mites die with the approach of summer, air-dry diapause eggs in dehydrated female cadavers can tolerate 70°C and hence survive the hot, dry summer period[11]. Diapause in *H. destructor* is terminated by exposure to heat, although embryo development can be arrested by post-diapause quiescence until autumn conditions necessary for survival (i.e.

following rainfall and a decline in temperature)[11,36]. There is no winter diapause period in *H. destructor*. The production of the filial generation (involving diapause or non-diapause eggs) is impacted by the maturity of host plant used in the early life stage of the parental generation prior to adulthood[11]. Therefore, the production of diapause or non-diapause eggs in *H. destructor* is a facultative response impacted by parental effects, similar to those documented for the winter diapause mite, *Panonychus ulmi*[22].

Diapause eggs of *H. destructor* can survive applications of pesticide as well as summer heat and desiccation[5,37], although the mechanisms involved are unclear. There are several synthetic chemicals registered in Australia to control this pest, but these only target the active stages of the mite (larvae, nymphs and adults), not eggs. Diapause eggs may survive better than active mites through protection provided by the egg cuticle, whereas non-diapause eggs hatch fast (<2 weeks, depending on temperature) and larvae are then exposed to residues of pesticides[36]. The number of diapause eggs produced by *H. destructor* dramatically impacts the population size in the following autumn[5]. The first generation of mites can cause serious feeding damage to crop and pasture seedlings because of the synchronicity between the germination of annual plants and the emergence of post-diapause *H. destructor*[38,39].

In order to minimize autumn population sizes, a carefully-timed chemical spray can be applied before the onset of diapause, as prescribed in a strategy developed in Western Australia based on assessments of 18 field populations[5]. This work suggested

diapause induction was best predicted by daylength (80.1% of variation explained), followed by the duration of long-term plant growing season (10.4%) and other factors (9.5%). Subsequently, a package known as Timerite® (www.timerite.com.au) was developed for Australian farmers, whereby the predicted spray date is geographically-specific but constant between years. In addition to daylength, the long-term plant growing season is used to adjust the predictions of diapause onset. This variable is estimated using rainfall and temperature data, averaged over 7 years[5]. This means the predicted onset date of diapause is constant in every year for each location.

Timerite® has proved to be highly successful in managing *H. destructor* in Australia, with population size reductions of 99% in the following autumn observed in parts of Western Australia[5,40]. However, Ridsdill-Smith[5] found the onset of diapause at some locations was not always constant between years. Furthermore, studies in eastern areas of Australia indicate the effectiveness of Timerite® may be less than in Western Australia[37,38]. The use of different approaches to identify the onset of diapause may contribute to inconsistent results, and uncertainty still exists about the cues responsible for inducing diapause in *H. destructor*. Firstly, diapause and non-diapause eggs are considered physically different and morphologically distinct due to a noticeably thicker chorion, which is seen as a dark rim surrounding the egg. The polar chorions of diapause and non-diapause eggs are in the range of 3.92-5.4μm (mean = 4.73 μm) and 1.6-2.32 μm (mean = 1.98 μm), respectively. The side-wall chorions of diapause and non-diapause eggs are in the range 2.56-4 μm (mean = 3.47 μm) and

1.28-2.32 µm (mean = 1.9 µm), respectively[41]. Morphological differences were used to identify the onset of diapause eggs of *H. destructor* when developing the Timerite® strategy[5]. A second approach is to consider the incubation period under a hatching test. For example, non-diapause eggs have been shown to hatch in 7.5-17.5 days at 11.3°C and 6-10 days at 16°C (never exceeding 20 days), while diapause eggs without heat exposure hatched in 114-331 days at 16°C[11,36]. A third approach is to focus on the timing of pesticide applications targeting field populations of mites. Researchers have been able to eliminate *H. destructor* populations (using pesticides) at regular intervals between winter and late spring, and successfully assess the resulting mite densities of the following autumn[37,38]. While spring treatments sprayed after the Timerite® date produced a marked increase in autumn mite numbers as expected, some diapause eggs were produced before the Timerite® date[37,38], suggesting additional factors are involved in diapause induction. In the absence of direct comparisons between approaches, it is unclear if morphological variation of *H. destructor* eggs accurately predicts diapause egg induction or egg hatching times observed in the field. There is also ambiguity around the specific role of various factors (*i.e.* daylength, temperature, moisture, host plants) in diapause induction[5,11,37,38]. For example, a field experiment that shortened the daylength exposure to 9 hours (using light-proof covers on field plots) was expected, but failed to, inhibit the diapause induction of mites with the approach of summer[11].

In this study, we undertook several experiments to improve our understanding of egg morphology and the environmental factors that induce diapause in *H. destructor*.

Specifically, we addressed the following questions. (1) How do environmental factors - daylength, temperature and soil moisture – contribute to diapause egg induction? (2) Can diapause and non-diapause eggs be confidently identified by morphology alone?

2 Materials and methods

2.1 Mite collections and microcosm experiments

Experiments were undertaken using a microcosm approach under semi-field conditions. This method has been used previously to determine numerous aspects of mite biology, including diapause, competition and plant host preferences[37,42–44]. In our experiments, clear plastic tubs (approximately 18 cm long, 12.5 cm wide and 26 cm high) with two gauze windows (approximately 10 cm long, 8 cm wide, for ventilation) were used as microcosms. Each tub contained sandy loam soil (1 L in total, consisting of 1 part sand and 4 parts loam). A mixture of pasture seeds was sown in mid-June 2016 at the following proportions: 40% vetch (*Vicia sativa* cv Rasina), 20% white clover (*Trifolium repens* cv. Storm), 20% subterranean clover (*Trifolium subterraneum* cv. Gosse), 10% phalaris (*Phalaris aquatica* cv. Holdfast), and 10% oats (*Avena sativa* cv. Williams). This mixture was selected because these plants were previously shown as suitable hosts for rearing mites in microcosms[42,45]. Seeds were obtained from Heritage Seeds (Victoria, Australia) and Smyth Seeds (Victoria, Australia). Following sowing, each tub was watered, enclosed with a clear plastic lid and placed in a shade-house. Tubs were watered as required thereafter.

Halotydeus destructor were collected using a vacuum method[38] in late-July 2016 at Bundoora, Victoria (-37.711364, 145.048763), from pasture dominated by legumes (>70%, mainly white clover with a few vetches (*Vicia* spp.) and medics (*Medicago* spp.)), some thick-bladed grasses (*Poaceae* spp.) and a few broad-leaf weeds (*Picris echioides* and *Arctotheca calendula*). The majority of original and microcosm hosts

were legumes to minimize the trade-offs of a host shift[44]. Based on predicted egg hatch timing[46], mites were estimated to be the second field generation. Mites were sorted in the laboratory and adults introduced into microcosm tubs (200 individuals per tub), followed by a 2-week incubation in a shade-house. Mites from the field (parental mites) were specifically collected to produce non-diapause 'winter' eggs, which later hatched resulting in the first filial generation (F1). Microcosms containing F1 eggs were then moved into temperature-controlled (CT) cabinets. Three different temperatures (11, 15 and 19 °C) and two daylengths (10/14 h and 16/8 h LD cycles) were tested in combination. For each combination, 6 microcosms experienced low soil moisture (dry treatment), and 5 microcosms experienced high soil moisture (moist treatment). This resulted in 12 environmental treatments (3 temperature x 2 daylength x 2 soil moisture) and a total of 66 microcosms. Soil moisture within each microcosm was measured twice a week with a soil probe (Gardman soil measure meter, Bunnings, Australia) and watered as required. Microcosms with dry and moist soil treatments were maintained at 1%-2.7% and 5.2%-10.2% (w/w water/soil), respectively. While in the shade-house, soil moisture within all microcosms was maintained at 5.2%-10.2%.

