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EFFECTS OF PHOTOPERIOD AND TEMPERATURE ON *Didinium nasutum* ENCYSTMENT

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ABSTRACT

Laboratory experiments were done to determine the effects of photoperiod and temperature on *Didinium nasutum* encystment. The cyst production in this freshwater ciliate was relatively independent of photoperiodic regimes (LL, LD or DD) at high temperatures (24-14ºC). When temperature decreased down to 3ºC, regardless of light conditions, population of the *Didinium nasutum* gradually started to form cyst so that after 72 hours nearly all observed individuals had transformed into cysts, even though there was still a fraction that never became encysted. The rate of the cyst production in DD (shortest daylength) was fastest, slow in LD and slowest in LL (longest daylength) conditions. This study suggests that temperature is a more critical cue than daylength in the cyst production of *Didinium nasutum*.

Key words: Encystment, freshwater ciliate, photoperiod, temperature, *Didinium nasutum*

INTRODUCTION

Response of some invertebrate such as *Gonyaulax polyedra* to photoperiod and low temperatures is by forming cyst (encystment) (Balzer, 1996). Encystment process is a mode of escaping harsh conditions, important for survival and appears to be a protective mechanism. It is a complex phenomenon in which the physiological response of the vegetative stage is modulated by more than one environmental cue (Sgrosso *et al*., 2001).

Under adverse environmental conditions, e.g. extreme high and low temperatures, oxygen depletion, pH reduction and/or when food is depleting many ciliates transform into a cyst (Calvo *et al*., 2003 & Foissner *et al*., 2005). In some dino-flagellates formation of cyst depends upon temperature and photoperiod (Sgrosso *et al*., 2001). Carter (1919) found encysted protozoa (*Amoeba*) mostly during winter months of the year. It has been suggested that the encystment is a regular phenomenon (Reid and John, 1978) as seen in *Crytostrombidium* that encystment generally occurs during a certain period of the seasonal cycle (Kim *et al*., 2002). In fact, according to Foissner *et al*. (2005) data on ciliates encystment are rather incomplete and only limited information concerning the precise factors which operate during the process of ciliates encystment ever known. Thus this study was to investigate the role of photoperiod and temperature in *Didinium nasutum* encystment under laboratory conditions. *D nasutum* (formerly *Vorticella nasuta*) is a Gymnostomatida freshwater ciliate, feeds principally upon *Paramecium*.

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MATERIALS AND METHODS

Didinium nasutum was collected from a pond inside the Faculty of Sciences, Shahrekord University, Shahrekord, Iran. The ciliates were fed using *Paramecium caudatum* (Iwadate and Asai, 1996) bacterised with *Klebsiella pneumoniae* under a photoperiod of 12:12 h light: dark (12L: 12 D) at room temperature (22-24ºC). For each part of the experiment eight sample tubes was selected and filled (1.5 ml) from stock culture. The initial density of *Didinium* and *Paramecium* were 45 and 4.6 x 10^3 cells per ml, respectively. Cysts were identified under microscope according to their shapes. The ciliates were fed at the end of each day by adding 0.5 ml *Paramecium* from its stock. The effects of photoperiod and temperature on *D.*

nasutum encystment were investigated in the following manner:

1. Photoperiod response experiment: The response of *D. nasutum* in forming a cyst was tested at room temperature under three different light conditions (LL, LD and DD). The LD, LL, and DD were defined as Light/Dark cycle, full photophase, and full scotophase, respectively The photoperiod provided by fluorescence tubes (Thorn, 36W, light white) was maintained at 12: 12 LD (lights on at 08:00 h, lights off at 20:00 h) with a light intensity of 4 μmol s^{-1} m^{-2} at the tubes during the photophase. All light was excluded during the scotophase. Eight sample tubes were used for each treatment, i.e. LL, LD and LD. The caps of the sample tubes were left open (aerated) and denoted as zero hour. And ciliate countings were then made at 12, 24, 36, 48, 60, and 72 hours later according to Salvadó & Gracia (1993). Sub-samples (20 μl each) of each tube were taken with a micropipette, and the numbers of *D. nasutum* (cysts, if any) were counted under optical microscope at x1000 magnification. A highly viscous medium containing 0.5% methylcellulose (Iwadate *et al*., 1997) was used to slow *D. nasutum* swimming and therefore facilitate the counting.