2.2 Diapause assessments using morphology and hatching tests

Once F1 mites had matured, sexes were identified by the color of the genital area, whereby red coloration and creamy-white coloration were used to identify males and females, respectively[26]. The diapause status of eggs of the second filial generation (F2) was morphologically identified from dissected F1 females (27-58 dissected females

were assessed from within each microcosm). Although some females containing no eggs were probably reproductively immature, each treatment included between 16-41 mature F1 females and 140-474 F2 eggs (see Supporting Table 1). We then performed hatching tests on the F2 eggs produced within each microcosm and compared the hatching results with the egg morphology. F2 eggs from each dissected F1 female were placed on moist filter paper in a petri dish. The total number of F2 eggs with and without a visible thicker chorion was recorded (all eggs fell into these two classes by thickness, although there was variation in the darkness of the thicker-chorion eggs). Petri dishes were sealed with Parafilm and incubated in a CT cabinet (15°C and 10/14 h LD) for 4 weeks. A 4-week incubation was used for the first hatching test because at this temperature all non-diapause eggs are expected to hatch (in < 2 weeks) but no diapause eggs (which hatch after 114 days) are expected to do so[36]. The vast majority of eggs with the thicker chorion failed to hatch after 4 weeks but hatched after a summer incubation period, demonstrating they were viable. Interestingly however, some unhatched eggs that did not have a thicker chorion, did hatch at a later date (see below), and are henceforth referred to as 'cryptic' diapause (CD) eggs because they are morphologically similar to non-diapause (ND) eggs (Supporting Fig.1).

2.3 Viability of eggs over summer

A second hatching test was performed to assess the viability of the CD eggs. Typical diapause (TD) eggs were also examined as the positive control. Despite removing eggs from the sheltered body of female mites, we found that 96% of the TD eggs hatched post-diapause, demonstrating that the viability of eggs is not negatively

impacted by this procedure. Petri dishes containing either CD and TD eggs were partly opened for 3 days to dehydrate eggs that had been placed on the moist filter paper. After 3 days, the paper was completely dry and both types of eggs shrunk and became somewhat bent and flattened. Air drying was necessary before long-term incubation because moistened diapause eggs are known to be more vulnerable to heat stress[36,47]. A subset of TD and CD eggs from within each treatment were then removed and dry incubated for 202 days (from November 2016 to mid-June 2017) in petri dishes over summer in a shade-house that was not temperature-controlled, ensuring eggs experienced natural day-length and temperature fluctuations, with temperatures frequently hotter than 30°C. This incubation period is somewhat longer than the expected diapause period in the field (typically around 5 months). Compared to diapause eggs which were expected to survive desiccation and the heat of summer, ND eggs have been shown to die from desiccation within 41-hours[41]. Even moistened ND eggs have high mortality if temperature is higher than 27.5°C[36].

In mid-June 2017, water was sprayed onto filter paper of each petri dish to moisten the eggs. After all eggs were re-moistened, the petri dishes were sealed and incubated for approximately 4 weeks (until mid-July) in a shade-house. Embryonic development of these eggs was observed approximately once per week. More than 70 % of all eggs developed into larvae, although for the purpose of analysis we defined a 'successfully hatched egg' as the development of an embryo to the deutovum (prelarva) stage following previous studies[11,36]. This overcame any potential issues associated with egg cannibalism by early-hatching larvae, as has been demonstrated in this species[41].

2.4 Data analysis

To assess diapause induction under different environmental conditions, two analyses were carried out: the first exploring factors influencing diapause generally (typical and cryptic); and the second focusing on factors influencing the newly discovered CD eggs. Firstly, female mites were categorized based on the number of eggs (N) dissected from a female: if $(N_{CD} + N_{TD})/N_{total}$ e 50% then classify as a female mite 'in diapause egg production' otherwise a female mite 'in non-diapause egg production'. This categorization was performed on individual mites, as their eggs were highly skewed towards being either all diapause eggs or all non-diapause eggs (Supporting Fig. 2). This phenomenon has been shown previously[11]. In less than 2% of cases, the proportion of non-diapause and diapause eggs within females was exactly 50%. In these instances, females were categorized as producing diapause eggs (although removal of these females and re-running the analysis did not alter the results). After mites had been categorized, a binomial logistic model was used to predict the status of egg production (diapause or non-diapause) of females with temperature, moisture, and daylength as categorical factors. Based on earlier literature, 10/14 h LD, 11°C and moist soil conditions were expected to inhibit diapause, and thus considered as reference factors in the model. Two-way interactions were tested between all explanatory variables. Following this, we explored factors that influence whether females produce TD or CD eggs. A similar analysis was performed on the subset of diapause mites classified as follows: if $N_{TD}/(N_{TD}+N_{CD})$ e 50% then classify as a female mite producing TD eggs' otherwise 'a female mite producing CD eggs'.

Females with no eggs were omitted from all analysis as they were unable to be categorized, likely due to reproductive immaturity. Two-way interactions between the explanatory variables were again tested but omitted here due to the low number of eggs and lack of variation in some categories leading to completely separated data when interactions were included. The relative importance of variables to the model fit was tested by omitting variables and comparing to the full model using the information criteria metrics AIC and BIC[48].

In *H. destructor*, non-diapause eggs are typically laid, while diapause eggs are retained within the body of females[33,36]. We examined the total number of eggs within female bodies that we categorized as described earlier using a one-way ANOVA and Scheffe's post-hoc tests, acknowledging that this does not provide a measure of female fecundity because some ND eggs would already have been laid.

The *glm* function as part of the *stats* library in the R language for statistical computing[49] was used to fit the binomial models and analyze the data.

3 Results

3.1 Environmental conditions influencing diapause

The effect of environmental factors on the incidence of diapause eggs in *H. destructor* are presented in Table 1 and Fig. 1. These results indicate (in order of importance) ambient temperature, soil moisture, and daylength each improve the model fit to observations. Two-way interactions between factors improved the model fit (df = 5, $Ç^2$ = 19.8, p = 0.001) and so were included in the analysis. The analysis revealed that main effects of higher temperature and dryer soil moisture significantly increased the production of diapause eggs (Table 1). The effect of daylength was significant, however the overall impact on diapause egg production was lower than temperature and soil moisture (Table 1). The effect of daylength was also not straightforward compared with temperature and moisture; longer daylength (16/8 h LD) decreased the diapause incidence at 19°C but increased the rate at 15°C (Fig. 1).

3.2 Differences in the induction and number of cryptic and typical diapause eggs

The proportion of diapause eggs that were classified as CD and TD within each environmental treatment are presented in Fig. 2, with the estimated effect of environmental factors (daylength, temperature and soil moisture) presented in Table 2. These show that daylength and temperature were important to predict diapause type, while the effect of moisture was marginal. Generally, higher temperatures and longer daylength were associated with mites producing TD eggs, rather than CD eggs (Fig. 2).

The average number of eggs extracted from the dissected females indicated that the females producing TD eggs contained more eggs than females producing ND and CD eggs ($p < 0.001$) (Supporting Fig. 3). Although females that produced CD eggs had slightly fewer total eggs compared with females that produced ND eggs, this difference was not significant ($p = 0.060$).

3.3 Viability of cryptic and typical diapause eggs

Although the color and shape of many CD eggs became bleached and collapsed during the desiccation period in the shade-house, 292 out of 387 (~75%) of the CD eggs hatched. By contrast, 577 out of 601 (~96%) TD eggs hatched. Although the CD egg is morphologically similar to ND eggs, unlike ND eggs, they were shown to be viable after exposure to summer conditions.

4 Discussion

For the first time, a distinct mode of cryptic diapause is described for *H. destructor*. This cryptic type represents a viable diapause egg despite lacking the thick chorion of typical diapause eggs, demonstrating three distinct types of eggs in *H. destructor*: non-diapause (ND, 'winter eggs'), cryptic diapause (CD) and typical diapause (TD). This has not been observed before in *H. destructor*[5,11,36,41], or in any arthropod pests of which we are aware. The lower hatch rate of CD eggs compared with TD eggs may reflect the lack of a deeper chorion leading to a weaker level of protection against hot and dry summer conditions. The difference in viability between both diapause types requires further study. However, the CD eggs and the interaction between environmental factors leading to their production is likely to be (at least partly) responsible for the inconsistency of results in previous studies.

Compared to ND eggs, diapause eggs of both types were induced by longer daylength, hotter temperature and drier soil (Fig.1 and Table 1). CD eggs were produced in relatively short-daylength and cooler conditions when compared to TD eggs (Fig. 2 and Table 2), indicating *H. destructor* has the ability to produce different types of diapause eggs in the lead up to spring. Early production of diapause eggs was noted in studies showing that post-diapause mites emerged after pesticides were applied according to the Timerite® dates[37,38,50]. In addition, we have found CD eggs when dissecting mites from the field well before the Timerite® date: we obtained 277 CD eggs, 24 ND eggs and no TD eggs from *H. destructor* collected on 15th September 2014 from a pasture paddock at Diamond Creek, Victoria (-37.676357, 145.131784).

The Timerite® predicted date for this location is 16th October, more than a month after our sample was taken.

This study shows the accuracy of the Timerite® date could be improved. Currently, the Timerite® date does not change between years, despite knowledge that the diapause date at a location can vary substantially (more than several weeks) between different years[5]. This study has revealed that there are key processes missing from the estimation process of the Timerite® date, such as temperature and moisture conditions, which were found to have a stronger effect on diapause than daylength. The incorporation of this new knowledge, as well as increasingly available dynamic climatic data on temperature and moisture that captures variation in conditions through years, has the potential to enhance Timerite®.

The production of CD eggs in *H. destructor* may reflect a bet-hedging strategy whereby some individuals of a generation enter diapause to adapt to uncertain environments[51]. It could also reflect an evolutionary strategy governing the hatching of autumn eggs after post-diapause quiescence, whereby the embryonic development of eggs is inhibited when temperatures exceed a threshold[36]. The threshold in Western Australia is known to be higher than in eastern Australia, likely because temperature (and rainfall patterns) in the eastern areas are more changeable and uncertain than those of Western Australia, indicating adaptive evolution[46]. Whether the threshold temperature varies between CD and TD eggs, or whether strategies differ between mites in Western Australia and eastern Australia, remains unclear.

In addition to bet-hedging, the two physiologically different types of diapause eggs may reflect phenotypic plasticity evolved under diversifying environmental selection[52]. Previous studies have found the number of ND eggs within a female mite is lower than that of diapause eggs, reflecting the fact females lay ND eggs but retain diapause eggs within their body[5,53]. Interestingly, our findings revealed that female mites producing CD (and ND) eggs carried fewer overall eggs than those females producing TD eggs. It is possible that CD eggs produced in relatively cooler temperatures are laid rather than being retained inside the mite body as is the case for TD eggs (which are also slightly larger than ND eggs)[5,41]. If this is the case, fewer resources would be required to produce CD eggs than the production and storage of TD eggs, thus offering a fitness advantage for a cryptic diapause strategy. Further studies are needed to understand the fertility, life span and fitness costs of females producing ND, CD and TD eggs.

Previous studies suggested that egg-laying is inhibited by high temperatures for diapause and ND eggs and therefore ND eggs may commence development inside the female body[41,54]. This phenomenon has been also observed in our research, especially when high temperature (19°C) accompanied the moist treatment. We clearly found that some eggs developed to the deutovum, or the larval stage, within female mites (https://youtu.be/er4I9v4IyiA), suggesting ovoviviparity in *H. destructor*. Ovoviviparity may be an evolutionary strategy utilized by *H. destructor* to protect ND eggs from future desiccation at high temperatures, recognizing ND eggs can die from

desiccation within 41 hours[41], which is insufficient time for embryonic development (>6 days)[11,36].

The effect of daylength was noted as being important for diapause induction by Ridsdill-Smith et al.[5]; however a shortened daylength did not reduce diapause responses in the approach of summer in the study by Wallace[11]. Our study revealed a daylength*temperature interaction reflecting the fact that the short daylength (14/10 h LD) did not have a marked effect at 11°C, but did at 15°C and 19°C. We found no marked effect of daylength on diapause egg production at 19°C under dry conditions, a finding that is congruent with the Wallace[11] study. Furthermore, differences were observed between individual mites that experienced the same treatment conditions. For example, under the 19°C/dry/10:4h LD treatment, some mites produced 100% diapause eggs while some produced 100% ND eggs. The 19°C and dry conditions are diapause-inducing, while a short-daylength (10/14 h LD) is expected to be diapause-inhibiting. Further studies are needed to understand whether these individual differences in sensitivity to various factors are heritable or represent non-genetic variability.

In addition to the individual differences in female mites observed in our study, evolutionary changes are probably involved in diapause characteristics of *H. destructor,* which entered Australia from South Africa over a century ago and have undergone genetic adaptation[28]. In comparison with South African populations, Australian populations have expanded into hotter and drier inland areas and have

become more thermoresistant[55–57]. Pesticide resistance against pyrethroids[58,59] and organophosphates[39] has also evolved in *H. destructor* populations in Australia. While the interaction between these evolutionary changes and diapause induction are unclear in *H. destructor*, pesticide resistance has been shown to influence photoperiodic diapause in the codling moth, *Cydia pomonella*, indicating pleiotropic trade-offs[60]. Geographical adaptation also influences the photoperiodic response of winter diapause in *Aedes albopictus*[61,62].

5 Conclusion

A novel mode of diapause in *H. destructor,* which is influenced by a complex of environmental factors has been identified in this study. Furthermore, we demonstrate that temperature and soil moisture play a critical role in diapause egg production in *H. destructor* which has implications for pest control. While a very useful management tool, the Timerite® model currently used by Australian farmers could be improved by incorporating real-time (or predicted) temperature and soil moisture information rather than long-term averages to allow predictions to account for variable environmental conditions between years. The presence of different types of diapause eggs raises the question of whether these reflect adaptive responses to different recurring environmental conditions and different levels of environmental variability. This could be further tested by comparing the incidence of diapause strategies across populations of mites given that *H. destructor* in Australia covers a wide range of climatic conditions and given there is evidence for adaptive differentiation in *H. destructor* populations[28,56,57].