2. Temperature response experiment: The protocols used for this experiment were followed as in Experiment 1 except that they were done under lower temperatures (15 and 3ºC).

Data were expressed in Means ± SE and the analysis of variance (ANOVA) was used to examine the level of significance. Differences at $p < 0.05$ were accepted as significant.

RESULTS

No cysts were found in any of the test tubes observed. All *D. nasutum* examined were motile and there was no evidence of encystment. These results showed that daylength may not be a cue in this ciliate.

The response of *D. nasutum* to temperatures and encystment was varied. When the temperature was kept at 15ºC, the results showed no encystment occurred in any of the tubes. However, at 3ºC, the process of encystment (roughly spherical cysts as revealed by the microscope) in the all sample tubes gradually started to take place. This was regardless of phtoperiodic regimes so that after 12 hours an average of 10% of individuals became necysted. This trend continued until 60 hours and at hour 72 nearly all observed individuals had transformed into cysts, even though there was still a fraction that never became encysted (Fig. 1). There was a significant difference in the number of cysts between three different conditions of light (df 2, 15, $F = 0.105$, $P = 0.901$). The rate of the cyst production in DD (shortest daylength) was fastest, slow in LD and slowest in LL (longest daylength) conditions. These results suggest that temperature is a more critical cue than photoperiod (daylength) in the cyst production of *D. nasutum*.

DISCUSSION

This is the first study to report some data on effect of photoperiod and temperature on the encystment in *D. nasutum*, indicating that daylength is not a single signal in the cyst production. Our data rather suggest that a low temperature (3ºC) is an important trigger for encystment. However, when the temperature was significantly decreased, the cyst was formed sooner in DD condition than in LD and/or LL conditions. This may indicate that in addition to temperature, daylength could be also a cue. While it was not shown, it can be argued that the low

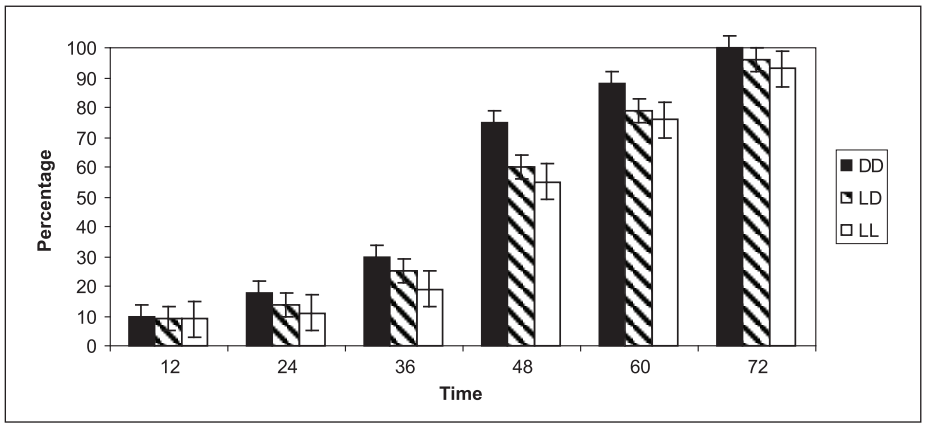

Fig. 1. The occurring of encystment in *D. nasutum* at 3ºC which is regardless of photoperiod. The numbers are average Mean ± SE.

temperature is the only involving factors of the cyst formation since coldness might have been overcome the effect of short daylength (DD).