Acknowledgements

Thanks to Josh Douglas, Kelly Richardson and Nick Bell for technical assistance, and Elia Pirtle for the graphical abstract. We also thank two anonymous reviewers for valuable comments on this manuscript. XC is supported by scholarships from the University of Melbourne (MIFRS, MIRS), the Australian Commonwealth Scientific and Research Organization (CSIRO) and the Taiwan Ministry of Education (GSSA). This research was supported by the Grains Research and Development Corporation.

References

1 Chippendale GM, Insect diapause, the seasonal synchronization of life cycles, and management strategies, Entomol Exp Appl **31**:24–35 (1982).

2 Denlinger DL, Regulation of diapause, Annu Rev Entomol **47**:93–122 (2002).

3 Hand SC and Podrabsky JE, Bioenergetics of diapause and quiescence in aquatic animals, Thermochim Acta **349**:31–42 (2000).

4 Belozerov VN, New aspects in investigations of diapause and non-diapause dormancy types in insects and other arthropods, Entomol Rev **89**:127–136 (2009).

5 Ridsdill-Smith J, Pavri C, De Boer E, and Kriticos D, Predictions of summer diapause in the redlegged earth mite, *Halotydeus destructor* (Acari: Penthaleidae), in Australia, J Insect Physiol **51**:717–726 (2005).

6 Lees AD, Diapause and photoperiodism in the fruit tree red spider mite (*Metatetranychus ulmi* Koch), Nature **166**:874–875 (1950).

7 Xue F, Spieth HR, Aiqing L, and Ai H, The role of photoperiod and temperature in determination of summer and winter diapause in the cabbage beetle, *Colaphellus bowringi* (Coleoptera: Chrysomelidae), J Insect Physiol **48**:279–286 (2002).

8 Wallace MMH, The ecology of *Sminthurus viridis* (Collembola) II. Diapause in the aestivating egg, Aust J Zool **16**:871–883 (1968).

9 Roberts JMK, Umina PA, Hoffmann AA, and Weeks AR, Population dynamics and diapause response of the springtail pest *Sminthurus viridis* (Collembola: Sminthuridae) in southeastern Australia, J Econ Entomol **104**:465–473 (2011).

10 Fife LC, Studies of the diapause in the pink bollworm in Puerto Rico, U.S. Dept. of Agriculture (1949).

11 Wallace MMH, Diapause in the aestivating egg of *Halotydeus destructor* (Acari: Eupodidae), Aust J Zool **18**:295–313 (1970).

12 Kroon A, Veenendaal RL, Bruin J, Egas M, and Sabelis MW, "Sleeping with the enemy"—predator-induced diapause in a mite, Naturwissenschaften **95**:1195–1198 (2008).

13 McMullen RD, The effects of photoperiod, temperature, and food supply on rate of development and diapause in *Coccinella Novemnotata*, Can Entomol **99**:578–586 (1967).

14 Masaki S, Summer Diapause, Annu Rev Entomol **25**:1–25 (1980).

15 Gill HK, Goyal G, and Chahil G, Insect diapause: a review, J Agric Sci Technol A **7**:454–473 (2017).

16 Belozerov VN, Diapause and quiescence as two main kinds of dormancy and their significance in life cycles of mites and ticks (Chelicerata: Arachnida: Acari). Part 1. Acariformes, Acarina **16**:79–130 (2008).

17 Beck SD, Photoperiodic induction of diapause in an insect, Biol Bull **122**:1–12 (1962).

18 Kroon A, Veenendaal RL, and Veerman A, Photoperiodic induction of diapause in the spider mite *Tetranychus urticae*: qualitative or quantitative time measurement?, Physiol Entomol **22**:357–364 (1997).

19 Hairston NG and Kearns CM, The interaction of photoperiod and temperature in diapause timing: a copepod example, Biol Bull **189**:42–48 (1995).

20 Usua EJ, Induction of diapause in the maize stemborer, *Busseola Fusca*, Entomol Exp Appl **16**:322–328 (1973).

21 Squire FA, Observations on the larval diapause of the pink bollworm, *Platyedra gossypiella*, Saund, Bull Entomol Res **30**:475–481 (1940).

22 Lees AD, Environmental factors controlling the evocation and termination of diapause in the fruit tree red spider mite *Metatetranychus ulmi* Koch (Acarina: Tetranychidae), Ann Appl Biol **40**:449–486 (1953).

23 Chippendale GM, Reddy AS, and Catt CL, Photoperiodic and thermoperiodic interactions in the regulation of the larval diapause of *Diatraea grandiosella*, J Insect Physiol **22**:823–828 (1976).

24 Xiao H-J, Mou F-C, Zhu X-F, and Xue F-S, Diapause induction, maintenance and termination in the rice stem borer *Chilo suppressalis* (Walker), J Insect Physiol **56**:1558–1564 (2010).

25 Belozerov VN, Seasonal adaptations in the life cycles of mites and ticks: comparative and evolutionary aspects, Trends in Acarology, Springer, Dordrecht, pp. 319–326 (2010).

26 Weeks AR, Fripp YJ, and Hoffmann AA, Genetic structure of *Halotydeus destructor* and *Penthaleus major* populations in Victoria (Acari: Penthaleidae), Exp Appl Acarol **19**:633–646 (1995).

27 Qin TK, Population genetics of redlegged earth mites *Halotydeus destructor* and *H. anthropus* (Acarina: Penthaleidae) from Australia and or South Africa, Bull Entomol Res **87**:289–298 (1997).

28 Hill MP, Hoffmann AA, Umina PA, Cheng X, and Miller AD, Genetic analysis along an invasion pathway reveals endemic cryptic taxa, but a single species with little population structure in the introduced range, Divers Distrib **22**:57–72 (2016).

29 Maclennan KE, McDonald G, and Ward SA, Soil microflora as hosts of redlegged earth mite (*Halotydeus destructor*), Entomol Exp Appl **86**:319–323 (1998).

30 Solomon ME, Behaviour of the red-legged earth mite, *Halotydeus destructor*, in relation to environmental conditions, J Anim Ecol **6**:340–361 (1937).

31 Annells AJ and Ridsdill-Smith TJ, Host plant species and carbohydrate supplements affecting rate of multiplication of redlegged earth mite, Exp Appl Acarol **18**:521–530 (1994).

32 Gaull KR and Ridsdill-Smith TJ, The foraging behaviour of redlegged earth mite, *Halotydeus destructor* (Acarina: Penthaleidae), in an annual subterranean clover pasture, Bull Entomol Res **86**:247–252 (1996).

33 Ridsdill-Smith TJ, Biology and control of *Halotydeus destructor* (Tucker) (Acarina: Penthaleidae): a review, Exp Appl Acarol **21**:193–223 (1997).

34 Umina PA, Hoffmann AA, and Weeks AR, Biology, ecology and control of the *Penthaleus* species complex (Acari: Penthaleidae), Exp Appl Acarol

34:211–237 (2004).

35 Halliday RB, Taxonomic background of the redlegged earth mite *Halotydeus destructor* (Tucker) (Acarina: Penthaleidae), Plant Prot Q **6**:162–165 (1991).

36 Wallace MMH, The influence of temperature on the post-diapausal development and survival in the aestivating eggs of *Halotydeus destructor* (Acari [illegible] **18**:215–229 (1970).

37 Umina PA and Hoffmann AA, Diapause and implications for control of *Penthaleus* species and *Halotydeus destructor* (Acari: Penthaleidae) in southeastern Australia, Exp Appl Acarol **31**:209–223 (2003).

38 Gower JMC, Hoffmann AA, and Weeks AR, Effectiveness of spring spraying targeting diapause egg production for controlling redlegged earth mites and other pests in pasture, Aust J Exp Agric **48**:1118–1125 (2008).

39 Umina PA, Lord A, Micic S, and Edwards O, Discovery and characterisation of field resistance to organophosphorus chemicals in a major mite pest, *Halotydeus destructor*, Pest Manag Sci **73**:1719–1724 (2017).

40 Ridsdill-Smith TJ, Hoffmann AA, Mangano GP, Gower JM, Pavri CC, and Umina PA, Strategies for control of the redlegged earth mite in Australia, Aust J Exp Agric **48**:1506–1513 (2008).

41 Norris KR, The aestivating eggs of the red-legged earth mite, *Halotydeus Destructor* (Tucker), Bulletin of the Commonwealth Scientific and Industrial Research Organisation (1950).

42 Umina PA and Hoffmann AA, Plant host associations of *Penthaleus species* and *Halotydeus destructor* (Acari: Penthaleidae) and implications for integrated pest management, Exp Appl Acarol **33**:1–20 (2004).

43 Umina PA and Hoffmann AA, Competitive interactions among four pest species of earth mites (Acari: Penthaleidae), J Econ Entomol **98**:307–316 (2005).

44 Cheng X, Umina PA, and Hoffmann AA, Influence of previous host plants on the reproductive success of a polyphagous mite pest, *Halotydeus destructor*, J Econ Entomol **111**:680-688 (2018).

45 Thackray DJ, Ridsdill-Smith TJ, and Gillespie DJ, Mass rearing *Halotydeus destructor* (Tucker) (Acari / : Penthaleidae) for u *Trifolium subterraneum* (L.) for mite resistance, Aust J Exp Agric **37**:343–349 (1997).

46 McDonald G, Umina PA, Macfadyen S, Mangano P, and Hoffmann AA, Predicting the timing of first generation egg hatch for the pest redlegged earth mite *Halotydeus destructor* (Acari: Penthaleidae), Exp Appl Acarol **65**:259–276 (2015).

47 James DG and O'Malley KJ, Oversummering of eggs of "*Halotydeus destructor*" Tucker (Acari: Penthaleidae): Diapause termination and mortality, Aust Entomol **18**:35 (1991).

48 Burnham KP and Anderson DR, Model selection and multimodel inference: a practical information-theoretic approach, 2nd edition, Springer Science & Business Media (2003).

49 R Core Team, R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/., 2017. https://www.gbif.org/tool/81287/r-a-language-and-environment-for-statistical-computing [accessed 4 December 2017].

50 Ridsdill-Smith TJ and Pavri CC, Controlling redlegged earth mite, *Halotydeus destructor* (Acari: Penthaleidae), with a spring spray in legume pastures, Crop Pasture Sci **66**:938–946 (2015).

51 Menu F, Roebuck J, and Viala M, Bet-hedging diapause strategies in stochastic environments, Am Nat **155**:724–734 (2000).

52 Furness AI, Lee K, and Reznick DN, Adaptation in a variable environment: phenotypic plasticity and bet-hedging during egg diapause and hatching in an annual killifish, Evolution **69**:1461–1475 (2015).

53 Ridsdill-Smith TJ and Annells AJ, Seasonal occurrence and abundance of redlegged earth mite *Halotydeus destructor* (Acari: Penthaleidae) in annual pastures of southwestern Australia, Bull Entomol Res **87**:413–423 (1997).

54 Norris KR, A population study of the red-legged earth mite (*Halotydeus destructor*) in Western Australia mites and notes on associated collembola, Melbourne / : Council for Scientific (1938).

55 Wallace MMH and Mahon JA, The distribution of *Halotydeus destructor* and *Penthaleus major* (Acari / : Eupodidae) in A land use, Aust J Zool **19**:65–76 (1971).

56 Hill MP, Hoffmann AA, Macfadyen S, Umina PA, and Elith J, Understanding

niche shifts: using current and historical data to model the invasive redlegged earth mite, *Halotydeus destructor*, Divers Distrib **18**:191–203 (2012).

57 Hill MP, Chown SL, and Hoffmann AA, A predicted niche shift corresponds with increased thermal resistance in an invasive mite, *Halotydeus destructor*, Glob Ecol Biogeogr **22**:942–951 (2013).

58 Umina PA, Pyrethroid resistance discovered in a major agricultural pest in southern Australia: the redlegged earth mite *Halotydeus destructor* (Acari: Penthaleidae), Pest Manag Sci **63**:1185–1190 (2007).

59 Umina PA, Weeks AR, Roberts J, Jenkins S, Mangano GP, Lord A, *et al.*, The current status of pesticide resistance in Australian populations of the redlegged earth mite (*Halotydeus destructor*), Pest Manag Sci **68**:889–896 (2012).

60 Boivin T, Bouvier J-C, Beslay D, and Sauphanor B, Variability in diapause propensity within populations of a temperate insect species: interactions between insecticide resistance genes and photoperiodism, Biol J Linn Soc **83**:341–351 (2004).

61 Lounibos LP, Escher RL, and Lourenço-De-Oliveira R, Asymmetric evolution of photoperiodic diapause in temperate and tropical invasive populations of *Aedes albopictus* (Diptera: Culicidae), Ann Entomol Soc Am **96**:512–518 (2003).

62 Urbanski J, Mogi M, O'Donnell D, DeCotiis M, Toma T, and Armbruster P, Rapid adaptive evolution of photoperiodic response during invasion and range expansion across a climatic gradient, Am Nat **179**:490–500 (2012).

Table 1. Table of model coefficients, standard errors, and goodness of fit measures for binomial models predicting the production of diapause eggs in female *H. destructor*.

	Full model	**No temperature**	**No moisture**	**No daylength**
(Intercept)	-2.14 (0.55)***	-0.90 (0.20) ***	-0.94 (0.26)***	-2.58 (0.52)***
Temperature = 15°C	0.86 (0.64)		0.29 (0.36)	1.84 (0.59)**
Temperature =19°C	2.59 (0.66)***		2.03 (0.43)***	2.74 (0.59)***
Moisture = dry	1.78 (0.60)**	1.08 (0.31)***		1.81 (0.58)**
Daylength = 16h	-1.28 (0.70)*	0.15 (0.29)	-1.07 (0.48)*	
15°C:dry	-0.63 (0.72)			-0.57 (0.69)
19°C:dry	-0.01 (0.81)			0.05 (0.77)
dry:16h	0.21 (0.59)	0.09 (0.45)		
15°C:16h	2.55 (0.67)***		2.44 (0.62)***	
19°C:16h	0.72 (0.77)		0.69 (0.65)	
AIC	387.82	468.82	418.67	398.57
BIC	426.54	484.31	441.90	421.80
Log Likelihood	-183.91	-230.41	-203.33	-193.29
Deviance	367.82	460.82	406.67	386.57
Num. observations	355	355	355	355

Reference levels of factors: Temperature = 11°C; Moisture = moist; Daylength = 10h. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$

Table 2. Table of model coefficients, standard errors, and goodness of fit measures for binomial models predicting the production of TD eggs in female *H. destructor*.