In most cases, a combination of factors is involved in the process of encystment and in this study these factors were low temperature and darkness. In fact, encystment is induced by adverse changes in the environment which protects the organism against unfavorable conditions. More than one environmental cue modulate encystment (Sgrosso et al., 2001) and different combination of factors may lead to different results. For example, Mast and Ibara (1923) examined the effect of temperature and food, but not photoperiod, on *Didinium nasutum* encystment. They found that the percentage of encystment was greater in cultures with food than those without food and it was greatest at 25-30ºC.

Cyst production (encystment) is a relatively common process occurring particularly amongst freshwater species of protists (Corliss and Esser, 1974; Leadbeater and Karpov, 2000). This phenomenon is a strategy against adverse and harsh conditions such as food shortage, oxygen depletion, pH reduction, high and low temperatures. Generally, alterations in one or more environmental variables trigger cyst formation (Fryxell, 1983; Sandgren, 1988). However in some protozoa such as *Dinobryon cylindricum* the process of encystment is endogenously controlled and depends upon cell density (Sandgren and Flanagin, 1986).

Little is known about the exact effect of environmental cues initiating encystment. Sgrosso *et al*. (2001) found that cyst formation in some dinoflagellates is regulated by combined effect of daylength, temperature and food concentration. In addition, the impact of food upon encystment of protozoa is inconclusive, with mixed results.

In a large number of ciliates a deficiency of food seems to cause encystment for example, in *Podophrya collini* (Root, 1915), *Vahlkampfia calkensi* (Hogue, 1915), *Pleurotrichia lanceolata (*Manwell, 1928; Penn, 1935), *Euplotes muscicola* (Faure-Fremeit *et al*., 1954), *Colpoda inflata* (Martin-Gonzalez *et al*., 1992), *Pelagostrombidium fallax* (Müller, 1996), and *Euplotes elegans* (Tomaru, 2002). Some other protozoa encyst in response to an excess of food such as *Blepharisma undulans* (Stolte, 1924), *Polytomella citri* (Kater and Burroughs, 1926). In the case of *D. nasutum* however, while Beers (1927, 1930) reported a deficiency of food can induce encystment, however it appears that neither absence nor presence of food cause *Didinium* to encyst, but rather the chemical contents of the water in which *Didinium* live has much to do with encystment (Mast and Ibara, 1923). The effect of nutrient limitation however, could be ruled out in the current study because amount of food during times in which the experiments were running was the same for the all test tubes. However, the chemical property of the water was not assessed in the current study and no conclusion can be made in this respect.

It appears that a species-specific temperature requirement must be met to induce encystment. Only at specific temperature and daylength conditions cyst production has been recorded (Sgrosso *et al*., 2001). For instance, Eren (1969) reported that presence or absence of light had no effect of *Peridinium* encystment, whereas in Alster *et al*. (2006) study light did stimulate encystment but only at specific temperature (20ºC). Therefore, our results may be compared with investigations of Alster *et al*. (2006) in that encystment in *D. nasutum* is not a photoperiodically process but rather under control of temperature, occurring at a low temperature (i.e. 3ºC).

The percentage of encysting cells varies and depends upon the species. Range of this figure reported in dinoflagellates varies between 1% (Pollingherd and Hickel, 1991) up to 100% (Olli and Anderson, 2002). Similarly, in this study *D. nasutum* (Exp. 2) encysted mostly after 72 hours (Figure 1). This finding is in agreement with Leadbeater and Carpov (2000), who found that in freshwater choanoflagellate *Desmarella moniliformis* under laboratory conditions and after 72 hours nearly all population produced cyst even though in ciliate *Pelagostrombidium fallax* only a fraction of population may encyst (Müller, 1996).

CONCLUSION

In summary, the results of our experiments show that under the experimental conditions, encystment of *D. nasutum* is mediated by a low temperature (3°C). The influence of other environmental cues such as salinity, dehydration, and pH has yet to be elucidated.

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