	Full model	**No temperature**	**No moisture**	**No daylength**
(Intercept)	-2.25 (0.65)***	-1.04 (0.35)**	-2.13 (0.57)***	-1.94 (0.63)**
Temperature = 15°C	1.07 (0.62)		1.05 (0.62)	1.41 (0.61)*
Temperature = 19°C	1.56 (0.61)*		1.53 (0.60)*	1.78 (0.60)**
Moisture = dry	0.15 (0.37)	-0.01 (0.36)		0.22 (0.36)
Daylength =16h	1.11 (0.35)**	1.21 (0.34)***	1.12 (0.35)**	
AIC	202.90	207.00	201.07	211.18
BIC	218.25	216.21	213.35	223.46
Log Likelihood	-96.45	-100.50	-96.54	-101.59
Deviance	192.90	201.00	193.07	203.18
No. observations (*n*)	159	159	159	159

Reference levels of factors: Temperature = 11°C; Moisture = moist; Daylength = 10h.
$^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$

Supporting Table 1. Table showing the sample sizes used in Fig. 1 and Fig. 2. Fig. 1 included the number of all F1 females and F2 eggs in each environmental treatment. ND eggs and the females in non-diapause-eggs production were excluded in Fig. 2.

Figure 1	**Temperature**	**Moisture**	**Daylength**	**Number of females**	**Number of eggs**
	11°C	dry	10h	41	389
	11°C	dry	16h	32	253
	11°C	moist	10h	30	361
	11°C	moist	16h	27	242
	15°C	dry	10h	35	330
	15°C	dry	16h	31	474
	15°C	moist	10h	35	326
	15°C	moist	16h	21	186
	19°C	dry	10h	23	256
	19°C	dry	16h	26	314
	19°C	moist	10h	25	263
	19°C	moist	16h	16	140
Figure 2	**Temperature**	**Moisture**	**Daylength**	**Number of females in diapause-egg production**	**Number of diapause eggs**
	11°C	dry	10h	18	152
	11°C	dry	16h	5	52
	11°C	moist	10h	2	43
	11°C	moist	16h	2	17
	15°C	dry	10h	16	178
	15°C	dry	16h	25	425
	15°C	moist	10h	8	65
	15°C	moist	16h	10	136
	19°C	dry	10h	20	239
	19°C	dry	16h	25	314

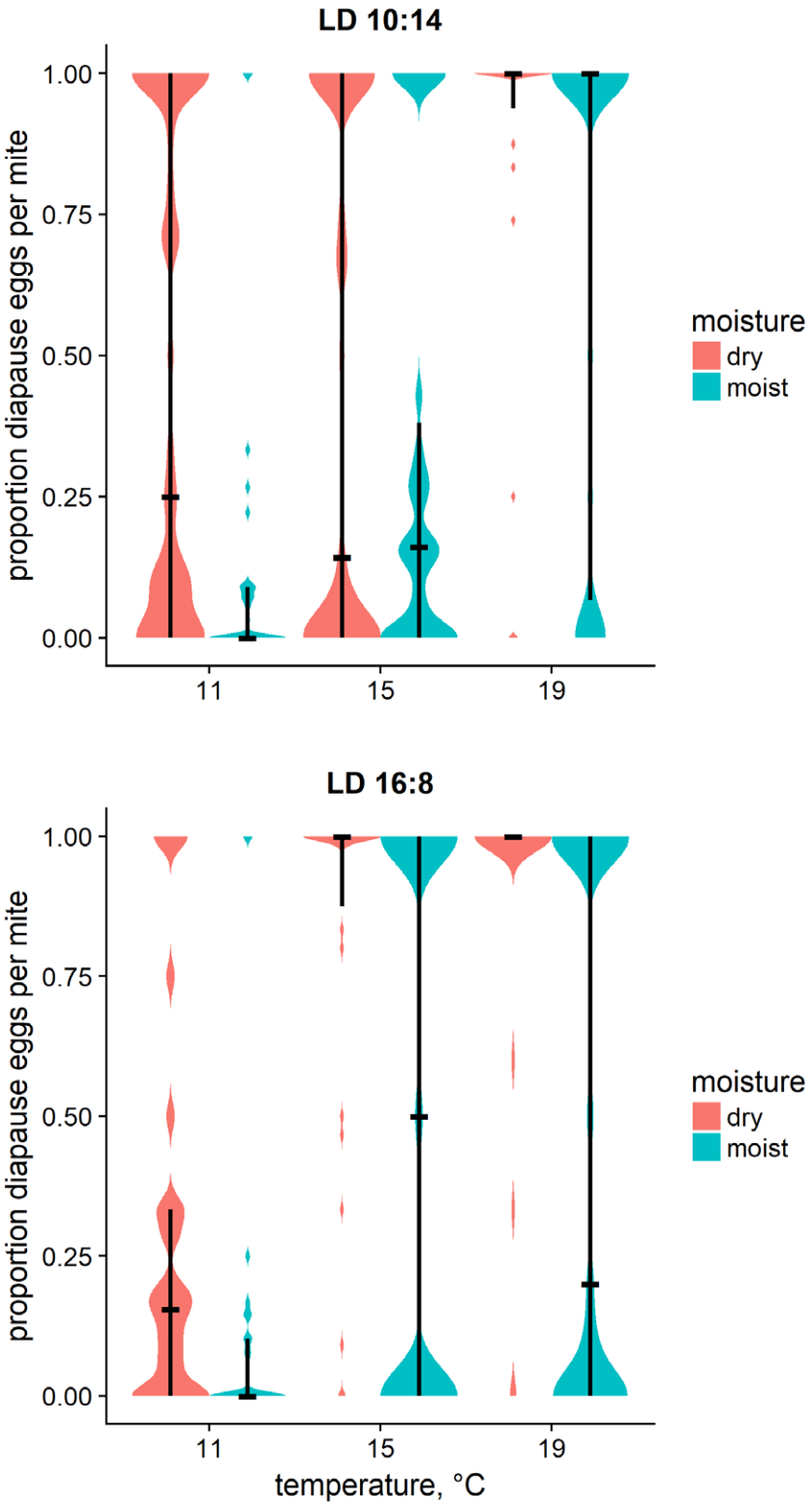


Figure 1.

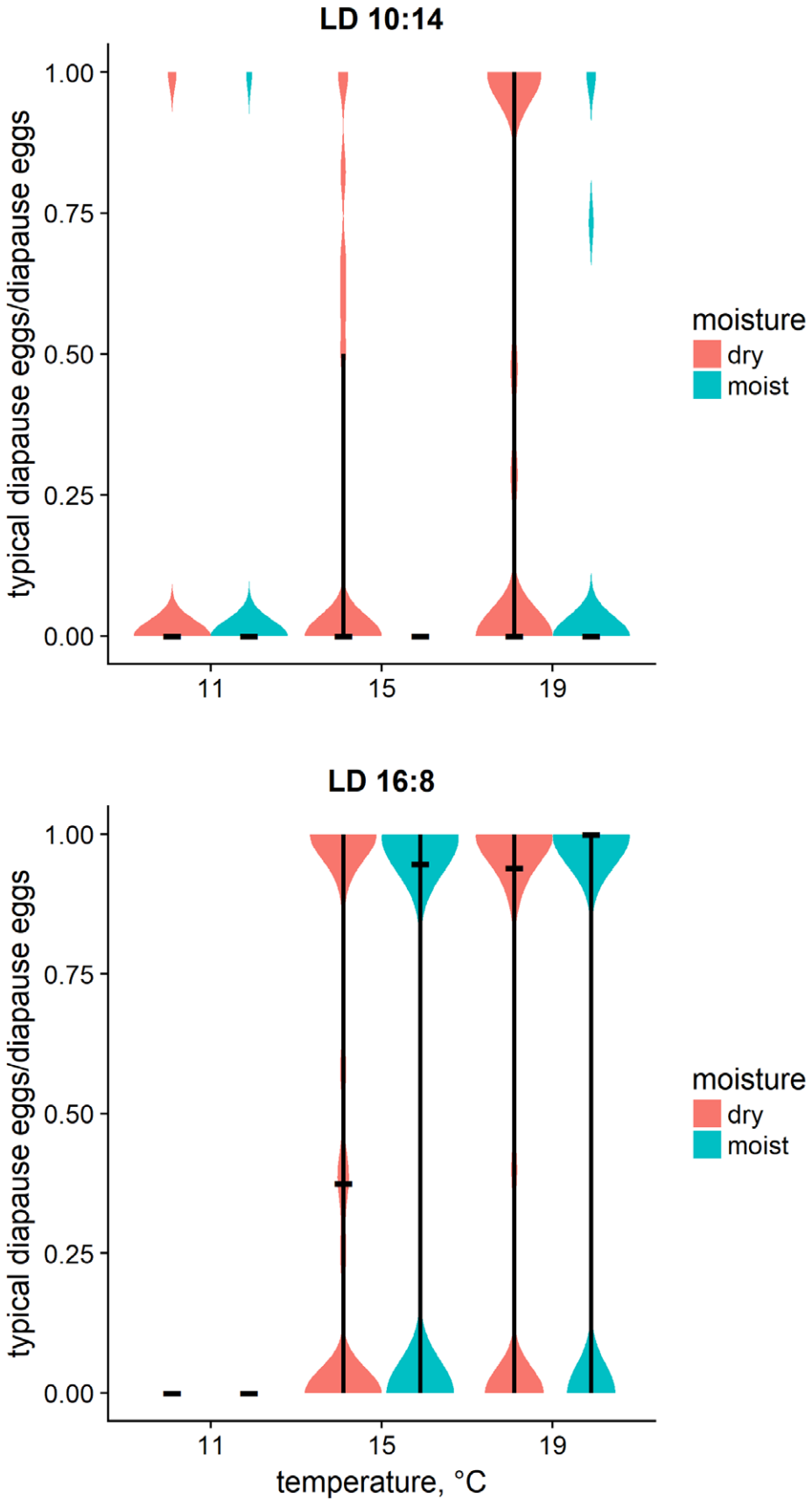
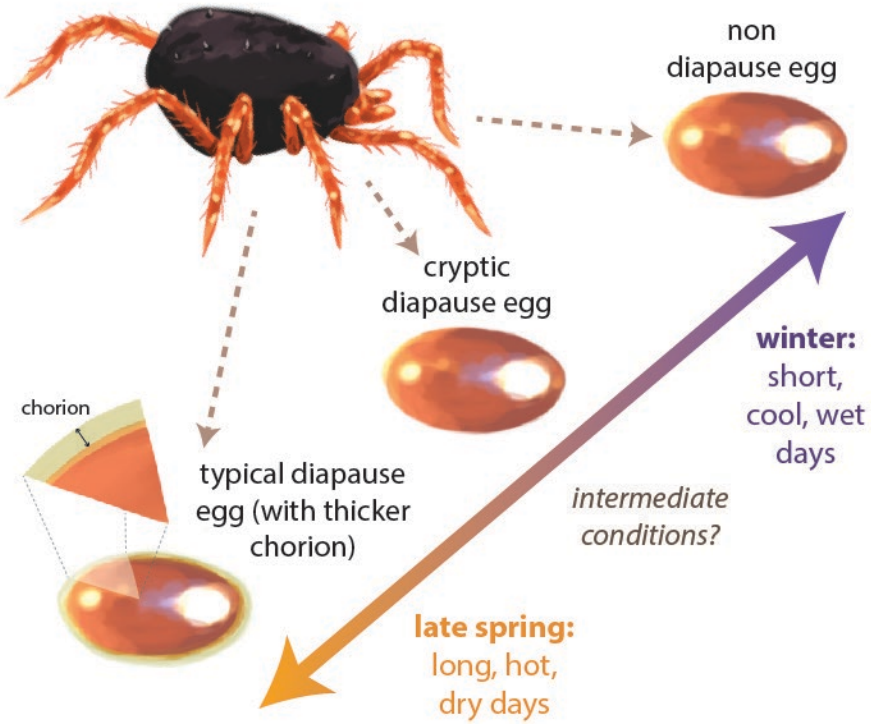
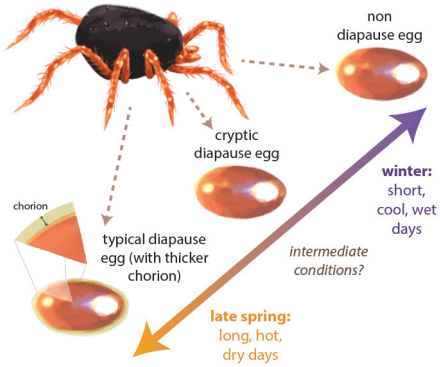


Figure 2.



Graphical Abstract: A new intermediate form of diapause is discovered in the mite, *Halotydeus destructor*, triggered by conditions between winter and late spring.

A new intermediate form of diapause is discovered in the mite, *Halotydeus destructor*, triggered by conditions between winter and late spring.



diapause mites3.jpg

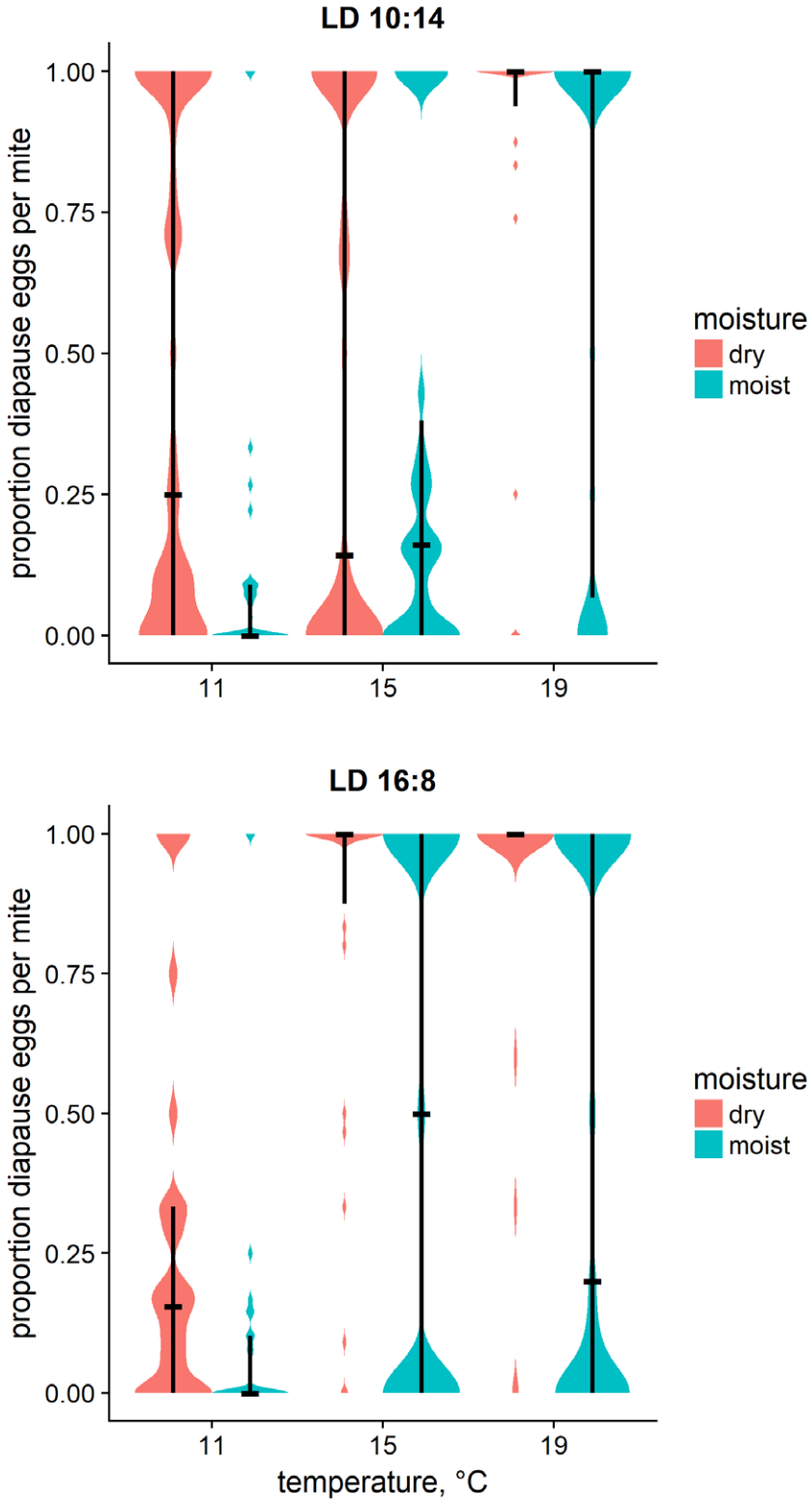


Figure 1.

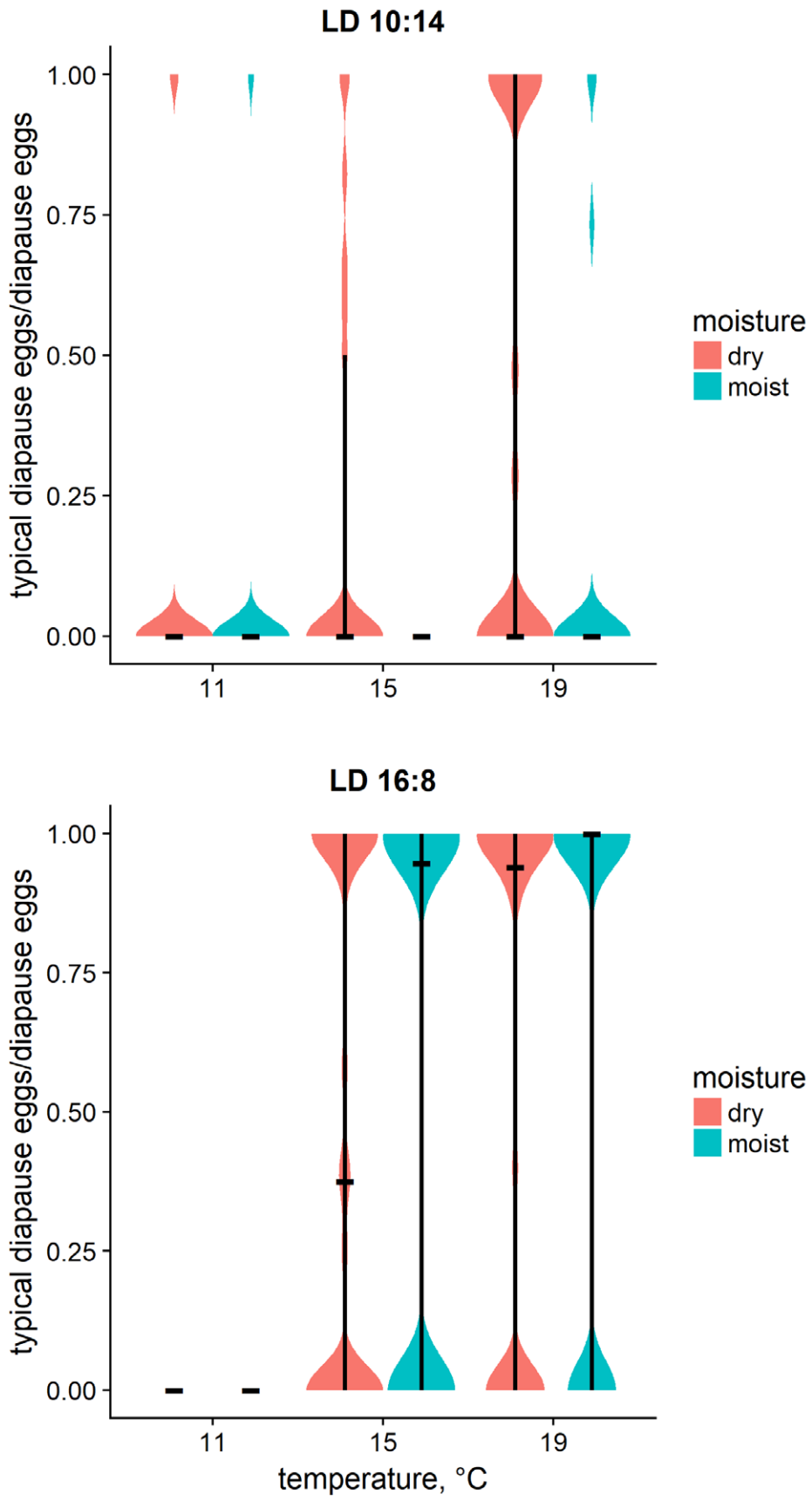


Figure 2.

Figure legends

Fig. 1. The diapause production status of female *H. destructor* for each treatment combination, as measured by the number of diapause eggs comprising the total number of eggs dissected from each mite. In the violin plots, the horizontal notch represents the median value for each group, while the vertical lines represent the inter-quartile range. Sample sizes of each treatment group are shown in Supporting Table 1.

Fig. 2. The proportion of TD eggs produced by female *H. destructor* for each treatment combination, as measured by the number of TD eggs comprising all diapause eggs dissected from each mite. Sample sizes of each treatment group are shown in Supporting Table 1.